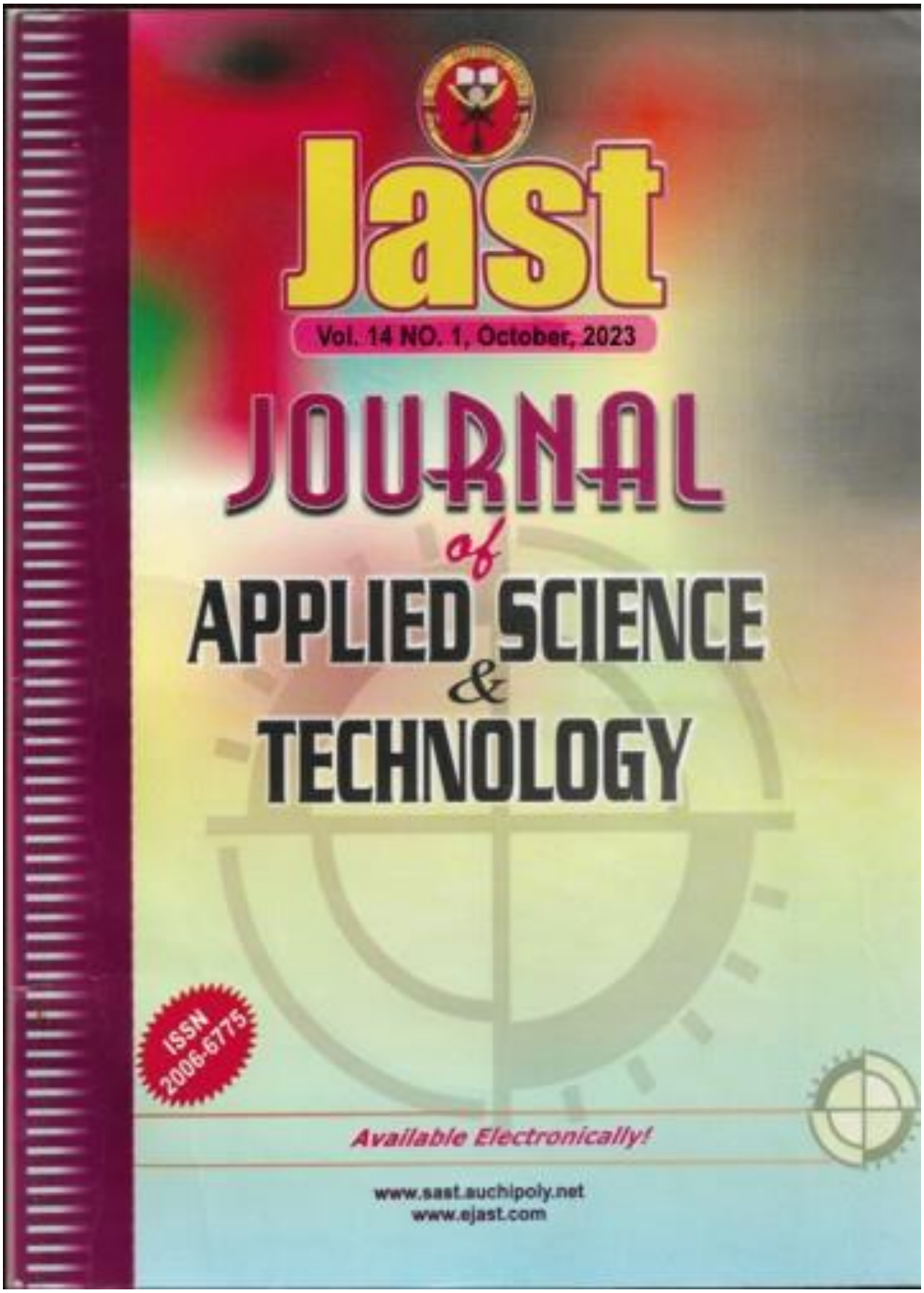
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UV-VIS SPECTROPHOTOMETRIC DETERMINATION OF CAFFEINE IN SOME SELECTED TEA SAMPLES

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ABSTRACT

Caffeine a stimulant, mild additive drug with its medicinal properties is a bioactive ingredient present in some teas and coffee. It occurs naturally in leaves, seeds or fruits of tea, caffeine, cocoa etc. it serves as a boost of energy or a feeling of heightened alertness when taking moderately, but at high does, it could bring about conditions of anxiety and depressive neuroses. In this study the quantification of the caffeine level of seven teas and coffee brands commonly sold in Auchi town was determined by UV/Vis spectrophotometric method. Chloroform was used as the solvent andconcentrations of caffeine measured at the wavelength of 274 nm. The results obtained for the selected tea and coffee samples were; 11.56 ppm, 30.22 ppm, 78.22 ppm for the Top tea, Richmond tea and Lipton tea respectively. While the results for the coffee were 138.34 ppm, 324.33 ppm, 348.22 ppm and 388.12 ppm for the Nescafe classic, Nescafe Malty, Nescafe original and Cowbell coffee respectively. Among the analyzed tea samples thehighest caffeine concentration was found in Cowbell Coffee (388.22 ppm) and the lowest in Top Tea (11.56 ppm). The results of this study gave preliminaryinformationaboutcaffeinelevelsin the oftenconsumedteasdrinksinNigeria. The Food and Drug Administration and Control recommended not more than five cups of tea or coffee containing caffeine (200 mg) per day

Keywords: caffeine, coffee, tea, UV-VIS Spectrophotometer

Introduction

Caffeine is a naturally occurring alkaloid methylxanthinefamily, found in the leaves,seeds and fruits of over 63 plants species worldwide It is a bitter white crystalline compound, with low solubility in water. Its chemical formula is $C_{18}H_{10}N_4O_2$, it systematic name is 1,2,3 trimethylxanthine. It has a molar mass of 194.19 g/mol and a density of 1.2 g/ml (Aurnand 1987).

Caffeine can befound in cola nuts, coffee beans, cocoa beans, tea leaves, mate leaves and otherkinds of plants (Andrews *et al.* 2007). While coffee and tea beveragesnaturallycontaincaffeineandotherm ethylxanthines,caffeineservesasan ingredient in many carbonated soft drink including colas, pepper-type beverages,and citrus beverages.

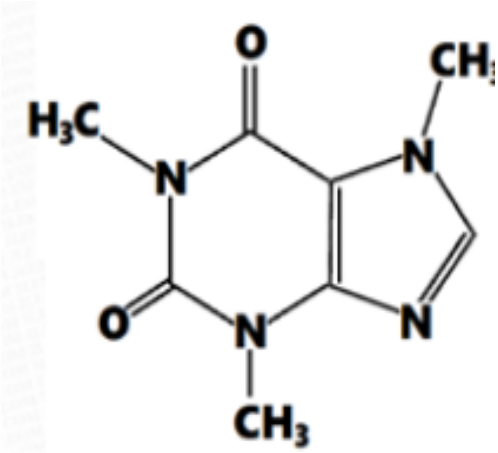

Figure 1 Structure of caffeine

Caffeine is regarded as a pharmacological active substance, it does not accumulate over the course of time and is normally excreted within hours of consumption (Baron and Roberts 1996) and has the ability to serve as a boost of energy or feeling of heightened alertness. It is often taking to stay awake longer, gives a pleasant stimulating feeling but a high dosage it may cause psychological symptoms such as anxiety and depressive neuroses. People with more psychological problem may have their symptoms exaggerated with excessive caffeine dose (Barone and Roberts 1996).)
High levels of caffeine consumption have been implicated in various disorder including gastric acid secretion, kidney malfunction, heart disease and disturbance of the central nervous system such as seizures and delirium. However, caffeine utilized as a co-adjuvant in many pharmaceutical formulations (Bispo *et al*., 2002)

The quality control of products containing caffeine for health and optimal performance and regulation should always be upheld

The reported caffeine content in main dietary sources varies significantly 93.0 – 163.5 mg per cup in instant coffee, 30.2 – 76.4 mg per cup in bag tea and 0.32-.0.54mg/l in dark sweet chocolate. These differences have been attributed to the variety of coffee bean or tea leaf, methods of preparations (i.e. the brewery of coffee and tea) volume of a cup and analytical methods utilized for caffeine determination. In the case of carbonated beverages, the variability occurs among brands, since most of the caffeine content in these products is added from other natural sources, less than 5% of the total present caffeine is from cola nut (Carmago *et al* 1999, Akinbile *et al* 2017)
Coffee, tea and drink with caffeine artificially stimulate the body and increase the heart rate, the artificial stimulation temporarily arouse the intellect and fatigue seems to disappears, but its short lived. The excess stimulation depletes the body of vital energy as it struggles to deal with poison that has entered its system (Khan *et al* 2006)
In this study selected tea and coffee sample were analyzed quantitatively for their caffeine level

Materials and Methods

Materials

The chemicals and reagents; chloroform, sodium carbonates, caffeine standards used in this study were of Analytical Grade product of Sigma- Aldrich (UK). A double

beam UV/Visible spectrophotometer (Jenway 6505), Quartz cuvette, Chemical balance (Metler Toledo AL240) measuring cylinder, magnetic stirrer, glass filter, beakers, thermometer, separatory funnel, funnel, ice bath, distilled water, chloroform (assay: 99.6%, Aldrich Germany), ethyl acetate (assay: 99%, Indian), caffeine (M.W. 194.19 g/mol, Aldrich Germany) were used at the course of the analytical procedure.

Seven brands of instant coffee and teas samples obtained from various selling outlets in Auchi were used for the study. The coffee and tea samples were kept at room temperature throughout the analysis. The caffeine levels were not indicated in their labels

Method

Extraction of Caffeine from Tea

The extraction of the caffeine was determined according to the method of Akinbile *et al.* (2017). The coffee and tea samples (2 g) were each weighed into conical flask,distilled water (20 mL) 2 added to each of the sample and the content were heated and allowed to boil for 10 min.Sodium carbonate (2g) was added to each sample to precipitate tannins. The samples were filtered and the filtrates concentratedto5 ml by heating, the concentrate was placed in a separating funnel, chloroform (5 ml) was added to extract the caffeine present in the samples. The lower layer containing the caffeine was separated and the caffeine content determined with UV-visible spectrophotometer. The extracts (0.1 ml) were mixed with chloroform (10ml) and placed in quartz cuvette. The Absorbance was measured at a wavelength of274 nm

Caffeine stock preparation and determination of Absorbance

Caffeine stock solution (100 ppm) was prepared by dissolving the caffeine (0.01g) in a volumetric flask containing chloroform (100 ml), from thecaffeine stock solution, 1 ppm, 5 ppm,10 ppm, 20 ppm and 25 ppm dilution were prepared. The absorbance of each was measured with a UV-VIS spectrophotometer (Janway 6505 model) at the wavelengthof274 nmusing quartzcurz

ResultsandDiscussions

Results

Table1:AbsorbanceoftheCalibration SolutionofCaffeine

No.	Concentration(ppm)	Absorbance
1	1	0.016
2	5	0.019
3	10	0.028
4	15	0.034
5	20	0.048
6	25	0.058

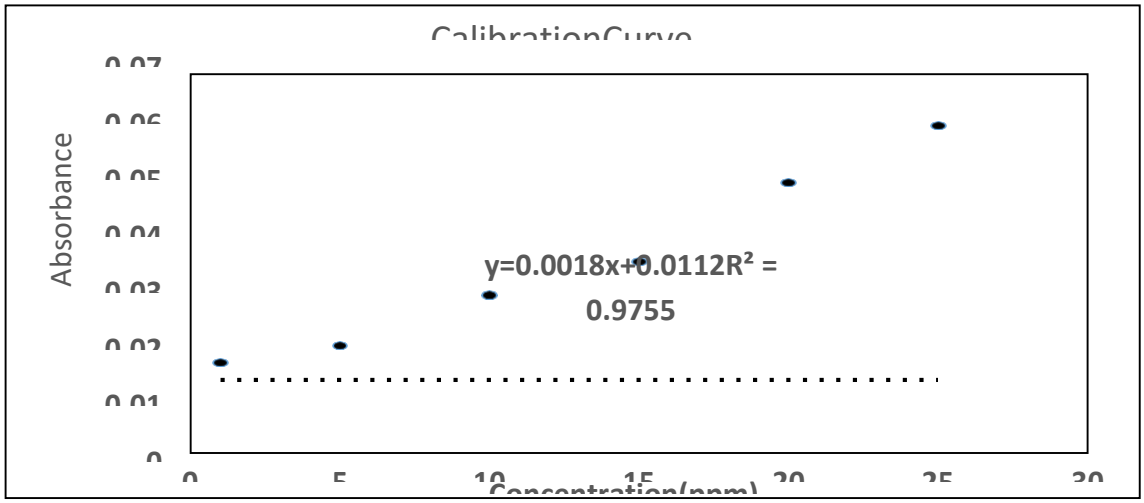

Figure1: Standardcalibrationcurveofcaffeine

Table2:CaffeineContentsofTeaandBeveragesSamples

Label	SampleName	3 Caffeine Concentration (ppm)	EFSA (ppm)
S1	Top Tea	11.56v	400
S2	RichmondTea	30.32	400
S3	LiptonTea	78.22	400
S4	NescafeClassic	138.34	400
S5	NescafeMalty	324.33	400
S6	NescafeOriginal	348.22	400
S7	CowbellCoffee	388.12	400

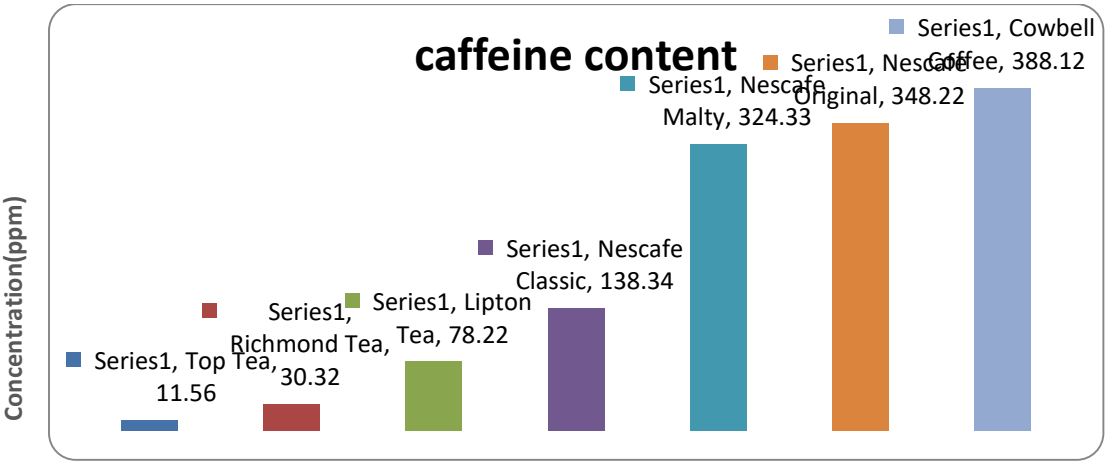

Figure2: Chartshowingcaffeinecontentintheteaandbeveragesamples

Discussion

The standard linear calibration curve obtained from the standard solutions of caffeine is presented in Table 1 and Figure 1. It showed a good linear relation between the absorbance and concentrations of standard solutions. Caffeine content levels in tea samples a represented and illustrated in Table 2 and Figure2. 4

The concentration of caffeine ın tea samples was in the range of 11.56 ppm and 78.34 ppm, while the coffee samples had values ranging from 324.33 ppm to 388.12 ppm. The highest caffeines concentration was measured in Cowbell Coffee and the lowest was measured inTop Tea. The results also indicated that the coffee samples had more caffeine content than the tea samples the results is in agreement with the study of Akinbile et al, (2017)but not in agreement with Wanyika et al, (2010) their finding reveals higher values of caffeine for tea than coffee samples obtained in Kenya market. As stated by Kaplan *et al.* (2011) growing conditions, processingconditionsandothervariablesaffect caffeinecontentandthatcertaintypesoftea contains somewhat more caffeine than other. Some of the factors that can have an effect on the amount of caffeine content include strength of the brew, growing conditions, processing techniques and other variables such as soil chemistry, altitude and position of leaf on the tea bush, type of plant and cultivation practices. Caffeine contention also varies widely depending on the type of bean and the method of preparation used (Heckman *et al*, 2010).

According to the European Food Safety Authority (EFSA) (2015), single doses of caffeine that do not raise safety concerns recommended for adults are up to 200 mg. When healthy adults consume caffeine at a dose of 400 mg throughout the day (about 5.7 mg/kg per day) they do not need to worry about safety (Vuletic *et al*, 2021). Since caffeine content depends on the type of tea and since portion size varies within and between countries one should be careful with caffeine intakes.

Conclusion and Recommendation

The results of this study gave preliminary information about the caffeine content in the selected teas and coffee. Caffeine was indicated to be present in the samples used but the amount present were not specified. The results obtained showed that the caffeine level were within the recommended limits. Caffeine content should be indicated on the product labels especially due to the great popularity and easy accessibility of

caffeine-containing beverages. Since caffeine can be a cause for potential health concerns, precise quantities stated on the labels of caffeinated beverages should be highlighted in the interest of those who drink them. It is necessary to work on raising awareness among those who drink caffeinated beverages about the amounts of caffeine they consume.

REFERENCES

Akinbile Babatunde, Temilola Oluseyi, Olayinka Kehinde Ofudje Andrew (2017). Determination and Characterization of Caffeine in Tea, Coffee and Soft DrinksbyDerivativeSpectrophotometryand High Performance Liquid Chromatography.Biomedicine and Nursing journal; 3 (2) page; 92-101

Aurnaud MJ (1987). The pharmacology of caffeine. Prog. Drug 31: 273.

Andrews, K., Schweitzer, A., Zhao, C., Holden, J.M., Roseland, J.M., Brandt, M., Dwyer, J.T., Picciano, M.F., Saldanha, L.G., Fisher, K.D., Yetley, E., Betz, J.M. and Douglass, L. (2007). The ca 5 content of dietary supple commonly purchased in the US: analysis of 53 products having caffeine containing ingredients. *Analytical and Bioanalytical Chemistry*, 389(1), 231-239. https://doi.org/10.1007/s00216-007-1437-2

Barone, J. J. - Roberts, H. (1996). Caffeine consumption. In *Food Chem Toxicol*, vol. 34, 1996, p. 119-129.

Bispo, M.S., Veloso, M.C., Pinheiro, H.L., De Oliveira, R.F., Reis, J.O., De Andrade, J.B.(2002). Simultaneous determination of caffeine, theobromine, and theophylline by highperformance liquid chromatography,*Journal of Chromatographic Science*, vol.40 (1), pp 45-8;

Camargo, M.C.R., Toledo, M.C.F(1999). HPLC determination of caffeine in tea, chocolate products and carbonated beverages, *Journal ofthe Science of Food and Agriculture*, vol 79 (13), pp 1861-1864;

European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies. (2015). Scientific opinion on the safety of caffeine. EFSA Journal, 13(5),4102.https://doi.org/10.2903/j.efsa.2015.4102

Heckman, M.A., Weil, J. and Gonzales de Mejia, E. (2010). Caffeine (1,3,7 trimethylxanthine) infoods: a comprehensive review on consumption, functionality, safety and regulatory matters. *Journal of Food Science* 75(3), R77-R87. https://doi.org/10.1111/j.17503841.2010.01561.x

Kaplan E, Holmes J H, Sapeika N (1974). Caffeine content of tea and coffee. S. Afr. Med. J., 48: 510-511

Vuletić, N., 2Bardić, L. and 1Odžak, R. (2021). Spectrophotometric determining of caffeine content in the selection of teas, soft and energy drinks available on the Croatian market. Food Research,vol5(2)325-330(April,2021)Journal.homepage:http://www.myfoodresearch.com

Wanyika H. N, Gatebe, E. G., Gitu, L. M., Ngumba E. K. and Maritim C. W (2010) Determination of caffeine content in teas and instant coffee brands found in Kenyan Market. African Journal of Food Science Vol. 4(6), pp. 353 – 358. Available online http://www.academicjournals.org/aj ISSN 1996-0794 ©2010 Academic Journals

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ANTIBACTERIAL POTENTIAL OF NOTABLE ENDOPHYTIC ISOLATES FROM (*Bryophyllum pinnata*) **LEAVES AGAINST** STREPTOCOCCUS AND KLEBSIELLA

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ABSTRACT
The antibacterial potential of notable Endophytic isolates from *Bryophyllum pinnata* leaves against *Streptococcus* and *Klebsiella* was carried out in Microbiology Laboratory, Auchi Polytechnic, Auchi. Plants are known to contain bioactive compounds that can be explored and used in the treatment of infection. The study investigated the antibacterial potential of notable Endophytic isolates from *Bryophyllum pinnata* leaves against *Streptococcus* and *Klebsiella*. The results of this study showed that no zone of inhabitation was noticed for Endophytes from Nutrient broth and Sabouraud broth for *Klebsiella*, the zone of inhibition was zero (0) while zone of inhibition for *Streptococcus* on Nutrient broth was 15mm and 12mm on Sabouraud broth. The Endophytic bacteria isolated was *Bacillus subtilis* and *Endophytic fungi* isolated was *Fusarium species.*

Keyword: *Bryophyllumpinnata,*Endophyte, Streptococcus, Klebsiella.

Introduction

Plants play important roles in discovery associated with new beneficial therapeutic agents andhave received significant focus because of their bio- active substances like antioxidants, hypoglycemic and hypolipidemic factors. Plants have invariably been exemplary source ofdrugs and a number of currently available drugs happen to be derived directly or indirectlyfrom them. This natural source has received considerable attention for discovery anddevelopment of leads as new drug molecules, because of its diversity. Rural people depend onherbal and traditional medicines to cure their diseases as medicinal plants are easily availablein their surroundings and have low cost with increased efficacy and reliability. According toWorld Health Organization (WHO) 80% of the population rely on traditional medicine as asource of primary healthcare needs (Nagaratna, Prakash and Hegde, 2015).

The use of traditional medicines and medicinal plants in mainly developing countries asremedial agents for the maintenance of health has been broadly observed. Modern day pharmacopoeia However, contains at least 25% drugs derived from plants and many others, which are synthetic analogues, built on prototype chemical substances isolated form plants.

Involvement in medicinal plants as a re-budding health assistance has been fuelled with therising charges of prescription drugs in the safeguarding of personalized health and well being and the bio prospecting of new plant derived drugs. Medicinal herbs area source of chemical compounds such as alkaloids, glycosides, saponin, oleoresins, sesqueterpine,lactones and oils. These biologically active ingredients are used for the prophylacticpurposes and for the different infectious diseases. Due to the presence of medicative properties, medicinal

plants have been used in wide area of the world. Many diseases like malaria, epilepsy, diarrhea, dysentery, fungal and bacterial infections have been treated by folklore medicines (Aprioku, 2018).

Bryophyllum pinnatum belongs to the family Grassulaceae an erect, succulent, perennial shrubthat grows about 1.5m height and reproduced from seeds and also vegetatively from leafbubbils. It is an introduced ornamental plant that is now growing as weed around plantationcrops. *Bryophyllum pinnatum* is commonly known as air plant, never die, miracle leaf, love plant. It is used in folk medicine in tropical Africa, tropicalAmerica, India, China, and Australia. It is well known for itswound healing and haemostatic properties. Traditionally, it isused for medicinal purpose for treatment of various ailmentsviz. anthelmintic, immunosuppressive, hepatoprotective, anti-nociceptive, anti-inflammatory, anti-diabetic,nephroprotective, antioxidant, antimicrobial, analgesic,anticonvulsant, neuropharmacological and antipyreticactivities. In South Eastern Nigeria, this herb is used tofacilitate the dropping of the placenta of a newly born baby (Ojewole, 2019).The plant leaf is mildly exposed to heat and the juiceextracted and applied to the baby's placenta on daily basis.The crushed leaves as well as the extracted juice are mixedwith palm oil and rubbed on abscesses. It is usually appliedexternally (Afzal and Kazmi, 2017).

Endophyte is an endosymbiont, often a bacterium or fungus, that lives within a plant for at least part of its life cycle without causing apparent disease. Endophytes are ubiquitous and have been found in all species of plants studied to date; however, most of the endophyte/plant relationships are not well understood. Some endophytes may enhance host growth, nutrient acquisition and improve the plant's ability to tolerate abiotic stresses, such as drought and decrease biotic stresses by enhancing plant resistance to insects, pathogens and herbivores. Although endophytic bacteria and fungi are frequently studied, endophytic archaea are increasingly being considered for their role in plant growth promotion as part of the core microbiome of a plant Das *et al.* (2019). Endophytes were first described by the German botanist Johann Heinrich Friedrich Link in 1809 They were thought to be plant parasitic fungi and they were later termed as "microzymas" by the French scientist Béchamp. There was a belief that plants were healthy under sterile conditions and it was not until 1887 that Victor Galippe discovered bacteria normally occurring inside plant tissues. Though, most of the endophytic studies reports the mutualistic relationship of bacteria and fungus, Das *et al.* (2019) reported about endophytic virome and their probable function in plant defense mechanisms.

Most endophyte-plant relationships are still not well understood. However, recently it was shown that endophytes are transmitted from one generation to another via seeds, in a process called vertical transmission. Endophytes and plants often engage in mutualism, with endophytes primarily aiding in the health and survival of the host plant with issues such as pathogens and disease, water stress, heat stress, nutrient availability and poor soil quality, salinity, and herbivory. In exchange the endophyte receives carbon for energy from the plant host. Plant-microbe interactions are not strictly mutualistic, as endophytic fungi can potentially become pathogens or saprotrophs, usually when the plant is stressed. Endophytes may become active and reproduce under specific environmental conditions or when their host plants are stressed or begin to senesce, thereby limiting the amount of carbon provided to the endophyte Das *et al.* (2019).

Endophytes may benefit host plants by preventing other pathogenic or parasitic organisms from colonizing them. Endophytes can extensively colonize plant tissues and competitively exclude other potential pathogens. Some fungal and bacterial endophytes have proven to increase plant growth and improve overall plant hardiness.

MATERIALS AND METHOD

Sample Collection and Processing of Life Plant (*Bryophyllum Pinnata*) Leaves

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Four (4) fresh leaves were collected from *Bryophyllum pinnata* from a residential area in Iyekhue girls Auchi, Edo State and placed in a sterile polyethylene bag which was transported to the laboratory immediately for analysis.

Study Area

The study was conducted at the Department of Biological Sciences Laboratory Technology at Federal Polytechnic, Auchi. Auchi is located in the northern part of Edo State in Nigeria, specifically at latitude 07°04'N and longitude 06°16'E. The town has a population of over 500,000 people, as per the 2015 population census, and is situated in the south-south geographical zone of Nigeria. It is approximately 130 km away from Benin City, the capital of Edo State. Auchi is the headquarters of Etsako West Local Government Area and has experienced growth due to rural-urban migration. The town is bounded by Jattu to the north, Aviele to the south, Iyakpi to the east, and Owan Local Government Area to the west. Additionally, Auchi is the home of the Federal Polytechnic, Auchi, in Edo State, Nigeria.

Materials

Petri dish, beaker, conical flask, Bijou bottles syringe, detergent, distilled water, foil paper, cotton wool, mythylated spirit, ethanol, electronic weighing balance, masking, tape, cock borer, permanent marker, microscope, measuring cylinder, Bunsen burner, slides, metylene blue, nutrient Agar, Potato Dextrose Agar, Sabouraud Agar, Nutrient broth, Sabouraud broth.

Preparation of Nutrient Agar

Preparation of Leaves for Inoculation

Healthy leaves was plucked and placed into a beaker and washed with detergent over running water for 30 minutes. Two beaker was washed with detergent, one was rinsed with ethanol and ethanol was poured into it 50ml and topped with distilled water 150ml. the other beaker as filled with jik 40ml and was topped with distilled water to 100ml. the solution of ethanol and water was used to rinsed the leaves then the water was drained off. The solution of jik and water was also used to rinse the plant simultaneously.It was rinsed simultaneously 7 times to wash the leaves.

The beaker containing leaf was covered with foil paper to avoid external contamination. A sterile syringe was used to pick one leaf out of the beaker and placed on a petri dish and was divided into four pieces. One place was placed on the prepared nutrient Agar plate and was inoculated over flame aseptically. The process was repeated twice on petri dish containing nutrient Agar and Potato Dextrose Agar. The petri dish containing nutrient Agar and PDA was sealed and labelled. Petri dish containing NA was kept in incubator for 24hours and PDA was kept at room Temperature for 3 days. After 24hour it was removed from the incubator and endophytes growth was observed. After 3 days the PDA was cecked and endophytic growth was observed.

14g of nutrient Agar powder was weighed using a weighing balance and dispensed into a beaker 500ml of distilled water was measured using a measuring cylinder and dispensed into the beaker containing the agar powder, it was stirred to dissolve for 10 minutes. The mixture was transferred into a conical flask and the neck of the flask was corked with cotton wool wrapped in aluminium foil. It was autoclaved at a temperature of 121^0c and pressure of 15Psi for 15-20 minutes. The sterilized agar was allowed to cool to about 450^0c and then aseptically poured into petri dishes and allowed to cool.

Preparation of Potato Dextrose Agar

9.75g of potato dextrose agar powder was weighed using a weighing balance and dispensed into a conical flask 250ml of distilled water was measured using a measuring cylinder and dispensed into a beaker containing the agar powder. It was stirred to dissolve for 10 minutes. The mixture was transferred into a conical flask and the neck of the flask was corked with cotton wool wrapped in aluminium foil. It was autoclaved at a temperature of 121^0c and pressure of 15psi for 15-20 minutes. The sterilized agar was allowed to cool to about 45^0c and then aseptically poured into petri dishes and allowed to set.

Preparation of Nutrient Broth

Fourteen (14) grams of nutrient agar was weighted and dissolved in 500ml distilled water according to manufacturers instruction. The weighted powder was poured into a clean beaker containing measured distilled water and decanted. Filter paper was placed on the orifice of a conical flask and nutrient agar was poured gradually to filter the solution. The filtered solution became the nutrient broth and was poured into bijou bottles and sterilized.

Preparation of Sabouraud Broth

16.25g of Sabouraud Agar was weighed and dissolved in 250ml distilled water according to the manufacturer's instruction. The weighted powder was poured into a clean beaker containing measured distilled water and decanted. Filer paper was placed on the orifice of conical flask and the solution was poured gradually to filter it. The filtered solution became Sabouraud broth and was poured into bijou bottles and sterilized

Identification of Endophytes

Identification of endophytes from the leaves was done by carrying out catalase test and gram staining techniques

Catalase Test

Principle: Catalase degrade hydrogen peroxide and release oxygen which is detected as effervescence. Procedure: 1 or 2 drops of hydrogen peroxide was pipette on clean slider. A few colonies of pure isolates was emulsified on the slide for few seconds. A gas bubble indicates a positive reaction.

Gram Staining

Twenty four hours old culture of the isolates was prepared separately on a clean grease free slide and heat fixed. The smear was stained with crystal violet for 60 seconds. The stain were washed off respectively with water and Gram iodine (a mordant) was applied on the slides for 30 seconds. The slides were immediately rinsed with water. Acetone was added to the slides and rinsed immediately a counter stain which is made up of 0.5% Safranin red was added for another 60seconds. After rinsing of the counter stain, the slides were examined unde,r the microscope using oil immersion objectives lens for the primary colour and the result was recorder

Inoculation of Edophytes

Nutrient broth and Sabouraud broth was poured into separate bijou bottles and sterilized. The Endophytes was then picked with sterilized wire loop. And was inoculated into the bijou bottles, masking tape was used to cover the bottles orifice and was labelled. Nutrient broth containing endophytes as placed in incubator for 24 hours and Sabouraud broth was placed in room Temperature for 3 days to produce metabolites which will be used for antimicrobial analysis.

Collection of Organism

Microorganisms (*Streptococcus species* and *Klebsiella species*) were both collected from Auchi cottage hospital. Nutrient Agar was prepared and poured in bijou bottles in slant and was allowed to gel and was used to collect microorganisms from the cottage laboratory.

Sensitivity of Endophytes Bacteria

After 24 hours growth was observed on nutrient broth, nutrient Agar was prepared and poured into petri dishes and allowed to gel, cock borer was used to boreholes and the prepared Agar, *Streptococcus* and *Klebsiella* was both streaked separately on the surface of the Agar plate and 0.1ml of the endophytes from nutrient broth was poured into the hole and was sealed with masking tape and foil paper and placed inside the incubator for 24 hours

Sensitivity for Endophytic Fungi

After 3 days growth was observed in sabouraud broth and cock borer was used to bore holes on the Agar surface, *Streptococcus and Klebsiella* was streaked separately on different petri dish on the surface of the plate and 0.1ml of Endophytes from Sabouraud broth was poured into the hole and was sealed with masking tape and foil paper and placed in room temperature for 3 days.

RESULTS AND DISCUSSION

Result

The result obtained from the analysis is presented as follow;

Table 1: Activity of Edophytic bacteria of *Bryophyllum pinnatum* on *Streptococcus and Klebsiella* on nutrient broth.

Test Organism	**Zone of Inhibition**	**Endophytic Bacteria**
Streptococcus specie *Klebsiella specie*	15mm Nil	*Bacillus subtilis*

Table 2: Activity of Endophytic fungi of *Bryophyllum pinnatum* on *Streptococcus and Klebsiella* in Sabouraud broth

Test Organism	**Zone of Inhibition**	**Endophytic Fungi**
Streptococcus species	12mm	

Klebsiella species	Nil	*Fusarium species*

Discussion

No zone of inhibition was noticed for Endophytes from nutrient broth and Sabouraud broth for *Klebsiella*, the zone of inhibition was zero (0) zone of inhibition for *Streptococcus* was 15mm on Sabouraud broth. Catalase test was positive (+). Endophytic isolates identified was *Bacillus subtilis* as Endophytic bacteria.

Zone of inhibition was witnessed for Sabouraud broth for *Streptococcus* to be 12mm and for *Klebsiella* was zero (0) there was no zone of inhibition. Endophytic fungi identified was *Fusarium species*

Table 1 shows the activity of Endophytic bacteria of *Bryophyllum pinnata* on St*reptococcus* and *Klebsiella* on Nutrient broth. There was a clear zone of inhibition noticed on Streptococcus specie, 15mm zone of inhibition was noticed on bacterial Endophytes, this results shows that the Endophytes is very effective against *Streptococcus* and should be used in curing disease caused by the *Streptococcus species* like cough. *Klebsiella* on the other hand showed no zone of inhibition which indicates that the Endophytes from Nutrient is not effective against it and cannot be used to cure disease caused by *Klebsiella species*.

Table 2 shows that the activity of Endophytic bacteria of *Bryophyllum pinnata* on *Streptococcus* and *Klebsiellla* on Sabouraud broth, there was no zone of inhibition noticed on *Klebsiella specie*, 12mm zone of inhibition was noticed on bacterial Endophytes on Sabouraud broth, this result shows that the Endophytes is not effective against *Klebsiella* and the Endophytes against *Streptococcus* is effective compared to *Klebsiella. Streptococcus* showed a clear zone of inhibition from both Nutrient broth and Sabouraud broth and should be advised to be used as antibiotics against disease caused by *Streptococcus species*

CONCLUSION

Antibacterial potentials of notable endophytic isolates from *Bryophyllum pinnata* leaves against *Streptococcus* and *Klebsiell*a has been established. Antibacterial activity of the extracts was carried out using agar cup diffusion method. The extracts therefore showed no antibacterial activity against the isolates at all the four concentrations tested. The results of this study proofs that no zone of inhibition was noticed for Endophytes from nutrient broth and sabouraud broth for *Klebsiella*, the zone of inhibition was zero (0) while zone of inhibition for *Streptococcus* was 15mm on Sabouraud broth. Catalase test was positive (+). Endophytic bacterial isolates obtained was *Bacillus subtilis* .

RECOMMENDATIONS

1. It is recommended that the use of *Endophytes from the leaves* should be massively encouraged in drugs just as antibiotics. Also, the Endophytes of the leaf should be combined to further strengthen their antibacterial properties.
2. Furthermore, further studies should be conducted on Endophytes from *Bryophyllum pinnatum* against broader microbial isolates.

REFERENCES

Afzal, M. and Kazmi, I. (2017). A Novel AntimicrobialPhenanthrene Alkaloid from Bryopyllum pinnatum. *E-Journal of Chemistry*, 8(3), 1456-1461.

Ahamefula, U. (2017). Antiulce of Methanolic Extract of *Bryophyllum pinn* *Biol*

Aprioku, J. S. (2018). Effects of Aqueous *Bryophyllum pinnatum* Leaf Extract of Haematological, Renal and Sperm Indices in Wistar Rats. *Indian Journal of Pharmaceutical Science*, 1(2) 203-211.

Gwehenberger, B. (2020). Effect of *Bryophyllum* pinnatum versus fenoterol on uterine contractility. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, 13(2)5-9

Jain, V. C. (2019). Antioxidant and antimicrobial activities of *Bryophyllum calycinum* salisb leaf. *Pharmacologyonline*, 1: 393-405.

John, S. (2016). Basella rubra and Bryophyllum: a promising treatment for stone (pathari) disease. 4(3): 16-17:2.

Majaz, Q. A and Tatiya, A. U. (2018). A photochemical and pharmacological review. *International Journal of Research in Ayurveda and Pharmacy*, 2(5):1478-1482.

Nagaratna, A., Prakash, L and Hegde, A. (2015). Comprehensive review on parnabeeja(*Bryophyllum pinnatum*(lam.) Oken. *Journal of Ethnopharmaceucology*, 230-237.

Ojewole, O. (2019). Antihypertensive properties of *Bryophyllum pinnatum* (lam) oken} leaf extracts. *American Journal of Hypertension*, 15(4): 56-66.

Plangger, N. (2019). Intravenous tocolysis with *Bryophyllum pinnatum* is better *Obstetrics & Gynecology and Reproductive Biology*, 124:168-172.

RossiBergmann,B.(2017).Immuno suppressive effect of the aq extract of *Kalanchoe pinna* mice. *Journal of Chemistry.* 3(3).15-22.

Salahdeen.H.M.(2019).Neuropharmacologic al Effects of Aqueous Leaf Extract of *Bryophyllum Pinnatum* in Mice. *African Journal of Biomedical Research*, 9 (6):101 – 107.

Thorat, S. S and Shah, R. R. (2018). Mohite SA, Patel NR. A review on *Bryophyllum pinnatum*. *International Resources Journal of Pharmaceucy.* 8(12):1-3

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EFFECT OF MUNICIPAL SOLID WASTE ON THE GROWTH OF AFRICAN WATERMELON (*Cucumeropsismannii*)

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ABSTRACT

This study investigated the effect of municipal solid waste dump soil on the growth of *Cucumeropsismannii*. The pristine soil (top soil) collected from the biological garden at 0-10cm depth on a random sampling basis were pulled into a composite soil sample (stock soil) and put into 23 by 20.2cm perforated plastic bucket to a weight of 5.5kg. The municipal solid waste dumps soil (top soil).Collected from the waste dump site from 0-10cm depth on a random sampling basis were pulled into a composite soil sample (stock soil) and put into 23 by 20.2cm to a weight of 5.5kg . Concentrated soil (control soil) 0% was represented by the stock soil (pristine soil). While concentrated soil 100% was represented by the municipal solid waste dump soil obtained from the waste dump site. 50% concentrated soil was obtained by mixing the pristine soil (control soil) with municipal solid waste dump soil obtained from the solid waste dump site in the ratio 1:1. The seeds were sown to an approximate depth of 3cm in the soil samples. Five seeds per bucket with three replicates per treatment were used. The entire set up was left under the prevailing environment condition for 30days.Percentage seedling emergence was lowest in control (26.60%) when compared to 50% municipal solid waste dumped soil (33.30%) and 100% municipal solid waste dump soil (53.30%). The lowest plant height (5.83cm) was obtained from control while the highest value (6.33cm) was obtained from 100% municipal solid waste dump soil. Leaf area was lowest in control soil (62.18cm^2) when compared to 50% municipal solid waste dumped soil (84.5cm^2) and 100% municipal solid waste dumped soil (1100.00cm^2). These result implies that the municipal solid waste dump soil supported the growth and development of *Cucumeropsis mannii***.**

Keywords: *Cucumeropsis mannii,* Solid waste, Soil

INTRODUCTION

In developing countries such as Nigeria have open dumpsites as common practice due to the low budget for waste disposal and lack of political will. A good amount of the city garbage is dumped in low lying areas which poses serious threat to groundwater resources and soil (Akinbile, 2012; Agamuthu and Fauziah, 2011). Many studies show evidence of seriousness of hazards caused by open waste dumping ultimately affecting the plant life on the planet leading towards an irreversible erosion trend unless the present land use pattern is checked (Phil-Eze, 2010). Solid waste pollutants serve as an external force affecting the physicochemical characteristics of soil ultimately contributing towards the poor production of vegetation (Christensen *et al.,* 2014). Nigeria is generally faced with rapid deterioration of environmental conditions due to the conventional system of collection and dumping of solid wastes. Therefore, waste management has become a major concern in cities. Little efforts have been made in order to improve the waste collection and disposal facilities.

Cucumeropsis mannii, commonly known

as white melon seed is a member of the cucubitaceae family. The plant is aspecies [1]Eguasa , H. Uof melon native to tropical West-Africa where its cultivation is usually associated to banana plant, corn and cassava (Fomekong *et al.,* 2008). It is consumed largely as thickener of traditional soup called egusi soup in Nigeria, Republic of Benin and pistachio soup in Coted'Ivore (Koffi *et al.,* 2008; Hanno and Susanne, 2010). The seed constitute about 44% oil (Badifu and Ogunsua, 1990). It therefore represents a very good source of lipase. Despite its agronomic and cultural (traditional medicine) importance, the plant lack attention from research and development so that it is categorized as orphan crop (Loukou *et al.,* 2007). The Limit of proper knowledge of other possible utilization of the seed apart from consumption as food and in traditional medicine is a major deterrent to its wider production, which should result to increased income for the local farmers. Finding its use as a source of industrial material (source lipase) would encourage its production and therefore improve the local economy. Their nutritional value is associated with the presence of sugars, protein, fats, vitamins, organic acids, and mineral compounds, whose content is affected by different variability factors (Malik *et al.,* 2014;Koubala *et al.*, 2016). Melon fruits also contain numerous secondary metabolites (including antioxidants) (Alagar Raja *et al*., 2015, El-Din Ibrahim and El-Masry, 2016), which allows us to include them in the group of health-promoting vegetables. The surface area cultivated with melon has increased in the last four years in many countries, as has cultivation using low-quality saline waters in some semiarid regions (Botía *et al.,* 2018). Semiarid regions with a shortage of rain and scarce good quality waters must make use of low quality water for irrigation. This water comes from aquifers or from seawater intrusion in coastal areas. The use of saline water sources may be limited by the salt tolerance of the crop. In many coastal areas of southern Italy where the groundwater contains high concentrations of NaCl, there is an increasing tendency among farmers to apply saline-sodic water for irrigation. Successful melon cultivation does, however, occur in Sicily's Pachino region, an area affected by salinity in the province of Siracusa; the quality of these cultivated melons is good despite the salinity. Studies of the effect of increasing salinity on key agronomic and economic parameters in this crop are scarce, but there is increasing attention from researchers. Several authors (Botía *et al*., 2005; Mendlinger and Pasternak, 1992; Mangal *et al.,* 2018; Shannon and Francois, 1978) who have studied melon tolerance to salinity during the full crop cycle or at particular phenological phases (del Amor *et al.,* 2019) have concluded that the melon is sensitive, to moderately sensitive, to salinity. These studies focused on crop yield response, on melon quality characteristics. (Botía *et al.,* 2005; Shannon and Francois, 2018). Greenhouse growing conditions were also examined (Nukaya *et al.,* 1980). Specific experimental results, however, are lacking for growth analysis, the salinity tolerance of melon (cv. Tendral) and the effects of salinity on melon preservability. Preservability is, for this type of melon, an essential and important characteristic being the main European producers (respectively 35%, 34%, and 13% of the total harvest). *C. melo* is characterized by significant genetic and phenotypic variability, which offers great possibilities of using this species in molecular breeding (Garcia-Mas *et al.,* 2012; Pavan *et al.,* 2017). Naudin's taxonomy divides *C. melo* into a single wild variety, *C. melo* var. agrestis, and cultivated varieties: cantalupensis, inodorus, conomon, dudaim, flexuosus and momordica (Liu, 2004).

MA MATERIALS AND METHODS

Stu Study Area

The study was conducted at the Department of Biological Sciences Laboratory Technology at Federal Polytechnic, Auchi. Auchi is located in the northern part of Edo State in Nigeria, specifically at latitude 07°04'N and longitude 06°16'E. The town has a population of over 500,000 people, as per the 2015 population census, and is situated in the south-south geographical zone of Nigeria. It is approximately 130 km away from Benin City, the capital of Edo State. Auchi is the headquarters of Etsako West Local Government Area and has experienced growth due to rural-urban migration. The town is bounded by Jattu to the north, Aviele to the south, Iyakpi to the east, and Owan Local Government Area to thewest.

Additionally, Auchi is the home of the Federal Polytechnic, Auchi, in Edo State, Nigeria.

Materials

The plant materials used for this study was *cucumeropsismanni*. The seeds were obtained from a local market in Auchi and in one purchase enough seeds were gotten for the study.

Municipal solid waste dump soil was obtained from a dumpsite located in Auchi pristine soil (top soil) was obtained from the biological garden of Department of Science Laboratory Technology, Auchi Polytechnic, Auchi.

Methods

Seed Viability Test

Seeds were placed in a bowl of water and left for 20 minutes. Submerged seed were collected and used while the ones that remained afloat were discarded.

Soil Treatment

The pristine soil (top soil) collected from the biological garden at 0-10cm depth on a random sampling basis were pulled into a composite soil sample (stock soil) and put into 23 by 20.2cm perforated plastic bucket to a weight of 5.5kg. The municipal solid waste dump soil (top soil) collected from the waste dumped soil from 0-10cm depth on a random sampling bases were pulled into a composite soil sample (stock soil) and put into 23 by 20.2cm to a weight of 5.5kg .concentrated soil (control soil) 0% was represented by the stock soil (pristine soil). concentrated soil 100% was represented by the municipal solid waste dump soil obtained from the waste dump site. concentrated soil 50% was obtained by mixing the pristine soil (control soil) with municipal soil waste dump solid method Meobtained from the solid waste dump site in the ratio 1:1. The seeds were sown to an approximate depth of 3cm in the soil samples. Five seeds per bucket with three replicate per treatment were used. The entire set up was left under the prevailing environmental condition for 30days.

Seedling Emergence

Percentage seedling emergence was the total number of seedlings per bucket divided by the total number of seeds sown (5) and multiplied by one hundred. This was taken up daily to the 15^{th} days. Seeds which failed to sprout after 15 days were regarded as non-viable.

Measurement of Height

The height was measure with meter rule from the soil level to the terminal bud. Measurement was taken on a seven days interval up to 30days after sowing.

Measurement of Leaf Area

The leaf area was determined by measuring the length and width (at the widest point) of each leaf. The product of this was multiplied by a correction factor of 0.75 to cater for leaf shape (Wath, 1973).

Measurement of Leaf Number

The number of leaves per plant was physically counted on a 7days interval up to 30days after sowing.

RESULT AND DISCUSSION

Results

Table 1: Effect of municipal solid waste dumpsite on seedling emergence of *Cucumeropsis mannii*

MSW Concentration	seedling emergence (%) in days				
	3	6	9	12	15
100%	0.00	13.30	46.60	53.30	53.30
50%	0.00	13.30	20.00	26.60	33.30
control	0.00	0.00	6.60	20.00	2 6.60

Table 2: Effect of municipal solid waste dumpsite on the height of *Cucumeropsis mannii*

MSW Concentration	Height (cm) in days				
	3	**7**	**14**	**21**	**28**
100%	2.33	4.83	5.66	5.66	6.33
50%	0.83	3.16	4.83	4.83	5.86
control	0.66	2.83	3.16	3.16	5.83

Table 3: Effect of municipal soil waste on dumpsite the leaf area of *Cucumeropsis mannii*

MSW Concentration	Leaf area (cm^2) in days			
	7	**14**	**21**	**28**
100%	8.00	22.87	76.00	110.00
50%	4.73	21.37	46.50	84.50
control	2.30	16.23	46.12	62.18

Table 4: Effect of municipal solid waste on dumpsite on the leaf number of *Cucumeropsis mannii*

MSW Concentration	Leaf number in days			
	17	**14**	**21**	**28**
100%	1.30	5.00	6.30	7.60
50%	0.60	3.00	4.60	7.30
control	0.60	2.60	3.60	6.00

Plate A: *Cucumeropsis mannii* at 30 days after sowing in control and treated soil

DISCUSSION

Table 1 shows the result of seedling emergence of *cucumeropsis mannii* when sown in municipal solid waste dump soil. It was observed that percent seedling emergence of *Cucumeropsis mannii* was lowest when sown is control (26.60%) than in 50% municipal solid waste dump soil (33.30%). The highest percent seedling emergence (53.30%) was observed in 100% municipal solid waste dump site. This result is in agreement with Ogbeibu *et al.*, (2003). Who stated that the used of dumpsite soil on farmland is common practice in urban and sub-urban countries such as Nigeria because of the belief that decayed and composited wastes enhance soil fertility.

Table 2 and plate A shows the result of the plant height when *cucumeropsis mannii* was sown in municipal solid waste dump soil. It was observed that percent plant height of *cucumeropsis mannii* was lowest in control (5.83cm) than in 50% municipal solid waste dump soil (5.86cm). The highest plant height was observed in 100% municipal solid waste dump soil (6.33cm).

Table 3 shows the result of leaf area of *cucumeropsis mannii* when sown in municipal solid waste dump soil. It was observed that the leaf area of *cucumeropsis mannii* was lowest in control (62.18cm^2) thanin 50% municipal solid waste dump soil (84.5cm^2). The highest leaf Area is (110.00 cm^2) was observed in 100% municipal solid waste dump soil.

Table 4 shows the result of the leaf number of *cucumeropsis mannii* sown in municipal solid waste dump soil. It was observed that the leaf number of *cucumeropsis mannii* was lowest control soil (6.00) than in 50% municipal solid waste dump soil (7.30).The highest number was observed for 100% municipal solid waste dump soil (7.60).

Conclusion and Recommendation

This study shows the performance of *Cucumeropsis mannii* on soil amended with municipal solid waste dump soil. It was observed that the percent seedling emergence plant height, leaf area, and leaf number increased in municipal solid waste dump soil. This implies that municipal solid waste dump soil support the growth and development of *Cucumeropsis manniii.* It is therefore recommended that the application of municipal solid waste dump soil for plant growth should be encouraged.

References

Achudume, T. G and Olawale, D. J. (2007). Modifications of polyuronides and hemicelluloses during muskmelon fruit softening. *PhysiologiaPlantarum,* 76:303-305

Agamuthu, I and Fauziah, R. (2011). Delayed leaf senescence in ethylenedeficient ACC-oxidase antisense tomato plants: molecular and physiological analysis.*The Plant Journal.,* 7: 483-489.

Alagar, R, M., Sahithi, G., Vasanthi, R., Banji, D., Rao, K. N. V and Selvakumar, D. (2015). Study of phytochemical and antioxidant activity of Cucumismelo var. agrestis *fruit. J. Pharm. Phytochem.,* 4(2): 303-306.

Alloway, P and Ayres, R. (2017). Effect of mulching with film of different colours made from original and recycled polyethylene on the yield of butterhead lettuce and celery. *Folia Hort. Ann.,* 19(1): 25-35.

Anikwe, B. R. (2009) .The nature of genetic divergence in relation to breeding system in crop plants.*Indian Journal of Genetics,* 26:188-198.

Badifu, C and Ogunsua, C. (1990). Morphological evaluation and comparison of Hungarian and Turkish melon (*Cucumismelo L.*) germplasm. *ScientiaHorticulturae,* 124(2): 170-182.

Botía, Y., Sa'ar, U., Distelfeld, A., Katzir, N., Yeselson, Y., Shen, S and Schaffer, A. A. (2005). Development of sweet melon (*Cucumismelo L.*) genotypes combining high sucrose and organic acid content.*J. Amer. Soc. Hort. Sci.,* 128(4): 537-540.

Christensen, B. B. Bassang'na, G., Yapo, B. M and Raihanatou, R. (2014). Morphological and biochemical changes during muskmelon (Cucumismelo var. Tibish) fruit maturation. *J Food Nutr Sci*., 4(1): 18-28.

El-Din Ibrahim, M. E and El-Masry, H. G. (2016). Phenolic Content and antioxidant activity of cantaloupe (Cu cumismelo var. cantalupensis) and food application. *Int J Nutr Food Sci.,* 5(1): 16-24.

Hoagland, D. R and Arnon, D. I. (1950). The water culture method for growing plants without soil. California Agricultural Experimental Station Circular, 347: 1-32.

Koubala, B. B., Bassang'na, G., Yapo, B. M and Raihanatou, R. (2016). Morphological and biochemical changes during muskmelon (Cucumismelo var. Tibish) fruit maturation. *Journal Food Nutrition Science.* 4(1): 18-28.

Liu, I. A. (2014). Evaluation of different muskmelon (Cucumismelo) cultivars and production systems in oman. *International.Journal Agric Biol.*, 11(5): 596-600.

Loukou, M. R., Koohkan S., Fanaei, H. R and Pahlavan M. R. (2017). Application of Artificial Neural Networks to predict the final fruit weight and random forest to select important variables in native population of melon (*Cucumismelo L.*). *Scientia Horticulturae.* 181: 108-112.

Mangal , A., Jaskani M. J., Khan I. A., Ahmad S., Ahmad R., Luo S and Ahmad N. M. (2018). Genetic diversity of Pakistani guava (*Psidiumguajava L.*) germplasm and its implications for conservation and breeding. *Scientia Horticulturae,* 172: 221-232.

Mbagwu, E. (2019). Mulching methods and their effects on the yield of tomato (Lycopersiconesculentum, Mill.) in the zeta plain. *Agricult Forest*, 52(14): 17-33.

Nukaya, E., Murty, B. R and Arunachalam, V. (2014). The nature of genetic divergence in relation to breeding system in crop plants.*Indian Journal of Genetics*, 26: 188-198.

Ogbeibu, J., Rosa, R., Kosterna-Kelle, E., Zaniewicz-Bajkowska, A and Panasz, M. (2013). The effect of transplanting date and covering on the growth and development of melon (*Cucumismelo L.*). ActaAgrobot., 70(2): 1699.

Okecha, D. J. (2010). Irrigation scheduling to increase muskmelon fruit biomass and soluble solids concentration. *Hort. Sci*., 41(2): 367-369.

Pavan, N., Villegas, D., Casadesus, J., Araus, J. L and Royo, C. (2017). Spectral vegetation indices as nondestructive tools for determining durum wheat yield. *Agronomy Journal*, 92: 83-91.

Phil-Eze, A. (2010). Yield potential and fruit quality of scallop squash (Cucurbitapepo L. var. patissoninaGreb. f. radiataNois.) cultivars grown for processing. Acta Agrobot., 68(3): 261-266.

Piccolo, K and Mbagwu, H. (2017). Agro-morphological characterization and assessment of variability in local germoplasm of *Cucumismelo L*. in Tunisia.*Journal of Biodiversity and Environmental Sciences*, 3(12): 198-20

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ISOLATION AND IDENTIFICATION OF BACTERIA FROM DRIED TOMATOES, LOCALLY MADE TOMATOES PASTE AND SPOILT TOMATOES

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ABSTRACT

Tomatoes (*Solanum lycopersicum*) is regarded as one of the most important vegetable crop, grown all over the world and Nigeria in particular, after onions and pepper. It has been noticed that tomatoes easily get deteriorated on time due to its high moisture content, microorganisms and biochemical reactions are responsible for it spoilage even though this fruit harbor lots of benefits. One of the most well-known tomatoes eating benefits is its lycopene content a vital anti-oxidant that helps in the fight against cancerous cell formation as well as other kinds of health complications and disease. This research work aim on the comparative study on the bacteria load of spoilt tomatoes, locally made tomatoes paste and dried tomatoes. The tomatoes used in the study were obtained from Uchi market in Auchi, Edo State, Nigeria and were taken to the laboratory for microbiological analysis. The product was divided into 3 portion/samples (Dried, paste and fresh which was allowed to undergo spoilage) and they were subjected to culturing before isolation and identification of organisms were done. Bacteria loads were counted and compared between the three samples. It was noticed that the fresh tomatoes has the highest bacteria load of 4.0 $x10^3$ because the fresh tomatoes was left to undergo spoilage while the sample that was dried had the least count of bacteria which is 1.8 $x10^3$ because of its reduced moisture content. From the study the bacteria isolated includes *Bacillus spp, Enterobacter spp, Lactobacillus spp,Staphylococcus aureus* and *Escherichia coli.*

Keywords:isolation, identification, tomatoes, bacteria, drying and spoilage

INTRODUCTION

Tomato (*Solanum lycopersicum*) is a perennial plant but usually grown as an annual plant. This plant is dicots,, and regarded as one of the most important vegetable plant grown all over the world and Nigeria in particular, after onions and pepper. It is reported that this plant can reach up to 3 meter, and grows as a series of branching stems, with a terminal bud at the tip that does the actual growing. The stems are somewhat weak and often require staking or support such as a cage, while the leaves are about 10 to 30 cm long and unevenly impair pinnate compound with variously indented or lobed margins. It has lateral buds that take over and grow into other, fully functional, vines (Peet, 2008).

Botanically, tomato is a fruit, however, it has much lower sugar content than other fruits and is therefore not as sweet (Ghosh, 2009). Typically served as part of a salad or main course of a meal, rather than as dessert, it is considered a vegetable for most culinary purposes. One exception is that tomatoes are treated as a fruit in home canning practice.

The tomato cultivars differ a great deal in size, shape and colour. There are also yellow, orange, green and brown varieties of tomatoes. The shape can varies from small cherry tomatoes, pear shaped tomatoes to large irregular shaped beefy tomatoes. The shape, size and colour of tomato decide their market value. *Solanum lycopersicum* can be consumed as paste, puree, pulp, juice and ketchup as well as sliced in salad.

They are a good source of vitamin C and vitamin A equivalents (in the form of β-carotene) and provide some vitamin E, folic acid, potassium and other trace

elements. One of the most well-known eating benefits of tomatoes is its lycopene content, which is a vital anti-oxidant that helps in the fight against cancerous cell formation as well as other kinds of health complications and diseases such as it has been noticed that diets that include tomato have been linked with reduced risk of obesity and some neurological diseases including Alzheimer's disease (Sato *et al*., 2012). This crop is cultivated in a lot of countries and Nigeria is been ranked as the second largest producer of tomato in Africa
and also the 13th in the world. This produce contain high moisture content of 65-95% (w/w) water which have make their handling, transportation and marketing a problem especially in the tropics (Garg *et al*., 2013).

Due to lack of post-harvest enterprise and poor post-harvest storage plans, Nigeria is unable to meet its domestic demands for tomatoes even though it ranks on the world tomato production hierarchy due to high moisture content that has led to the quick perishable attribute and high load of microorganisms such as *Escherichia coli, Staphylococcus aureus, Bacillus spp,* on this crop rendering it unfit for consumption after being left for a long period of time without preservation. These microorganisms are active spoilage agents of fresh and preserved produce which render produce poisonous by their activities. They are affected by temperature, moisture, oxygen concentration spoilage organisms and the presence or absence of growth inhibitors. Fresh tomatoes can become infected through injuries caused by careless handling during harvesting and by disease insect, or other animal damage.

Tomatoes due to it fast deteriorate capacity after harvest and easy microbial infestation requires preservation and/or processing in other to extend its shelf life.
Drying tomatoes is one of the easiest known preservation methods. The amount of time it takes to dry tomatoes depends on the tomato variety, the air's humidity during the drying process, the thickness of the tomato slices, or pieces, and the efficiency of the dehydrator or oven. Traditionally, drying has been used as a method of preserving foodstuffs in Nigeria and other developing countries (Akinyele *et al.,* 2012) and its basically removes moisture from food and this inhibits the growth of bacteria and fungi. Moreover, it slows down the enzyme action without deactivating them. These factors ensure that food does not spoil easily and hence, makes drying an effective food preservation technique. Since tomato is seasonal, preservation is important to minimize wastage and spoilage during the tomato producing season and to ensure that maximum nutritional contents of the fruits are retained. The success of such effort would lead to the development of a suitable and usable technology profile that would ensure the optimization of the production of good quality dried tomato fruits at small and medium scale levels.
Hence, this study, therefore, aims at determine the effect of drying (sun drying) and converting of tomatoes into paste under hygienic condition, and the effect on in reducing the microbial loads on tomatoes and also extending its shelf live for consumption, with a view to reducing wastages and increasing the income generation of rural farmers.

MATERIALS AND METHOD

Purchase of tomatoes fruits

The tomatoes used for the study was obtained from Uchi market in Auchi, Edo State, Nigeria and was divided into three groups. The first group was subjected to drying (sun drying), the second group was converted to paste and was done under a hygienic condition while the last was not process but left in it fresh state and it was allowed to undergo spoilage. From each groups little samples were taken and the microbial load were analyzed and pour plate method was used.

Drying Of Tomatoes

Sun drying was used in this process. The tomatoes were washed thoroughly with clean water, sliced into smaller sizes, arranged properly in a clean big tray and then covered with a clean net to prevent flies before it was placed under the sun to dry. This process was done for three (3) days until dryness of the sample was obtained.

The tomatoes were properly washed with clean water and then blended using a clean blender after which it was poured into a pot and boiled until the moisture in it drastically reduced and it becomes paste.

For it packaging the paste was introduced into a clean glass jar and sealed properly before the enclosed jar was then introduced into a pot of water and allowed to heat up for some minutes thereby reducing and killing any microorganisms that could have gotten into the enclosed jar.

Sterilization and preparation of Agar

All glass wares and materials used for processing of the tomatoes were washed and sterilized using a hot air oven while the media was sterilized using an Autoclave at 121^0C for 15 minutes. Nutrient Agar was used for the cultivation of bacteria and it was prepared according to manufacturer's instructions. Serial dilution was used to obtain the aliquot and pour plate method was used in plating. This was done under a sterile environment. After plating the loaded petri plates were kept in the incubator for 24 hours at 37^0C. After 24 hours of incubation the morphological characteristics of the organism was observed, gram reaction and biochemical test were done on the isolates.

Bacteriological Analysis

Colonies from the primary plates were aseptically picked with a sterile wire loop and transferred into freshly prepared sterile nutrient agar plate, with a streaking technique such that discrete colonies appear at the ends of streaked lines after incubation. The subculture plates were incubated at 37°C from 24 hours to 48 hours. Discrete colonies from the subculture plates were aseptically transferred and streaked on slant and incubated for another 24 hours at 37°C (Akinmusire, 2011)

Identification Tests

Identification tests were done using the standard method given by Sherman (2005) these tests include Gram stain, Coagulase test, Catalase test, Indole test

Gram Reaction

This reaction differentiates the Gram positive from the Gram negative bacteria due to differences in their cell wall structure. A sterile wire loop was used to obtain a colony of the isolate on the plates and emulsified on a clean glass slide, it was smeared and air dried completely. The fixed smear was covered with crystal violet stains for 60 seconds and rinsed with clean water. Iodine was then used on the smear for 60 seconds and rinsed off with clean water. It was decolorized with acetone alcohol and flushed out with sterile clean water lastly the smear was then covered with safranin stain for 1 minutes and rinsed with clean water. The back of the slide was wiped clean and allowed to air-dry after which the smear was examined microscopically, first with the X40 objective and the oil immersion objective to examine the bacteria.

Catalase Test

This test is used to differentiate those bacteria that produce the enzyme Catalase, such as Staphylococci from non-catalase producing bacteria such as Streptococci. A smear of the bacterium was made on a slide using sterilized wire loop. About 2 drops of 3% hydrogen peroxide was added

Production of dried tomatoes

A clean blender was used in blending the sun dried tomatoes, and a powder like result was obtained. The grinded dried tomato was then kept in a clean glass container and properly sealed.

Production of paste tomatoes And Packaging

onto the suspension on the slide. The production of gas bubbles indicates a positive reaction.

Indole test

5ml of peptone water was dispensed into a testtube and the organism was introduced into the medium and was incubated for 24 hours at 37^0C. After incubating the medium was shake properly and allowed to mix well and then 3 drops of KOVACS reagent was added to the medium and it was observed for pink colouration.

Coagulase test

A drop of saline was placed on each on a slide; a wire loop was used to pick the colony and was emulsified on the saline. A drop of human plasma was add to it and mixed gently. A positive result gives clumping of the organism within 10 seconds while for a negative result no clumping of the organisms.

RESULTS AND DISCUSSION

RESULTS

Table 1: Total bacteria Count

S/N	Sample	No. of Colonies CFU/ml
1	Dried tomatoes	1.8×10^3
2	Paste tomatoes	2.6×10^3
3	Fresh tomatoes that has undergone spoilage	4.0×10^3

The presence of bacteria per ml of sample taken from each samples and their Colony Forming Units (CFU) after incubation are displayed in (Table 1) the fresh tomatoes had the highest Colony Forming Units of 4.0×10^3 (CFU/ml) while dried tomatoes showed the lowest CFU of 1.8×10^3 CFU/ml) for bacteria.

Table 2 : shows the Morphology and gram's staining characteristics of the isolated bacteria while the Biochemical test for isolated organisms is seen in table

Table 2: Morphology and gram's staining characteristics

Sampl	Colour	Shape	Elevation	Margin	Cell shape	Gram's Reaction
DT_1	Off white	Circular	Flat	Entire	Rods in chain	+
DT_2	White	Circular	Raised	Entire	Cocci	+
PT_1	White	Circular	Raised	Entire	Rods	+
PT_2	Off white	Circular	Flat	Entire	Rods in chain	+
FT_1	Grayish	Circular	Raised	Entire	Rods	-
FT_2	Golden yellow	Circular	Convex	Entire	Cocci	+
FT_3	Off white	Circular	Flat	Entire	Rods in chain	+

KEYS

DT = Represent bacteria isolated from dried tomatoes sample

PT = Represent bacteria isolated from paste tomato sample

FT = Represent bacteria isolated from fresh tomato sample that has undergone spoilage.

Table 3: Biochemical Reaction on Isolate

Isolate	Catalase	Oxidase	Indole	Coagulase	Microorganism
DT_1	+	-	-	-	*Bacillus spp*
DT_2	+	-	-		*Enterobacter spp*
PT_1	-	-	-	-	*Lactobaccllus spp*
PT_2	+	-	-	-	*Bacillus spp*
FT_1	+	-	+	-	*Escherichia coli*
FT_2	+	-	-	+	*Staphylococcus aureus*
FT3	+	-	-	-	*Bacillus spp*

KEYS

DT = Represent bacteria isolated from dried tomatoes sample

PT = Represent bacteria isolated from paste tomato sample

FT = Represent bacteria isolated from fresh tomato sample that has undergone spoilage.

Discussion

In this study the bacteria identified are *Bacillus spp, Enterobacter spp, Lactobacillus spp,Staphylococcus aureus* and *Escherichia coli.Bacillus spp,Staphylococcus aureus* and *Escherichia coli,* were isolated from fresh fruits that has undergone spoilage This study collaborates with findings of Larka, (2004) who demonstrated that microorganisms are primarily responsible for tomato deterioration. And also the findings of Ukaoma et al., (2020) *Bacillus spp* was one of the organisms isolated from spoilt tomatoes. This could be acquired through post-harvest probably during transportation from the farm to the market or other route.

Also from the study the total bacteria count from the fresh tomato fruits that has undergone spoilage was high compare to other samples which was 4.0x 10^3 while dried tomato had the least with 1.8 x 10^3 and paste tomatoes came after it having a bacteria load of 2.6 x 10^3. This could be an indication of unhygienic handling of tomato fruits either during harvesting or during transportation of the fruits from the farm to the market by careless and unsanitary handling of tomato by farmers,' retailers or even the consumers.

Staphylococcus aureus was isolated from the fresh tomatoes samples examined which is a known micro flora of the nostrils, skin and hands of man. It might have originated from the traders bodies, contaminated air or even the final consumer.

Conclusion

From this study convention of tomato into paste or drying could be a good way to prevent spoilage associated with tomatoes due to its high moisture content which

allows microorganisms to thrive well in it. And this could arrest most of the microorganisms coming from the farm during harvesting, post-harvest, during storage or in transit, also by the careless handling of by traders and consumers.

Households could process tomatoes fruit locally either into paste or its dried form to reduce deterioration of tomato and also extending shelf life. Moreso, Agencies should effectively monitor the production of processed tomatoes past, dried tomatoes powder to make sure industries adhere strictly to safety regulation

Recommendations

From the study the following recommendation were made.

- Spoilt tomatoes consumption should be discouraged at all means.
- Fresh tomatoes before spoilage could be converted to paste tomatoes or could be dried to extend it shelf life because the presence of microorganisms on the surface of tomato fruits has an adverse effect on their shelf life. and also to prevent deterioration of product, but more research should be done on the available nutrient content that would be available after this process (drying and cooking to obtain pate)
- Finally sanitary measures should be taken from production to consumption because it has been noticed that most isolated microorganisms could be gotten from any stage in this process
- More studies should be conducted on the effect of cooking and drying on the nutritional content of processed tomatoes

REFERENCES

Akinmusire, O. O. (2011). Fungal species associated with the spoilage of some edible fruits in Maiduguri, Northern Eastern Nigeria. Advances in Environmental Biology,5 (1). 157-161

Akinyele, B. J. and Akinkunmi, C. O. (2012). "Fungi associated with the spoilage of berry and their reaction to electromagnetic field, "*Journal of Yeast and Fungal Research,* vol. 3, no. 4, pp. 49–57.

Garg, R. K. Batav, N. Silawat, N. and Singh, R. K. (2013). "Isolation and identification of pathogenic microbes from tomato puree and their delineation of distinctness by molecular techniques," *Journal of Applied Biology & Biotechnology*, vol. 1, no. 4, pp. 24– 31.

Ghosh, A. (2009). Identification of microorganisms responsible for spoilage of tomato (Lycopersiconesculentum) fruits .*Journal of Physiology*, 1(6), 414-41.

Larka, B.S. (2004). Integrated approach for the management of soft rot (pectobacterituim carotovorum of radish seed crop). Argon. 20:128-129.

Peet, M. (2008). Effect of high temperature on pollen characteristics. Growth and fruit set in tomato (Lycopersicon esculentum). *Journal America society horticulture science*. 121:514-519.

Sato, S., Tabata, S., Hirakawa, H., Asamizu, E., Shirasawa, K., Isobe, S., Kaneko, T., Nakamura, Y. Shibata, D. (2012). Nature. **485** (7400): 635–641.

Ukaoma, A. A., Duru, M., Ezeanowai, C., Nwachukwu, N. (2020). Microorganism associated with the spoilage of four different varieties of tomato fruits. (*Lycopersicon esculentum*) Volume 7, Issue 3.

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PROXIMATE ANALYSIS, PHYTOCHEMICAL SCREENING, AND ANTI BACTERIAL ACTIVITY OF CASTOR OIL SEED EXTRACT (*Ricinus communis*).

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ABSTRACT

The determination of proximate analysis, phytochemical screening, and anti-bacterial activity of castor oil seed, *Ricinus communis* yielded interesting results. Proximate analysis reveals the presence of ash (2.00±0.03%) crude protein (0.25±0.03%), carbohydrate (1.16±0.00%), fibre (4.59±0.02%) and lipid (92.00±0.03%). Qualitative phytochemical screening of the castor oil seed extract confirms the presence of glycosides, reducing sugar.The castor oil seed extract was tested against seven pathogenic bacteria isolates;*Bacillus subtilis*,*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*,*Klebsiella pneumonia*,*Enterobacter aeruginosa*, and *Proteus mirabilis*.The extract exhibited antibacterial activity with *Staphylococcus aureus* having the highest sensitivity with 29.00±0.1 mm mean diameter zone of inhibition while *Enterobacter aeruginosa* had the least sensitivity with12.00±0.4mm mean diameter zone of *inhibition* .*Escherichia coli* and *Pseudomonas* aeruginosa had equal sensitivitywith same mean diameter zone of inhibition of 20.00±0.5mm and 20.00±0.0mm.. *Bacillus subtilis* had the second highest sensitivity with mean diameter zone of inhibition of 25.00±0.2mm *while Klebsiella pneumonia* had third highest sensitivity with a mean diameter zone of inhibition of 21.00±0.6mm and *Proteus mirabilis* had the second lowest sensitivity with a mean diameter zone of inhibition of 17.00±0.4mm.Statistical significance of the study was carried out using SPSS package(version17.0).Analysis of variance(ANOVA) one way and coefficient of variation were used to establish statistical significance (α=0.05) among the parameters studied.The castor oil seed extract possess excellent medicinal and therapeutic properties.

Keywords: proximate analysis, phytochemical screening, antibacterial activity, castor oil.

INTRODUCTION

Nuts are very often high in nutrients because they are the source of energy for the new plant. Most nuts contain a considerable quantity of fat and vitamins and are rich in essential amino acids. The high energy density makes nuts a very filling food.

Several epidemiological studies have revealed that people who consume nuts regularly are less likely to suffer from coronary heart disease (CHD)(KellyandSebate,2006).Although nuts contain various substances thought to possess cardioprotective effects, scientists believe that their omega 3 fatty acid profile is at least in part responsible for the hypolipidemic properties found in castor oil Consequently,dietitians frequently recommend nuts be included in diet prescribed for patient with insulin resistance problem such as diabetes mellitus type 2 (Josse *et al.*,2007).A study found that people who eat nut live two to three years longer than those who do not.(Fraser and Shavlik,2001).

Nuts contain the essential fatty acids linoleic and linolenic acid, and the fats in nuts for *the* most part are unsaturated, including monounsaturated fats.

A seed is an embryonic plant enclosed in a protective outer covering called the seed coat, usually with some stored food.It is a characteristic of spermatophytes.Nuts and seeds offer protein, healthy fats, fiber, vitamin E, magnesium and many other nutrients. The castor oil seed is a triglyceride in which approximately 90 percent of fatty acid chain are ricinoleate, oleate and linoleate are the other significant component.

Castor oil and its derivatives are used in the manufacturing of soaps,lubricants,hydraulic and brake fluid,paints, dyes coatings,inks,cold resistant plastics,waxes and polishes,nylon,pharmaceutical and perfumes.(Mutlu,2010).

Therapeutically, modern drugs are rarely given in a pure chemical state, so most active ingredients are combined with excipients or additives. Castor oil or castor oil derivative such as Kolliphor EL(Polyethoxylated castor oil,anonionic surfactant)is added to many modern drugs,including:Miconazole,antifungal agent,Paclitaxel, a mitotic inhibitor used in cancer chemotherapy(Micha *et al.*,2006).Sandimmune(Cyclosporine injection, USP) an immunosuppressant Ricin have the potential to be used in the treatment of tumors, as a "magic bullet" to destroy target cells(Spooner *et al*.,2006).Because ricin is a protein, it can be linked to a monoclonal antibody to target malignant cells recognized by the antibody.The major problem with ricin is that if its native internalization sequences are present in a therapeutic agent then the drug will be internalized by,and kill, untargeted non-tumorous cells as well as targeted malignant cells. Modifying ricin may sufficiently lessen the likelihood that Bioactive phytochemical constituents such as alkaloids,anthocyanins,flavonoids,phenoli

drug widely used in connection with organ transplant to reduce the activity of the patients immune system,Nelfinavirmosylate an HIVprotease inhibitor.(Zhany *et al*.,2001).

Nuts and seeds benefit our health by providing a source of dietary fibre.Fibre is a specialized type of carbohydrate found in plant–based foods.It does not breakdown as it passes through the digestive tract, and the undigested fibre add bulk to your stool to promote regular bowel movements. (O'Grady *et al.,* 2019). Fibre also helps slow the rate of digestion.

A diet rich in nuts and seeds also helps keeps you healthy as you age by preventing diseases. People who regularly consume nuts tend to weigh less than those who rarely eat nuts, as well as face a lower risk for weight gain in the future.Nut consumption also correlates with a reduced risk of type 2 diabetes (Kendall *et. al.*,2010).Castor seeds(*Ricinus communis*)have been found in Egyptian tombs dating back to 4000BC,the slow burning oil was used mostly to fuel lamps. Cleopatra is reported to have used it to brighten the whites of her eyes. It is regularly given to children orally for de-worming (Tunaru *et al.,* 2012).

the ricin component of these immunotoxins will cause the wrong cells to internalize it, while still retaining its cell-killing activity when it is internalized by the targeted cells. A promising approach for ricin is to use the non-toxic B subunit (a lectin)as a vehicle for delivering antigens into cell, thus greatly increasing their immunogenicity. Use of ricin as an adjuvant has potential implications for developing mucosal vaccines.(Spooner *et al.*,2006).

c compounds ,tannins,terpenoids,and so on as well as vitamins that produc

e specific beneficial physiological and pharmaceutical functions in human body.(Edeoga *et al*.,2005;Jaime *et al.*,2010;Gulcin,2012).Flavonoids are low molecular weight secondary polyphenolic metabolites present in plants characterized by their flavan nucleus.(Chae *et al.*,2013;Thiakarathna and Rupasinghe,2013).There are over 700 characterized flavonoids and have been shown to be responsible for the flavor and colour pigment intensities in flowers, fruit and leaves(Samappito and Butskhupl,2010;Thiakarathna and Rupasinghe,2013). Flavonoid have been found in many food product such as colour intense fruits, red wine and in beverages.(Samappito and Butskhupl,2010;Kycli,2011).

Tannins are groups of plant polyphenolic high molecular weight secondary metabolites that have been use by human for decades. The name tannins came from the French word "tan" meaning the bark of the Holm Oak tree use for tanning (ability to darken colour) and have been found to be present not only in the Oak tree but in the leaves of castor oil. (Frutos *et al.*,2004).Tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins. The astringency from tannins is that which causes the dry and puckery feeling in the mouth following the consumption of red wine, strong tea, or an unripened fruit (Ashok and Upadhyaya, 2012).

Alkaloids are the largest in these groups of secondary chemical plant metabolites and are made principally of ammonia compound comprising of nitrogen bases that are synthesized from amino acid building blocks with various radicals replacing one or more of the hydrogen atoms in the polypeptide motifs. Nitrogenous atoms in alkaloids (as 1^0, 2^0or 3^0amines) facilitate their alkaline properties, making them turn red litmus paper to blue. The presence and exact location of these amines basically determines the alkalinity of alkaloids. (Doughari, 2012)

$$Ash\ Content = \frac{Weight\ of\ Ash}{Sample\ Weight} \times \frac{100}{1}$$

M METERIALS AND METHODS

Sample preparation

Castor oil seeds were purchased from Uchi Market, Auchi, Edo State, Nigeria.290.1g of castor oil seed were oven-dried at 160^{o}C and then blended into powder using laboratory blender. Extraction of the oil was carried out using Soxhlet apparatus.The powdered sample was extracted using kfdmethanol(analytical grade).Thereafter, the extracted oil(weighing45.64g)was concentrated in vacuum and the oil was stored in a dried closed glass container until further analyses were carried out. The herbarium number for *Ricinus communis* (Castor oil) seed was determined after utilizing two morphological structures; the leaves and seed at Paxherbals herbarium in the Pax Herbals Diagnostic and Research Laboratory, Ewu, Edo State, Nigeria. The herbarium number is PAX696.Proximate analysis, qualitative phytochemical screening and anti-bacterial evaluation were performed.

AS PH CONTENT DETERMINATION

1g of sample was added to pre-weighed porcelain crucible. The sample was incinerated in the muffle furnace at 560^{o}C.The porcelain crucible containing the sample was then removed from the muffle furnace cooled in a desiccator, it was weighed again. Final result was expressed in percentage. (AOAC, 1990)

CRUDE PROTEIN DETERMINATION

4g of sample was weighed and put in a Kjedhal flask and a tablet of Kjedhal catalyst (selenium) was added.10ml of Conc.H_2SO_4 to the sample. It was then allowed to digest for 2 hours. Thereafter, 0.2ml of 30% hydrogen peroxide was added after digestion, allow to cool at room temperature. (AOAC, 1990).

FIBRE DETERMINATION

Defatted Sample (1g) was placed in a glass crucible and attached to the extraction unit.150ml boiling1.25% tetraoxosulphate (vi) acid solution was added.The sample was digested for 30mins and then the acid was drained out and the sample was washed with boiling distilled water. Afterwards, 1.25% sodium hydroxide solution (150ml) was added. The crucible was oven-dried at 110^{o}C for1hr and was cooled in the desiccator and was weighed.The digested sample was put in the crucible and weighed. Then put in the oven for 3hrs at105^{o}C.The sample was put in a desiccator to cool and weighed(W1).It was then transferred to the muffle furnance for1hr.It was kept in a desiccator to cool,and then reweighed(W2).The extracted fibre was expressed in percentage of the original undefatted sample and calculated.(AOAC,2023)

$$\%\ Fibre = \frac{W1 - W2}{Weight\ of\ Sample} \times \frac{100}{1}$$

LIPID DETERMINATION

5g of the sample was weighed into an extraction thimble.50ml solvent (petroleum ether) was added. It was subjected to extraction using a Soxhlet apparatus for 30mins.The weight of the flash was taken before the extraction, the solvent was evaporated and the weight of the flask+extracted lipid was subtracted from the weight of flask to the weight of fat . (AOAC,2023)

$$\%\ Fat = \frac{Extracted\ Fat}{Sample\ Weight} \times \frac{100}{1}$$

Or

Organism Source

The organisms were clinical isolates which includes: *Bacillus subtilis,Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa,Klebsiella pneumonia,Enterobacter aeruginosa, and Proteus mirabilis*.They were obtained from the Department of Medical Microbiology, University of Benin Teaching Hospital(UBTH).Benin- City, Nigeria. All the organisms were checked for purity at Pax Herbal Diagnostic and Research Laboratory,Ewu by a Microbiologist,Mr Kingsley.C.Ezenwa and maintained at 4^0C in slants of nutrient agar.

Preparation of extract stock concentration for antimicrobial susceptibility testing.

A test stock concentration of 50% was prepared by dissolving 5ml of the oil in 5ml of Dimethyl sulfoxide (DMSO) into a universal specimen collection bottle.

AN ANTI-MICROBIOLOGICAL EVALUATION OF CASTOR OIL SEED EXTRACT

The oil extract was assayed for its antimicrobial activity using the agar diffusion technique by Kirby–Bauer as described by Isu and Onyeagba (1998) and Ibekwe *et.al.*,(2001).The sterile Mueller Hinton agar plates were seeded with standardized broth culture of test organisms containing 10^8cfu/ml equivalent to 0.5Mcfarland standards(NCCLS)and 6mm diameter holes were made with sterile cork borer and the 500µl containing 50% of the extracted castor oil were dispensed into bored holes with aid of a micropipette and allowed to stand for about 1hr on a bench in laminar flow cabinet chamber. They were incubated at 37^{o}C for 24hrs and observed for clear zone diameter of

inhibition against the various organisms. The zones diameters were measured with a transparent ruler and were recorded in millimeters (mm).The assay was done in duplicates.Sterilized discs were soaked in Dimethyl sulfoxide (DMSO) as negative control 2mg/ml of Ampicillin– cloxacillin (Ampiclox) as positive control.

Preparation of the inoculums

A loopful of the test organism was taken from their respective agar slants and sub–cultured in to testubes containing Mueller Hinton Broth. The testubes were incubated for 18hrs at 37^{o}C. The obtained microorganism is the broth were standardized using normal saline to obtain a population density of 10^{8}cfu/ml.

Preparation of media

33g of Mueller Hinton Agar were weighed into conical flask.1000ml of deionized water was added and capped with a cotton wool. The media were boiled to dissolution and then sterilized at 121^{o}C for 15mins.The media were allowed to cool for 45^{o}C and 20ml of the sterilized medium was poured into sterile Petri dishes and allowed to cool and solidify.The plates were labeled with the test microorganism (each plate with a test microbe). The microbes were spread evenly over the surface of the medium with the acid of a glass spreader.The plates were dried at 37^{o}C for 30mins respectively.

and solidify.The contents of the mean inhibitory concentration (MIC) test tubes

then sub-cultured into the media and incubated at 37^{o}C for 24hrs and observed for colony growth. The mean bactericidal concentration (MBC) was the plate with the lowest concentration of extract and without colony growth.

Can be used. The formation of reddish brown precipitate and orange precipitate respectively indicate

Test for Reducing Sugars and Glycosides

Minimum Inhibitory Concentration-Broth Dilution Method

The minimum inhibitory concentration of the compound(i.e. the extracted oil),was carried out using macro broth dilution technique as described by Boron and Fingold (1990).The MuellerHinton broth was prepared according to the manufacturers instruction. 9ml of each broth was dispensed into separate test-tube and was sterilized at121^{o}C for 15minutes and then allowed to cool. Various volumes of the oil were picked from the 50% stock concentration to range from100, 200,300, 400,500, and 600 microliters (µL) respectively 2mgl/ml, 4mg/ml, 6mg/ml, 8mg/ml, 10mg/ml, 12mg/ml respectively of the castor oil concentration. The standardized inoculums (0.1ml) of the microbes were inoculated into the different concentrations of the extracted oil in the broth.The test tubes of the broth were incubated at 37^{o}C for 24hrs and observed for turbidity.The lowest concentration which showed no turbidity is the Minimum Inhibitory Concentration. (MIC).

Minimum Bactericidal Concentration-Macro Broth Dilution Method.

Fresh Mueller Hinton Agar was prepared, sterilized at 121^{o}C for 15mins and poured into sterile Petri-dishes and left to cool

(that is the test tubes that showed no growth)were

PHYTOCHEMICAL SCREENING OF CASTOR OIL SEED EXTRACT.

Test for Alkaloids

Wagner's reagent or Dragendoff's reagent

the presence of alkaloids. (Trease and Evans, 1989**)**

Fehling's solution A and B was used. The formation of dense red precipitate

indicate the presence of reducing sugar and glycosides**. (**Trease and Evans**,** 1989**)**

Test for Flavonoids

Alkaline-acid test was used. An intense yellow colour was observed, and it changes to colourless on addition of dilute tetraoxosulphate (vi) acid which indicates the presence of flavonoids.(Trease and Evans,1989)

Test for Steroids

2ml acetic anhydride was added to 2ml ethanolic extract with 2ml tetraoxosulphate (vi) acid. The change of colour from violet to blue indicates the **Te** presence of steroids.(Trease and Evans,1989) **Test for Saponins**

Frothing test was used. The formation of 10ml layer of foam or froth indicate the presence of saponins.(Trease and Evans,1989)

Test for Terpenoids

Brady's reagent was used. The formation of orange precipitate indicate the presence of terpenoids.(Trease and Evans,1989)

Test for Tannins

2ml of plant extract was treated with 1% ferric chloride. The formation of brownish green or bluish-black precipitate indicates the presence of condensed or hydrolysable tannins respectively. (Trease and Evans, 1989)

Test for Anthraquinones

To 2ml of the extract, 2ml of 25%ammonia solution was added and shaken. A cherry–red solution indicates the presence of anthraquinones. (Trease and Evans, 1989)

RESULTS AND DISCUSSIONS TABLE1:Proximate Analysis of Castor Oil seed extract

PARAMETER	**RESULT(%)**
Ash	2.00±0.03
Crude protein	0.25±0.03
Carbohydrate	1.16±0.00
Fibre	4.59±0.02
Lipid	92.00±0.03

Values are expressed as mean triplicates ±S.E.M

TABLE2: Qualitative Phytochemical Screening of Castor Oil seed extract

PARAMETER	**RESULT**
Glycosides	++
Cardiac glycosides	-
Saponin	-
Flavonoids	-
Phenolic compound	-
Hydrolysable Tannin	-
Condensed Tannin	-
Phlobatannin	-
Terpenoid	-
Alkaloid	-
Polysaccharide/Starch	-
Reducing sugar	++
Steroid	-

(++) indicates presence,(-) indicates a

TABLE 3: Antibacterial Activity, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Castor oil(*Ricinus communis*)extract on some pathogenic bacterial isolates.

Isolates	**Mean zone Diameter of Inhibition(mm)**			**(50%v/v)**	
	Castor oil	**Ampiclox**	**Dimethylsulfoxide DMSO)**	**(MIC)**	**(MBC)**
Bacillus subtilis	25±0.2	19	0	100	200
Escherichia coli	20±0.5	0	0	200	300
Enterobacter aeruginosa	12±0.4	0	0	400	500
Klebsiella pneumonia	21±0.6	0	0	100	200
Pseudomonas aeruginosa	20±0.0	17	0	100	200
Staphylococcus aureus	29±0.1	19	0	100	200
Proteus mirabilis	17±0.4	0	0	300	400

Values expressed are in mean duplicates±SEM

DISCUSSION

Percentage of lipid content (92.00±0.03%).The high percentage lipid content showed that the plant. (*Ricinus communis*) has the potential of yielding considerable amount of oil. The carbohydrate content in castor oil seed was found to be 1.16±0.00%.This value indicates the presence of carbohydrate which is a good source of energy for both man and livestock. The crude protein obtained in this experiment was found to be 0.25±0.03%.This value The results obtained from the proximate analysis of the oil of castor plant indicate a high reveals the presence of crude protein which is a very good class of food needed by man and livestock.

In this research,the ash content was found to be 2.00±0.03%.It could be seen that high percentage of ash content mean high minerals while low content of minerals is indicated by low percentage of ash content. Minerals are good in the body; thus needed by man and livestock. Determining the ash content in food samples is part of the proximate analysis necessary for nutritional evaluation. This ensures the safety of foods, making sure there are no toxic minerals present.

The fibre content was found to be 4.59±0.02%.It is very imperative to dehull castor seeds before analysis begins and it is applied to animal feed; particularly in the monogastric feeding system. High fibre content influences the utilization of other nutrient by the animal.(Komarova *et al.*,1995 ;Aderibigbe *et al.*,1997).

Glycosides are molecules in which a sugar is bound to another functional group via a glyosidic bond. In this research,(castor oil seed)glycosides were found to be present which play numerous important roles in living organisms. Many plant store chemicals in the form of inactive glycoside. These can be activated by enzyme hydrolysis, which cause sugar part to be broken off, making the chemical available for use. Many such plant (castor plant) glycosides are used as medication in animals and humans. Poisons are often bound to sugar molecules as part of their elimination from the body. (Brito-Arias, 2007).Reducing sugars in the other hand

occur when its anomeric carbon is free. Sucrose need to be broken down in the stomach by acid into fructose and glucose before it can be passed to the body.Sucrose requires the body to expand energy to turn it into a reducing sugar that the body can use (which probably produces a lot of excess stomach acid which is not good for the stomach) while something like fructose can be passed directly to the cell for distribution to the liver or body for use as food. The castor oil extract inhibited the growth of all test isolates. Among the gram positive bacteria; *Staphylococcus aureus* and *Bacillus subtilis*,and gram negative bacteria; *Pseudomonas aeruginosa*,*Klebsiella pneumonia*,*Enterobacter aeruginosa*,*Proteus mirabilis* and *Escherichia coli*, *Staphylococcus aureus* with 29.00mm mean diameter zone of inhibition was found to have highest sensitivity to the extract and gram negative bacteria; *Enterobacter aeruginosa* was the least sensitive to the extract with 12.00mmm mean diameter zone of inhibition.The gram negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* were found to have equal diameter zone of inhibition of 20.00mm..Generally, the castor oil extract was more effective on gram positive bacteria than gram negative bacteria as observed in the Table 3. However, the result of the antibiotic (the controlled drug: Ampiclox) sensitivity assay revealed that castor oil extract was higher. The susceptibility of some of the organisms used may be due to their genetic make-up and absence of resistant transfer factor or their ability to produce different enzymes and toxins which maybe able to degrade some of the active components of the plants (Momoh *et al*., 2012).Gram positive bacteria used showed very good susceptibility action to the castor oil seed extract. The gram negative bacteria showed moderate susceptibility to the extracts probably due to their genetic make-up.The following bacterial isolates;*Bacillus subtilis* **,***Klebsiella pneumonia***,** *Pseudomonas aeruginosa* had a constant MIC value (2gml,50%v/v).*Staphylococcus aureus* with *Escherichia coli* had varying MIC value of 4mg/ml,50%v/v)with *Enterobacter aeruginosa* and *Proteus mirabilis* with MIC value of 8mg/ml,50%v/v.Though the mechanism of action of the extract was not studied.The presence of biologically active phytochemicals could be responsible for the antimicrobial activity of the castor oil seed extract. It is interesting to note that even crude extracts of the castor oil

showed good activity against Ampiclox resistant strains where modern antibiotic therapy has limited effect, also the oil has encompassing effects on both gram positive and gram negative bacteria. These low MIC values corroborates the findings of Andrews(2001)who said that minimum inhibitory concentration(MIC) are important in diagnostic laboratories to confirm resistance of micro-organism to an antimicrobial agent and also to monitor the activity of new antimicrobial agents.The antibacterial activity observed when the isolates were inoculated correlates with the findings of Mims and Playfair(1993)who said that minimum bactericidal concentration (MBC)is the lowest concentration of an antimicrobial agent required to kill a particular bacterium.Thus there is a need for detailed scientific study of traditional medical practices

to ensure that valuable therapeutic knowledge of some plants is preserved and also to provide scientific information on their efficacies.

CONCLUSION

This study on castor oil seed(*Ricinus communis*) extract highlighted interesting result as regards the proximate composition, qualitative phytochemical screening and antibacterial activity.Castor oil seed plays prominent role in nutrition and health because of its numerous,medicinal,therapeutic properties.It has also found its application in the industry where it is useful in the manufacturing of lubricants,hydraulic fluids, and coatings.

REFERENCES

Aderibigbe, A. O. Johnson, L.E., Makkor, H.P.S. Becker, K., and Fold, T.N. (1997) Chemical Composition and Effect of Heat On Organic Matter and Nitrogen Degradability and some Ant nutritional Components of Jatropha Meal. *Animal Feed Science Technol.* 67:223–243.

Andrews, J.M. (2001) "Determination of Minimum Inhibitory Concentrations. *Journal of Antimicrobial Chemotherapy*. 48(1)5-16

AOAC, (1990) Official Methods of Analysis. Association of Official Analytical Chemists.14th Ed. Washington D.C. Pp 223-225; 992-995.

AOAC, (2023). Official Methods of Analysis. 22nd Edition. Oils and Fats.981:11

Ashok, P.K. and Upadhyaya, K (2012) Tannins are Astringent. *Journal of Pharmacognosy and Phytochemistry* 1(3):45

Boron, J.E. and Fingold, S.M. (1990). Method for Testing Antimicrobial effectiveness In: Bailey Scotts Diagnostic Microbiology, 8th Edition, Missouri.

Brito-Arias, M. (2007) Synthesis and Characterization of Glycosides Springer. Pp 26251–26252.

Chae. C.S, Lee, J.H., Park, S.U., (2013) Recent Studies on Flavonoids and Their Antioxidant Activities. *EXCLI Journal* 12:226–228.

Doughari, J.H. (2012). Phytochemicals: Extraction Methods, Basic Structures and Mode of Action as Potential Chemotherapeutic Agents. Intech .doi:10.5772/26052

Edeoga, H.O., Okwu,D.E., Mbaebie, B.O. (2005):, Phytochemical Constituents of some Nigeria Medical Plants. *African Journal of Biotechnology* 4(7); 685–688.

Fraser, G.E, and Shavilk, D.J (2001). Ten Years of life: Is it a matter of

choice? *Arch Int Med 16*1(13):1645-1652

Frutos, P., Hervas, G, Giraldez, F.J, Mantecon, A.R. (2004) Review: Tannins and Ruminant Nutrition. *Spanish Journal of Agricultural Research.*2(2):191–202.

Gulcin, I. (2012) Antioxidant Activity of Food Constituents: An Overview. .*Archives of Toxicology*, ;86:345–391.

Ibekwe, V. I. Nnwayere, N.F, and Akujobi, C. O (2001), Studies on Antibacterial Activity and Phytochemical Qualities of Extracts of Orange Peels.*Int. J. Environ. Health and Human Dev*., 2(1):41-46.

Ibiyemi, S.O., Adepoju, T.O. Okanlawon, S.O., and Fadipe, V.O., (1992). *Emulsion Preparation and Stability Journal of Nutritional Science*13,(1-2),31–34

Isu, N.R. and Onyeaba, R.A. (1998). Basic Practical sin Microbial. 2nd Edition Fashen Communication, Okigwe, Nig. p137.

Jaime, G.C, Lurgi, C.P, Andrea, C.C, Fernando, M.S, Heidi S.S, Emilio H.U, Emma B.T, and Miren, A.I. (2010). Antioxidant Capacity, Anthocyanins, and Total Phenols of Wild and Cultivated Berries in Chile. *Chilean Journal of Agricultural Research,*70 (4):537–544.

Josse, A.R., Kendall C.W.C, Augustina, L.S.A, Ellis, P.R., and Jenkins, D.J.A (2007). Almonds and postprandial glycemia-a dose response study. *Metabolism* 56(3):400-404

Kelly, J.H, and Sebate, J (2006) "Nuts and coronary heart disease: an epidemiological perspective. *Br J. Nuts* 96:S61-S67.

Kendall, C.W.C, Amin, E., Jennifer, T., Korbua, S., David J.A., Jenkins, M.D, (2010) Health benefits of nuts in prevention and management of diabetes *Asia Pac. J Clin Nutr* 19(1):110116

Komarova, E. N., Vyskrebentseva, E.I and Trunova, T.I (1995). Lectin Activity in Winta Wheat Crowns During Hardening. *Russians J. Plant Physio* 42:543–546.

Kycli, P. (2011) Berry Phenolics: Isolation, Analysis, Identification and Antioxidants Properties. Pp13.

Micha, .J.P., Goldstein, B. H., Birk, C.L., Rettenm, M.A., Brown,J.V. (2006) Abraxane in the Treatment of Ovarian Cancer: The Absence of Hypersensitive Reaction. *Gynol Oncol* 100(2):437

Mims and Playfair,(1993). *Medical Microbiology* Mosby, Europe 31-35.

Momoh, A.O., Oladunmoye, M.K., and Adeolu T.T. (2012). Evaluation of the antimicrobial and phytochemical properties of oil from castor seeds (*Ricinus communis* Linn) *Bulletin of Environment, Pharmacology and Life Science*s1(10):21-27

Mutlu, H. (2010). Castor Oil as a Renewable Resource for the Chemical Industry. *European Journal of Lipid and Technology.* 112(1):10–30.

O'Grady, .J., O'Connor,. E.M., and Fergus, S. (2019) Dietary fibre in the era of microbiome science. *Alimentary pharmacology & therapeutics 49(5),506-515.*

Samappito, S., and Butskhupl. (2010), Analysis of Anthocyanins,

Flavonoids and Phenolic Acid Contents of by Ten Fruits and Antioxidants Activity. *Internatio*

Journal of Fruit Science. 10(3):264–280.

Spooner, R.A., Smith, D.C., Easton, A.J., Roberts, L.M., Lord, J.M, (2006)"Retrograde Transport Pathways Utilized by Virus and Protein Toxins *"Virol J* 3:26.

Thilakarathna, S.H, and Rupasinghe, H.P.V. (2013) *Flavonoids Bioavailability and Attempts for Bioavailability Enhancement*, *Nutrients 3*:368.

Trease, G.E and Evans, W.C. (1989) 13th Edition. Bailliere Tindall. Ltd. London.

Tunaru, S., Althoff, T.F., Nusing, R.M., Dienar, M. Offermanns, S. *(*2012*)*, Castor Oil Induces Lactation and Uterus Contraction Via Ricinoleic Acid Activating Prostaglantin EP3 Receptor. *Proceeding of National Academy of Sciences of United States of America* 109(23)9179–9184.

Zhang, K.E, Wu,. E, Patrick, A.K (2001). *Circulatory Metabolites of the Human Immunodeficiency Virus Protease Inhibitor Neffinavinin Humans:* Structural Identification, in Plasma, and Antiviral Activities. *Antimicrobs. Agent Chemother* 45(4):1086-1093.

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EVALUATION OF ANTI-NUTRITIONAL FACTORS, PHYSIOCHEMICAL AND ANTIOXIDANT ACTIVITIES OF CASTOR OIL SEED EXTRACT.

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ABSTRACT

The investigation of some biochemical parameters in castor oil seed extract gives interesting findings. Anti-nutritional factors showed the presence of oxalate (0.25±0.00%), phytic acid(1.80±0.03%),saponin(1.09±0.00%),alkaloid was absent. Physiochemical evaluation reveal the following, iodine value(3.52±0.03ml/g),acid value(20.20±0.03ml/g),saponification value(63.95±0.00meq/kgKOH),and peroxide value(100.00±0.03meq/kg).The castor oil extract exhibited excellent antioxidant properties from DPPH radical scavenging assay, with lowest mean percentage inhibition at 20µg/ml with a value of 89.79±0.88%and highest mean percentage inhibition at 100µg/ml with a value of 92.65±0.11% for standard (Vitamin C).The extract had the lowest mean percentage inhibition of 45.66±0.95% at 20µg/ml with highest mean percentage inhibition of 87.58±0.62% at 60µg/ml. Lipid peroxidation reveal a concentration-dependent activity for the standard (Vitamin E) with the highest mean percentage inhibition occurring at 100µg/ml with a value of 97.33±0.00% and lowest mean percentage inhibition at 20µg/ml with a value of 95.02±0.01%The lipid peroxidation activity for the extract reveal concentration- independent activity with the highest mean percentage inhibition recorded at 40µg/ml with a value of 79.75±0.02% while the lowest mean percentage inhibition was recorded at 80µg/ml with a value of 44.27±0.03%.Ferric reducing power (FRAP) assay reveal a concentration-dependent activity for both standard (Vitamin C) and the extract. Highest reducing power activity for the standard was recorded at 100µg/ml with a value of 1.048±0.051nm with lowest reducing power activity of 0.513±0.004nm at 20µg/ml while the extract also showed highest reducing power activity occurring at 100µg/ml with a value of 0.423±0.002nm and the lowest reducing power activity of 0.275±0.022nm at 20µg/ml. The castor oil seed extract possess excellent medicinal, therapeutic properties , and anti-oxidant properties. Statistical significance of the study was carried out using SPSS package (version17.0).

Keywords: *anti-oxidant studies, anti-nutrient, physiochemical evaluation, anti-nutritional factors, radical scavenging, lipid peroxidation.*

INTRODUCTION

A seed is an embryonic plant enclosed in a protective outer covering called the seed coat, usually with some stored food. It is a characteristic of spermatophytes. Castor oil and its derivatives are used in the manufacturing of soaps, lubricants, hydraulic and brake fluid, paints, dyes coatings, inks, cold resistant plastics, waxes and polishes nylon, pharmaceutical and perfumes.(Mutlu,2010).Nuts and seeds benefit your health by providing a source of dietary fibre. Fibre is a specialized type of carbohydrate found in plant–based foods. It does not breakdown as it passes through the digestive tract, and the undigested fibre add bulk to your stool to promote regular bowel movements. Fibre also helps slow the rate of digestion. Fibre is a form of functional food joining a growing list of examples of diet_microbe_host interactions which link microbe_host metabolic and immune cascades.(O'Grady *et al.*,2019).Nut consumption also

correlates with a reduced risk of type 2 diabetes.(Kendall, *et al*.,2010).Anti-nutritional factors are present in different food substances in varying amounts, depending on the kind of food, mode of its propagation, chemicals used in growing the crop as well as those chemicals used in storage and preservation of the food substances.(Thakur and Sharma,2019).Many traditional method of food preparation such as fermentation, cooking and malting increase the nutritive quality of plant food through reducing certain antinutrients such as phytic acid, polyphenol, and oxalate.(Hotz and Gibson,2007). Phytic acid interferes with the absorption of certain minerals. The presence of antinutrients in the extracted oil is typical of most legumes and oil seeds (Balogun and Fetuga,1986).

Detection of peroxide gives the initial evidence of rancidity in unsaturated fats and oils. Other methods are available but peroxide value is the most widely used. It gives a measure of the extent to which an oil sample has undergone primary oxidation, extent of secondary oxidation may be determined from panisidine test. High peroxide value of the oil sample shows that the oil is prone to rancidity and it is less stable. The value is a measure of the degree at making excellent soaps and cosmetics in industries. It enable us to classify the oil in the non-drying groups, the value could be used to quantify the amount of double bond present in the oil which reflect the susceptibility of the oil to oxidation which are useful in the manufacture of soaps and can be regarded as liquid oil. Thus, the oil may find application in the manufacturing of lubricants, hydraulic fluid and coating. (Ibiyemi *et al.,* 1992).The higher the iodine value, the more reactive, less stable, and more susceptible to oxidation and rancidification of the oil. It also expresses the degree of unsaturation. This can be used to check the level of oxidation deterioration of the oil by enzymatic or chemical oxidation, the value falls, within the free fatty acid of oil is expected to range between 0.00–3.00% before it find application in cooking. However, the free fatty acid can be modified to edible oil by subjecting it to refining and this will also improve its quality for industrial usage(Jumat *et al.*,2010).Saponins are naturally occurring substances with various biological effects. In the presence of cholesterol, saponins exhibit strong hypocholesterolemic effect. (Ikewuchi, 2012).They can also lead to hypoglycemia. (Barky *et al*., 2017).

Some organic acids can have antinutritional factors. Oxalic acid can form soluble(potassium and sodium)or insoluble(calcium ,magnesium, iron)salts or esters called oxalates that are commonly found in plants i.e. leafy vegetables or synthesized in the body(Akwaowo *et al*.,2000).Insoluble salts cannot be passed out of the urinary tract once processed through the digestive system. Calcium oxalate can have a deleterious effect on human nutrition and health by

accumulating kidney stones. (Olawoye and Gbadamosi, 2017).

MATERIALS AND METHODS

Sample preparation

Castor oil seeds were purchased from Uchi Market, Auchi, Edo State, Nigeria. 290g of castor oil seed were oven-dried at 160ºC and then blended into powder using laboratory blender. Extraction of the oil was carried out using Soxhlet apparatus. The powder sample was extracted using methanol (analytical grade).Thereafter, the extracted oil (weighing 45.00g) was concentrated in vacuum using a Rotary Evaporator and the oil was stored in a dried closed glass container until further analyses were carried out. The herbarium number for *Ricinus communis* (castor oil) seed was determined after utilizing two morphological structures; the leaves and seed at Paxherbals herbarium in the Paxherbals Diagnostic and Research Laboratory, Ewu.

ANTI-NUTRIENT DETERMINATION.

ALKALOID

5g of sample was weighed into a 250ml beaker and 200ml of 40% hydrochloric acid in ethanol was added and covered to stand for 4hrs.This was filtered and extract was concentrated using a water bath to one quarter of the original volume and concentrated ammonium hydroxide (10%) was prepared and10% ammonium hydroxide was added drop wise until it form a precipitate. Then filter using a filter paper and weigh the precipitate. (AOAC, 2023)

OXALATE DETERMINATION

To 1g of the sample, 75cm^3 of 1.5M H_2SO_4 was added. The solution was carefully agitated with magnetic stirrer for 1hr and then filtered using a filter paper. The filtrate (25cm^3) was collected and titrated against 0.1M $kMnO_4$ solution till a pink colour that persisted for 30sec appears.

% phytic acid = Ans × 1.19 × 100 = % (Lucas and Markaka, 1975)

SAPONIN DETERMINATION

The saponin in content of the extract was determined by double extraction gravimetric method described by (Harborne, 1984)

(Munro, 2000; AOAC, 2023).

LIPID DETERMINATION.

5g of the sample was weighed into an extraction thimble.50ml solvent (petroleum ether) was added. It was subjected to extraction using a Soxhlet apparatus for 30mins.The weight of the flash was taken before the extraction, the solvent was evaporated and the weight of the flask+ extracted lipid was subtracted from the weight of flask to the weight of fat. (AOAC, 2023)

$$\% fat = \frac{Extracted\ Fat}{Sample\ Weight} \times \frac{100}{1}$$

DE TERMINATION OF PHYTIC ACID

2.0g of the sample was weighed into a 250ml conical flask.100ml of 2%Con.Hcl was used to soak sample for 3hrs and filtered with a filterpaper.50cm^3 of the filtrate and 10cm^3 of distilled water was added in each case to give proper acidity.100ml of 0.3% ammonium thiocyanate solution was added into the solution as indicator and filtered with standard iron(iii)chloride,$FeCl_3$ solution containing 0.00195gFe/ml. Endpoint observed to be brown which persisted for 5mins.The percentage phytic acid was calculated as:

$$\text{Average titre value} = \frac{Final\ reading - Initial\ reading}{2}$$

% phytic acid = n × 1.19 × 100

Y– titre value × 0.00195

FIBRE DETERMINATION

Defatted Sample (1g) was placed in a glass crucible and attached to the extraction

unit. 150 ml boiling1.25% tetraoxosulphate (vi) acid solution added. The sample was digested for 30mins and then the acid was drained out and the sample was washed with boiling distilled water. Afterwards, 1.25% sodium hydroxide solution(150ml)was added. The sample was digested for 30mins.The base was drained out and the sample was washed with boiling water. Finally, the crucible was oven-dried at110^{o}C for 1hr and was cooled in the desiccator and was weighed. The digested sample was put in the crucible and weighed, then taken to the oven for 3hrs at 105^{o}C.The sample was put in a desiccator to cool and weighed (W1).It was then transferred to the muffle furnace for1hr.It was kept in a desiccator to cool, and then reweighed (W2).The extracted fibre was expressed in percentage of the original undefatted sample and calculated.(AOAC,2023)

$$\%\ fibre = \frac{W1 - W2}{Weight\ of\ Sample} \times \frac{100}{1}$$

IODINE VALUE DETERMINATION

Pipette out 10ml of fat sample dissolved in chloroform to an iodination flask labeled as "TEST." Add 20ml of iodine monochloride reagent in the flask, mix the contents in the flask thoroughly. Then the flask is allowed to stand for half an hour incubation in dark. Set up a blank in another iodination flask by adding 10ml chloroform to the flask. Add to the blank, 20ml of iodine monochloride reagent and mix the contents in the flask thoroughly incubate the blank in dark for 30 mins. Meanwhile, take out the TEST from incubation after 30 minutes and add 10ml of potassium iodide solution into the flask. Rinse the stopper and the sides of flask using 50ml distilled water. Titrate the "TEST" against standardized sodium thiosulphate solution until a pale straw colour is observed. Add about 1ml starch indicator into the contents in the flask, a purple colour is observed. Continue the titration until the colour of the solution in the flask turns colourless. The disappearance of the blue colour is recorded as the endpoint of the titration. Similarly, the procedure is repeated for the flask labeled "blank". Record the endpoint value of the blank.

Calculate the iodine number using the equation below:

Volume of sodium thiosulphate used = (Blank – Test) ml.

Iodine no of fat = Equivalent weight of iodine × Volume of $Na_2S_2O_3$ used × Normality of $Na_2S_2O_3$ Weight of fat sample used for analysis.

Normality of sodium thiosulphate ($Na_2S_2O_3$) = 0.1N (AOAC, 20

Unless otherwise specified, weigh accurately a quantity of the fatty oil and place it in a 250ml conical flask. Then add 50ml of ethanol–ether solution. Shake it well. If necessary, reflux the mixtures gently until the substance is completely dissolved. Titrate the solution with sodium hydroxide titrant until pink coloration can be observed which persists for 30s. Measure the volume of sodium hydroxide titrant used and calculate the acid value according to the following equation

$$Acid\ Value = \frac{V_{NaOH} \times 5.61}{W}$$

Where V_{NaOH} = volume of sodium hydroxide titrant used (ml)

W = Weight of the fatty oil being examined (g)

When the acid value is less than 10, it is suggested that a10ml semi–micro burette may be used for the titration. (AOAC, 2002)

SAPONIFICATIONVALUE DETERMINATION

Weight 1g of fat in a beaker and dissolved in about 3ml of the fat solvent (ethanol/ether mixture). Quantitatively transfer the contents of the beaker three times with a further 7ml of the solvent. Add 25ml of 0.5N alcoholic KOH and mix well, attach this to a reflux condenser. Set up another reflux condenser as the blank with all other reagents present except the fat. Place both the flasks in a boiling water bath for 30mins.Cool the flasks to room temperature. Now add phenolphthalein indicator to both the flasks and titrate with 0.5M Hcl. Note down the endpoint of blank and test. The difference between the blank and test reading gives the number of milliliters of 0.5N KOH required to saponify 1g of fat. (AOAC, 2002).Calculate the saponification value using the formula:

Saponification value or number of fat = mg of KOH consumed by 1g of fat
Weight of KOH=Normality of KOH x Equivalent weight x volume of KOH.
Volume of KOH consumed by 1g fat = (Blank–test) ml.

ID VALUE DETERMINATION

PE ROXIDE VALUE DETERMINATION

Detection of peroxide gives the initial evidence of rancidity in unsaturated fats and oils. Other methods are available but peroxide value is the most widely used. It gives a measure of the extent to which an oil sample has undergone primary oxidation, extent of secondary oxidation maybe determined from panisidine test. (AOAC, 2002)

FE RRIC REDUCING POWER (FRAP) ASSAY OF CASTOR OIL SEED EXTRACT

Ferric reducing power (FRAP) was determined by mixing various concentrations of plant extracts and standard (ascorbic acid solution) (20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, and 100µg/ml). 1 ml of methanol with phosphate buffer (2.5ml, 0.2M at pH6.6) and potassium ferricyanide [$K_3Fe(CN_6)$], 2.5ml. The mixture was incubated at 50^{o}C for 20mins. 2.5ml of 10% trichloroacetic acid (TCA) was added to mixture, which was then centrifuged at 300rpm for 10min at room temperature. 25ml of supernatant was mixed with 2.5ml distilled water and iron(iii)chloride ($FeCl_3$) (0.5ml,0.1%), and the absorbance of the reaction mixture was measured at 700nm as indicative of increased reducing power. (Benzie and Strain, 1999). The assay was performed in triplicates.

DPPH RADICAL SCAVENGING ANTIOXIDANT ASSAY OF CASTOR OIL SEED EXTRACT

The radical scavenging activity of the plant extract against 2, 2-Dipheny-1-picrylhydrazylradical (Sigma Aldrich) was determined by UV Spectrophotometry at 517nm. Radical scavenging activity was measured by a slightly modified method previously described. (Murphy *et.al.*, 2002). 20-100µg/ml of crude extract and vitamin C were prepared in methanol (Analar grade). 1ml of the extract was placed in a test tube followed by 2ml of 0.1mM DPPH in methanol. A control solution was prepared containing the same amount of methanol and DPPH. The radical scavenging activity was calculated using the following formula.

$$\% \text{ inhibition} = \frac{(A_b - A_a)}{A_b} \times \frac{100}{1}$$

Where A_b the absorption of the blank sample and A_a is the absorption of the extract.

LIPID PEROXIDATION ACTIVITY OF CASTOR OIL SEED EXTRACLipid peroxidation inhibition assay was carried out using slightly modified method described by (Ohkawa *et.al.*,1979).Liver homogenate was prepared from commercial available goat liver. The liver was washed several times with ice cold saline solution. A 10% liver homogenate was prepared using ice-cold KCl (0.15M) in a blender. Lipid peroxidation was initiated in 1ml of tissue homogenate incubated with various concentration of extracts (20–100µg/ml),by the addition of 0.1ml of iron (ii) tetraxosulphate (vi)(25µM,0.1ml of ascorbate,100µM) and 0.lml of KH_2PO_4 (10mM) and the volume was made up to 3ml with distilled water and incubated at 37^{o}C for 1hr. Then 1ml of 5% Tricloroacetic acid (TCA) and1ml of 0.67%Thiobarbituric acid (TBA)was added to this reaction mixture and the tubes were boiled for 30mins in a boiling water bath. This was centrifuged at 3500rpm for 10mins.The extent of inhibition of lipid peroxidation was evaluated by the estimation of Thiobarbaturic Acid Reactive Substance (TBARS) level by measuring the absorbance at 532nm. (Ohkawa *et al.*,1979).The experiment was done in triplicates and vitamin E was used as standard. The lipid peroxidation inhibition percentage was calculated using the formula below.

$$\% \text{ I inhibition} = \frac{(A_b - A_a)}{A_b} \times \frac{100}{1}$$

Where A_b the absorption of control and A_a is the absorption of the castor oil seed extraction

RESULTS TABLE 1: Anti-nutrients determination from Castor Oil Seed extract

PARAMETER	**RESULT (%)**
Oxalate	0.25±0.00
Phytic acid	1.80±0.03
Saponin	1.09±0.00
Alkaloid	–

Values expressed are mean triplicates ±S.E.M

TABLE 2: Iodine value, Acid value, Saponification value and Peroxide value for castor oil seed extract

PARAMETER	**RESULT**
Iodine value(ml/g)	3.52±0.03
Acid value(ml/g)	20.20±0.03
Saponification value(meq/kgKOH)	63.95±0.00
Peroxide value(meq/kg)	100.00±0.03

Values expressed are mean triplicates ±S.E.M

TABLE 3: DPPH Radical Scavenging Activity for Standard (Vitamin C)

VitaminC(STANDARD)	**CONCENTRATION**	**MEAN ABSORBANCE 1AT 517nm**	**MEAN % INHIBITION**
SA	20µg/ml	0.123±0.008	89.79±0.88
SB	40µg/ml	0.101±0.002	91.85±0.12
SC	60µg/ml	0.103±0.002	91.70±0.04
SD	80µg/ml	0.094±0.003	92.40±0.27
SE	100µg/ml	0.092±0.002	92.65±0.11

Values expressed are mean triplicates ±S.

TABLE 4: DPPH Radical Scavenging Activity for Castor oil seed extract.

SAMPLE	CONCENTRATION	MEAN ABSORBANCEAT 517nm	MEAN % INHIBITION
VA	20µg/ml	0.187±0.004	84.83±0.04
VB	40µg/ml	0.173±0.002	86.16±0.05
VC	60µg/ml	0.399±0.008	87.58±0.62
VD	80µg/ml	0.492±0.024	54.91±5.03
VE	100µg/ml	0.670±0.012	45.66±0.95

Values expressed are mean triplicates ±S.E.M.

TABLE 5: Lipid Peroxidation Activity of Castor oil seed extract

SAMPLE	CONCENTRATION	MEAN ABSORBANCEAT 532nm	MEAN % INHIBITION
VA	20µg/ml	0.135±0.001	76.82±0.23
VB	40µg/ml	0.118±0.004	79.75±0.67
VC	60µg/ml	0.121±0.001	79.23±0.12
VD	80µg/ml	0.324±0.001	44.27±0.01
VE	100µg/ml	0.152±0.001	73.95±0.11

Values expressed are mean triplicates ±S.E.M.

TABLE 6: Lipid Peroxidation Activity of Standard (Vitamin E)

SAMPLE	CONCENTRATION	MEAN % INHIBITION
SA	20µg/ml	95.02±0.01
SB	40µg/ml	96.82±0.02
SC	60µg/ml	95.64±0.00
SD	80µg/ml	94.30±0.00
SE	100µg/ml	97.33±0.00

Values expressed are mean triplicates ±

TABLE 7: Ferric Reducing Power (FRAP) Activity of Standard (Vitamin C)

STANDARD	**CONCENTRATION**	**MEAN ABSORBANCE AT 700nm**
SA	20µg/ml	0.513±0.004
SB	40µg/ml	0.680±0.008
SC	60µg/ml	0.794±0.006
SD	80µg/ml	0.903±0.011
SE	100µg/ml	1.048±0.051

Values expressed are mean triplicates ±S.E.M

TABLE 8: Ferric Reducing Power (FRAP) Activity of Castor oil seed extract.

SAMPLE	**CONCENTRATION**	**MEAN ABSORBANCE AT 700nm**
VA	20µg/ml	0.275±0.022
VB	40µg/ml	0.342±0.004
VC	60µg/ml	0.339±0.002
VD	80µg/ml	0.414±0.009
VE	100µg/ml	0.423±0.002

Values expressed are mean triplicates ±S.E.M

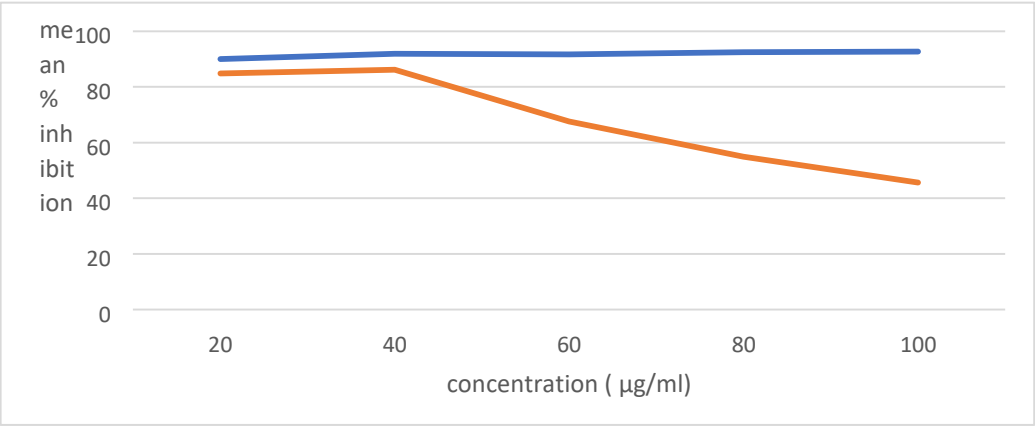

Fig1: DPPH Radical Scavenging Activity of both standard (Vitamin C) and castor oil seed extract

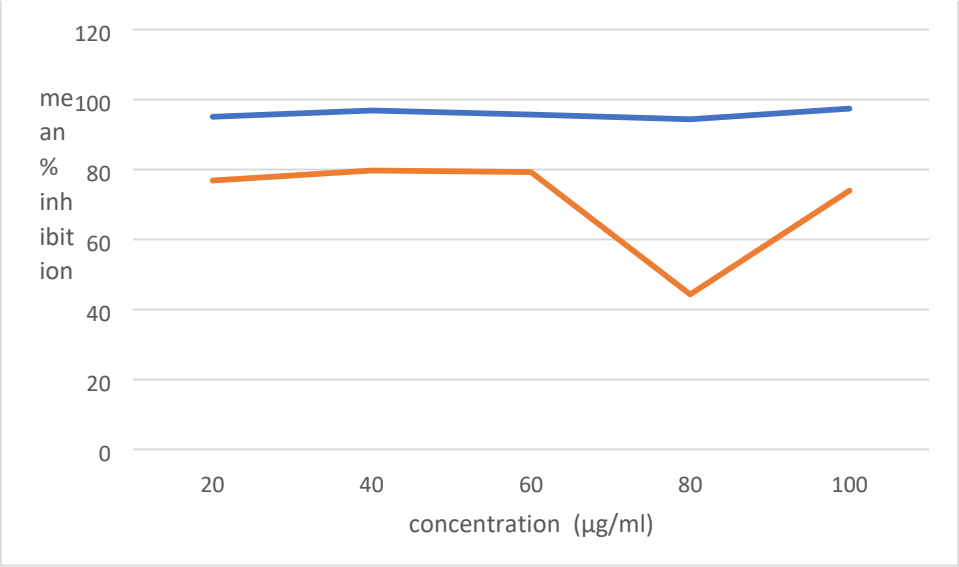

Fig2: Lipid Peroxidation Activity of both standard (Vitamin E) and castor oil seed extrac

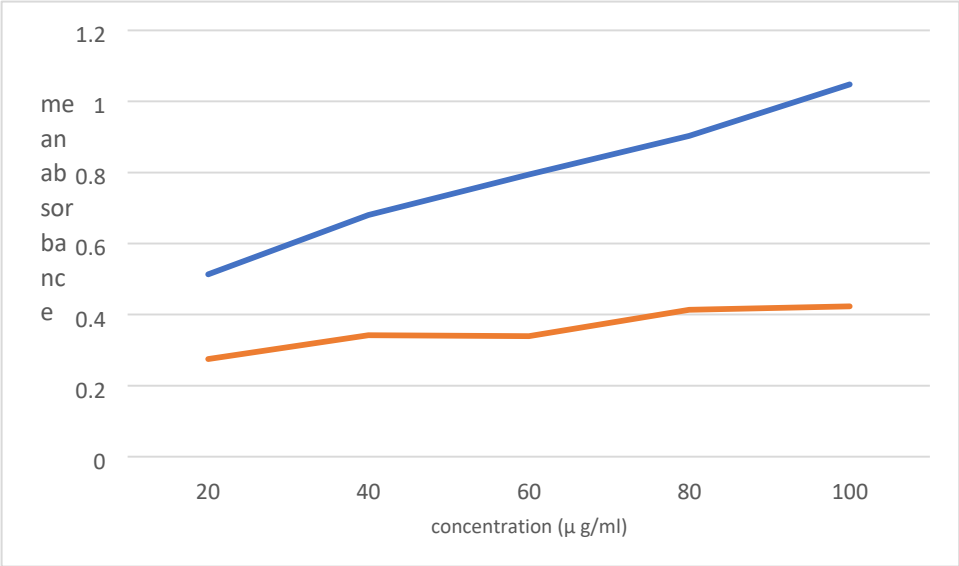

Fig3: Ferric Reducing Power Activity (FRAP) of standard (Vitamin C) and castor oil seed extract.

DISCUSSION

Anti-nutrient composition showed the presence of oxalate (0.25±0.00%), phytic acid (1.80±0.03%), saponin (1.09±0.00%) ,alkaloid was absent. Antinutrients are natural or synthetic compounds, that interfere with the absorption of nutrient. Many traditional methods of food preparation such as fermentation ,cooking and malting increase the nutritive qualitv of plant food through reducing cert 48 anti-nutrients such as phytic acid ,polyphenol ,and oxalate (Hotz and Gibson,2007). Phytic acid interferes with the absorption of certain minerals. The presence of anti- nutrients in the extracted oil is typical of most legumes and oil seeds (Balogun and Fetuga, 1986.)

Some saponins (steroid or triterpene glycoside compounds) can be used for

food while others are toxic. Saponins with a bitter taste are toxic in high concentrations and can affect nutrient absorption by inhibiting enzymes (metabolic and digestive) as well as by binding with nutrients such as zinc. Saponins are naturally occurring substances with various biological effects. In the presence of cholesterol, saponins exhibit strong hypocholesterolemic effect. (Ikewuchi, 2012). They can also lead to hypoglycemia.(Barky *et. al*.,2017).

Some organic acids can have antinutritional factors. Oxalic acid can form soluble(potassium and sodium) or insoluble (calcium, magnesium, iron)salts or esters called oxalates that are commonly found in plants i.e. leafy vegetables or synthesized in the body (Akwaowo *et al.,*2000).Insoluble salts cannot be processed out of the urinary tract once passed through the digestive system. Calcium oxalate can have a deleterious effect on human nutrition and health by accumulating kidney stones (Olawoye and Gbadamosi, 2017)

The peroxide value of castor oil was found to be 100.00±0.03meq/kg. The high peroxide value of the oil sample shows that the oil is prone to rancidity and thus less stable. The saponification value of castor oil was found to be 63.95±0.00mg/kgKOH. It shows that the extracted oil have soap making properties. The value is a measure of the degree at making excellent soaps and cosmetics in industries. The iodine value for castor oil is found to be 3.52±0.03ml/g. Since the value obtained is less than 100,it enable us to classify the oil in the non- drying groups, the value could be used to quantify the amount of double bond present in the oil which reflect the susceptibility of the oil to oxidation which are useful in the manufacture of soaps and can be regarded as liquid oil. Thus, the oil may find application in the manufacturing of lubricants, hydraulic fluid and coating (Ibiyemi *et. al.,*1992).The higher the iodine value, the more reactive, less stable, and more susceptible to oxidation and rancidification of the oil. It also expresses the degree of unsaturation. Acid value also help to ascertain if oils will be highly susceptible to rancidity as the presence of free fatty acids will encourage rancidity. The acid value obtained from castor oil was found to be 20.20±0.0003ml/g. This can be used to check the level of oxidation deterioration of the oil by enzymatic or chemical oxidation, the value falls, within the free fatty acid of oil is expected to range between 0.00–3.00%before it find application in cooking. However the free fatty acid can be modified to edible oil by subjecting it to refining and this will also improve its quality for industrial usage..(Jumat e*t al*,2010).

The graph (Fig.1) reveals a concentration–dependent percentage inhibition for the standard (Vitamin C) while the extract reveal a concentration–independent percentage inhibition. Nevertheless, antioxidant activity was observed at 20µg/ml and 40µg/ml of castor oil seed extract respectively. This finding was corroborated by Singh *et al*,(2010)who reported that *Ricinus communis* seed extracts showed the presence of methylricinoleate, ricinoleic acid, and 12-

octadecadienoic acid, and methylester primarily responsible for the antioxidant activity by free radical scavenging effect on DPPH and hydroxyl radical generated from hydrogen peroxide. Treatment of disease resulting from oxidative stress is one of the benefits of total antioxidant assay. The responsible chemical constituent of *Ricinus communis* which produce antioxidant activity are methylricinoleate, ricinoleic acid, 12–octadecadienoic acid and methylester (Jitendra and Ashish, 2012).

The graph (Fig.2) reveals the trends for both the vitamin E (standard) and the castor oil seed extract. Both vitamin E and castor oil seed extract show a concentration-independent activity. The castor oil seed extract had the lowest percentage inhibition at 80µg/ml(i.e.44.27%) while the highest percentage inhibition was observed at 40µg/ml (i.e.79.75%) respectively. The interesting discovering of castor oil extract as being effective against lipid peroxidation corroborates the findings of while the trend observed for the castor oil seed extract is a concentration-dependent activity, with highest activity occurring at100µg/ml and lowest activity occurring at 20µg/ml. However, the ferric reducing power of the castor oil seed extract was confirmed with increased activities occurring at 40µg/ml,80µg/ml,and100µg/ml respectively. The interesting trend on the ferric reducing power of the castor oil was also discovered by Singh *et al*,(2010)who reported that *Ricinus communis* seed extract showed the presence of methylricinoleate, ricinoleic acid,12octadecadienoic acid, and methylester primarily responsible for the antioxidant activity by lipid peroxidation Singh *et. al.*,(2010)who reported that in *Ricinus communis* seed extracts showed the presence of methylricinoleate, ricinoleic acid, 12–octadecadienoic acid, and methylester, Primarily responsible for the antioxidant activity in lipid peroxidation by ferricthiocynate method and free radical scavenging effect on DPPH and hydroxyl radical generated from hydrogen perioxide. Knowledge of lipid peroxidation will help in the treatment of diseases resulting from oxidative stress. The end products of lipid peroxidation are reactive aldehydes, such as malondialdehyde (MDA) and 4–hydroxynonenal (HNE), these can being known also as "second messenger of free radicals "and major bioactive marker of lipid peroxidation due to its numerous biological activities resembling activities of reactive oxygen species(Adams and Boekel,2008).

The graph (Fig.3) reveals a concentration-dependent activity for the standard (Vitamin C)

by ferric thiocynate method and free radical scavenging effect on DPPH and hydroxyl radical generated from hydrogen peroxide.

CONCLUSION

The study reveals excellent medicinal, therapeutic, ethnomedical and antioxidant properties of castor oil seed extract. The relevance of the assays performed cannot be overemphasized as the knowledge of anti-nutrients and anti-nutritional factors helps to inform the public of the dangers of consuming poorly processed food. The quality control assessment of the oil extract and the potent antioxidant properties contained in the castor oil seed extract.

REFERENCES

Adams, A.N.D and Boekel, M.A.J.S. (2008), Modification of Casein by lipid oxidation product ;Malondialdehyde. *J. Agric. Food Chemical* 56(4):1713-1719.

Akwaowo, E.U, Ndon, B.A, Etuk, E.U. (2000) Minerals and antinutrients in

Fluted pumpkin *(Telfairia occidentalis) Food Chem;* 70(2)235-40.

AOAC (2002) "PeroxideValue at Oils and Fats". Official Methods 965:33

AOAC (2023). Official Methods of Analysis. 22nd Edition. Oils and Fats. 981:11

Balogun,. A.H and Fetuga, B.L. (1986) Chemical Composition of Some Under Exploited Leguminous Crop Seeds in Nigeria. Relevance of acid value *J.Agric. Food chem*. 34;189192.

Barky, A, Hussein, S, AlmEldeen, Y. (2017) Saponins and their potential role in diabetes mellitus. *Diabetes Manag* 7(1):14858.

Benzie, I.F.F. and Strain, J.J. (1999). Ferric Reducing Antioxidant Power Assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid *concentration. Alternat. Med*. 1(2):189-192.

Harbone, J.B. (1984) A guide to modern techniques of plant analysis, Springer Dordrecht, Pp136.

Hotz, C., and Gibson, R.S. (2007) Traditional Food Processing and Preparation Practice to Enhance the Bioavailability of Micronutrients in Plant-based Diet *J. Nutrition 137*(4):1097– 1100.

Ibiyemi, S.O., Adepoju, T.O. Okanlawon, S.O., and Fadipe, V.O., (1992). Emulsion Preparation and Stability. *Journal* of *Nutritional Science*13(1-2):31–34.

Ikewuchi,.C.C.(2012).Hypocholesterolemic effect of an aqueous extract of the leaves of *Sansevieria senegambica* Baker on plasma lipid profile and atherogenic indices of rats fed egg yolk supplemented diet. *EXCLIJ* 11:346-56.

Jitendra, J. and Ashish, K.G. (2012). *Ricinus communis* Linn.A phytopharmacological review.*I nternational Journal of Pharmacy and Pharmaceutical Sciences* 4(4):1-5.

Jumat, S., Dina, A.M.N., Nazrizawati, A.T., Firdaus, M.Y.M., Noraihah .A. (2010): Fatty Acid Composition and Physiochemical of Malaysian Castor Bean *Ricinus communis* L. Seed Oil. *Sains Malaysians*. 39(5)761–764.

Kendall, C.W.C, Amin, E., Jennifer, T.Korbua, S., David J.A., Jenkins, M.D, (2010) Health benefits of nuts in prevention and management of diabetes *Asia Pac J Clin Nutr* 19(1):110116

Lucas, G.M and Markaka, P (1975) Phytic acid and other phosphorus compound of bean (*Phaseolus vugaris).J. Agric. Ed. Chem* 23:13-15.

Munro, A.B. (2000) Oxalate in Nigeria Vegetables. *Biol. Appl. Chem.*12(1):14-18.

Murphy, K.N.C., Singh, R.P., Japaprakasha, G.K (2002) *Journal of Agricultural and Food Chemistry* 50:509-591.

Mutlu, H. (2010). Castor Oil as a Renewable Resource for the Chemical Industry. *European Journal of Lipid and Technology*. 112(1):10–30.

O'Grady,. J., O'Connor, .E.M., and Fergus, S. (2019) Dietary fibre in the era of

microbiome science .*Alimentary Pharmacology & Therapeutics* 49(5):506-515.

Ohkawa, H., Ohishi, N.Yagi, K. (1979) Assay of Lipid Peroxides in Animal Tissues by thiobabituric Acid Reaction *Anal. BioChem*. 95:351–358.

Olawoye, B.T. and Gbadamosi, S.O (2017) Effect of different treatments on *in vitro* protein digestibility, antinutrients, antioxidant properties and mineral composition of *Amaranthus viridis* seed.Cogent Food and Agriculture 3(1):1 – 34.

Singh, R.K., Gupta, M.K., Singh, A.K., and Kumar, S. (2010). Pharmacognostical investigation of *Ricinus communis* stem. *International Journal of Pharmaceutical Sciences and Research* 1(6):89-94

Thakur, A., and Sharma, .V. (2019). An Overview of anti-nutritional factors in foods. *International Journal of Chemical Studies* 7(1):2472-247

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CONSTRUCTION AND PERFORMANCE EVALUATION OF A REMOTE CONTROL BASED HOME AUTOMATION SYSTEM

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Abstract

This study presents a report on the construction and performance evaluation of a remote control-based home automation system. This research describes in detail the construction and performance evaluation of effective remote-control based home automation system that can, coordinate, control and monitor home appliances via infra-red signal from remote control system. In this research, notable components were employed for specific function such as remote, transformation, switching, comparator etc. other components such as resistor, Vero board, soldering lead etc. were carefully and specifically used to actualize the workability of this construction. The home appliances are switched ON/OFF using infrared remote control without actually going near to the switch board or regulator. This electronic system is fast, simple, efficient approach to control and coordinate our home appliances, and works with a great compelling result.

Key words: home automation system, Microcontroller, Remote control system, DTMF Decoder.

Introduction

Home automation systems are dominating rapidly. They are used to provide comfort, convenience, quality of life and security for residents. Home automation system can be designed and developed by using a single microcontroller which has the ability to control and monitor different interconnected appliances such as power plugs, lights, temperature and humidity sensors, smoke, gas and fire detectors as well as emergency and security systems. One of the greatest advantages of home automation system is that it can be controlled and managed easily from an array of devices such as smart phones, hand held remote control system, tablet, desktop and laptop. The rapid growth of wireless technologies influences us to use Smartphone to remotely control and monitor the home appliances around the world (Dickey et *al*., 2012).

Today we are living in 21st century where automation is playing important role in human life. Home automation allows us to control household appliances like light, door, fan, air condition, television, etc. It also provides home security and emergency system to be activated.

Home automation not only contributes to reduce human efforts but also energy efficiency and time saving. The main objective of home automation system is to help handicapped and old aged people to coordinate and control their home appliances and to alert them in critical situations. The home automation features in one's home to help promote security, comfort, energy efficiency, and convenience. Another benefit of home automation systems is the amount of labour, time, energy and materials are saved.

The increasing ubiquity of heterogeneous computing devices such as laptop computers, palms, mobiles etc. shows that users prefer a ubiquitous access of a system rather than to be uncomfortably forced to go physically to the nearest control point. Remote control saves time and everybody is aware of this, it also provides increased security and flexibility. For example, if the user receives a SMS saying that there was an intrusion, he/she can connect to the internet and watch the video cameras inside the house to see what happens, another example could be the possibility to turn on the heaters from the distance using a mobile, laptop or PDA so as soon as the user reaches the house it will be hot already, this could be really useful especially in cold countries. As a matter of fact, security will always be a main priority in all families, and prevention is better than cure (Grill et *al*., 2009) .

In this study, we construct a remote based home automation with the aims to remotely control and co-ordinate security lighting system, to minimize power loss that lead to effective and efficient use of electrical power and time wastage, to control and monitor multiple lighting system using remote control system, and to eliminate the need of being physically present in any switch location for the lighting system to be switched ON or OFF within a house and office.

Research Methodology

- **Circuit Development and Procedure**

The block diagram of this research is depicted below in the figure1.0. This block diagram gives outline detail description of how this work was implemented and the various steps involved in it. From the block diagram, the infra- red remote control system serves as the transmitter from which the user sends commanding signal (code) and instructions to the control section. The received signal by the control section is in infra- red format which is the microcontroller processes and carries out the operation connected to ULN2003 which amplified the signal that triggers the relay. The relay switches ON or OFF as instructed by the microcontroller in the control section, the different appliances connected to the home automation.

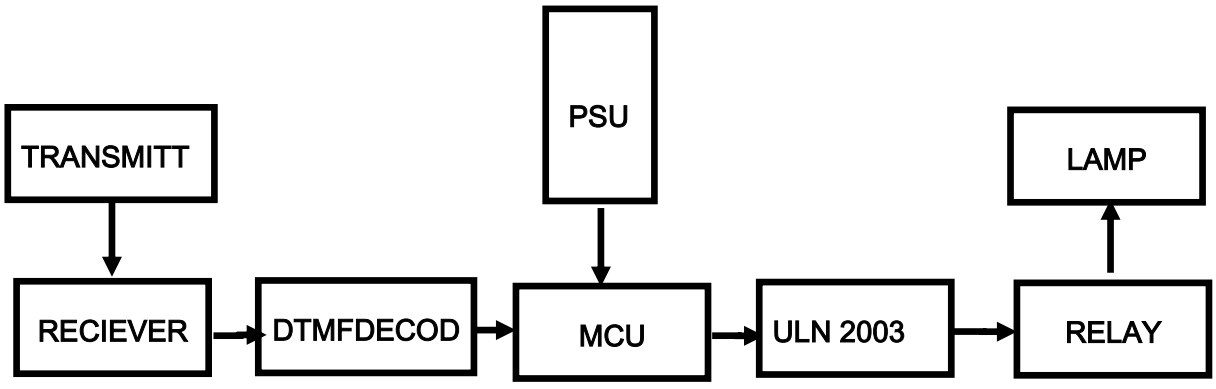


Figure 1.0 a block diagram of a remote based home automation

- **Power Supply**

Basically all electronic circuits are biased with voltage supply. In this research, 5V is employed to bias the various electronic circuit components used in the

construction. Since the supply from various power authorities in Nigeria is 220V in order to provide our circuits with appropriate voltage supply different electronic circuit have to be made based on design requirement. Here are the different stages of circuit design:

- **Transformer**

  In this research, a step down transformer of 220/12V output was employed. The step down transformer helped to step down the supplied voltage to 5V required for the designed electronic circuit.
- **Rectification**

  The home automation circuit needs a dc supply, so the ac output from the transformer secondary winding is used to drive a bridge rectifier circuit to convert voltage signal to dc. A bridge rectifier circuit basically has four diodes connected as shown below to provide rectification.. In this research, bridge rectifier circuit is employed for rectification of ac to dc.

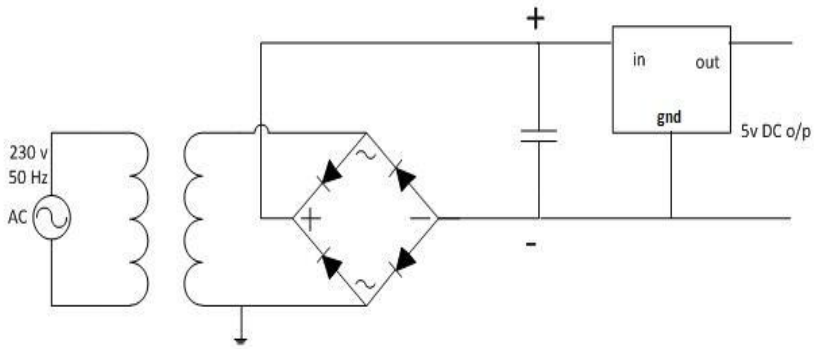


Figure 2.0. a Bridge Rectifier Circuit.

- **Filtering Circuit**

  In this research, a capacitor was connected to the output of the bridge rectifier circuit in order to filter out element of ac ripple from the electronic circuit.
- **Regulation of Voltage**

  A voltage regulator (IC7812) was employed to stabilise the pulsating dc output from the smoothing circuit.
- **Control Circuit**

  The control circuit basically consists of all the electronic circuit components and integrated circuits, the design is as follows:

  DTMF Decoder

A DTMF (MT8870) was employed in this electronic circuit as the receiver IC of commanding signal from the remote control system.. The DTMF takes a number code from the number pad and converts it to DTMF (dual tone multi frequency) signal and then a DTMF decoder converts the DTMF signal to digital code that can be fed to the microcontroller.

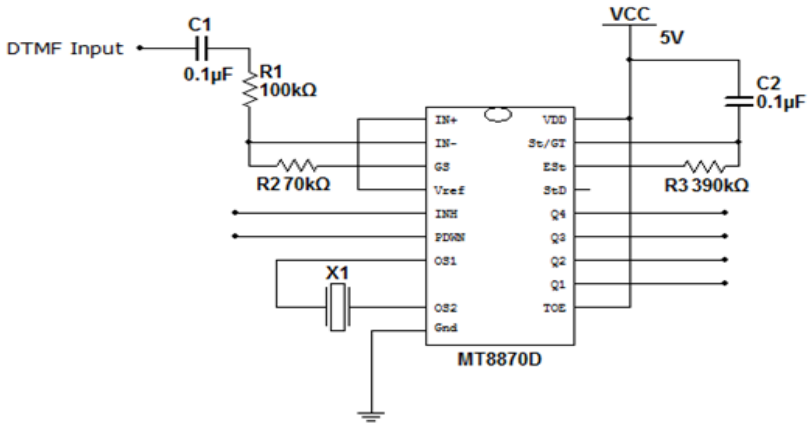


Figure 3.0. DTMF Decoder IC (MT8870**)**

8051 Microcontroller

An atmel AT 89S52 was programmed to take digital output from the DTMF decoder through pins of port P1 and then process to give the appropriate output through the pin of port P0 to the relays connected to switch ON/OFF of the particular appliances.

ULN2003 (Darlington Array IC)

An ULN2003 was used in research and it serves as current amplifier. It amplifies the output current from the DTMF and provided amplified output drive the relay (Das, et *al*. 2013)

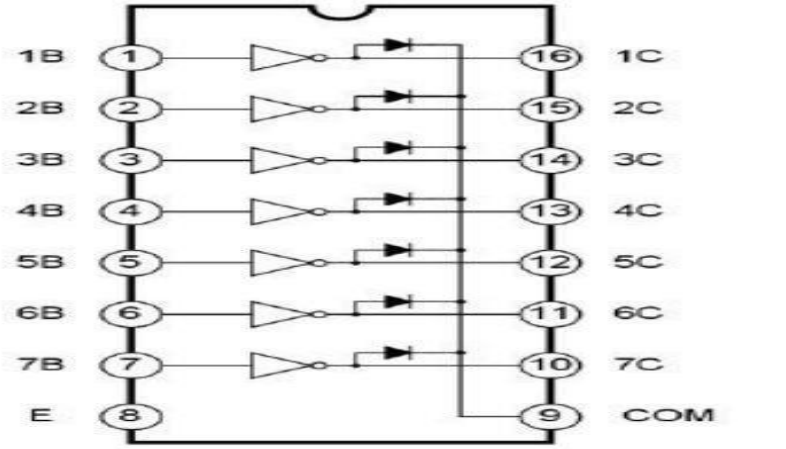


Figure 4.0 ULN2003 IC Logic Diagram

Relay

The relay is the final stage of this remote based home automation system which plays a very prominent role to control the final appliances. A relay was used to isolate the lamp electrically.

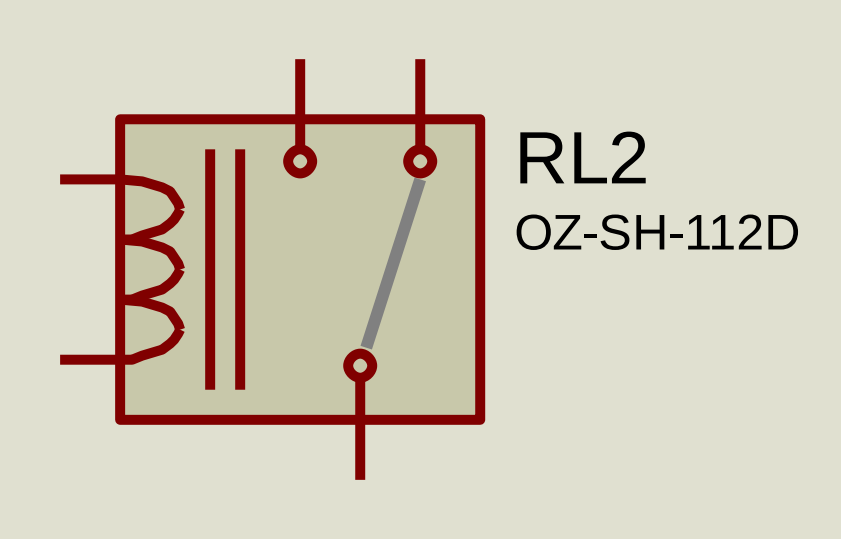


Figure 5.0. A relay

- **Principle of Operation Remote Controller Based Home Automation**
  The remote controller based home automation is an electronic circuit system that can be used to control and co-ordinate any electrical gadget by sending commanding signal to it. The system will faithfully respond to every command made from your remote control system and will electrically switch the connected load ON or OFF base on the imputed instructions by the users.
  It employs the fundamental principle of converting infra-red signal from remote control system into a command output to operate the relays. The infra- red signal receiver acts as sensor and is permanently attached with the internal control circuit of the unit. It senses, receives the signal and then sends it to the DTMF. The DTMF in turn converts the signal into digital signal and then sends it to the microcontroller. Now the microcontroller will process the digital signal then send instruction to the relays which then switch on or off the load.
- **Construction of a Remote Based Home Automation System**
  In the cause of development of this electronic circuit, each component and material was tested before configuration to know the values of the components and to know their working conditions, so that there would not be any problems after the components had been soldered or configured on the Vero- board. Point to point continuity test was carried out; the some faults were traced and corrected.

The development this system was first carried out on bread circuit board in order to as certain or confirm the workability of the circuit diagram. Then the board layouts of electronic circuit components were done on the vero circuit board and they were soldered with the aid of soldering iron and soldering lead.

- **Testing**

In the cause of soldering and testing, point to point continuity test was carried out; the following faults were traced and corrected:
The diode across the relay was bad and it was replaced with new one.
This programming written in

microcontroller was wrong and then it was reprogrammed.

- **Configuration of the Infra Red Remote Control System**

Address pin of the Infra Red receiver connection

Include the infra-red receiver library by ken sheriff

Include the infra-red receive header

Connect the data pin of the infra-red receiver to pin 9 of the microcontroller.

Declare the pin 9 of the microcontroller as receive pin

Enable the print option.

Open the serial monitor of the ARDUINO IDE.

Point the remote to the infra-red receiver and press any button.

Hexadecimal numbers were shown in the serial monitor representing the unique code of each button.

- **Coding of the Relay**

Declaring the address pin

Include Infra-red relay header file

Declare the pin using int x= pin number

Enable the pins as output

Communication between microcontroller and infra red receiver

Copy the hex number of each remote button to the required area or declare the hexa decimal numbers as code1,code2 and code3.

Use if statement to interswitch state.

Verify and upload into the microcontroller.

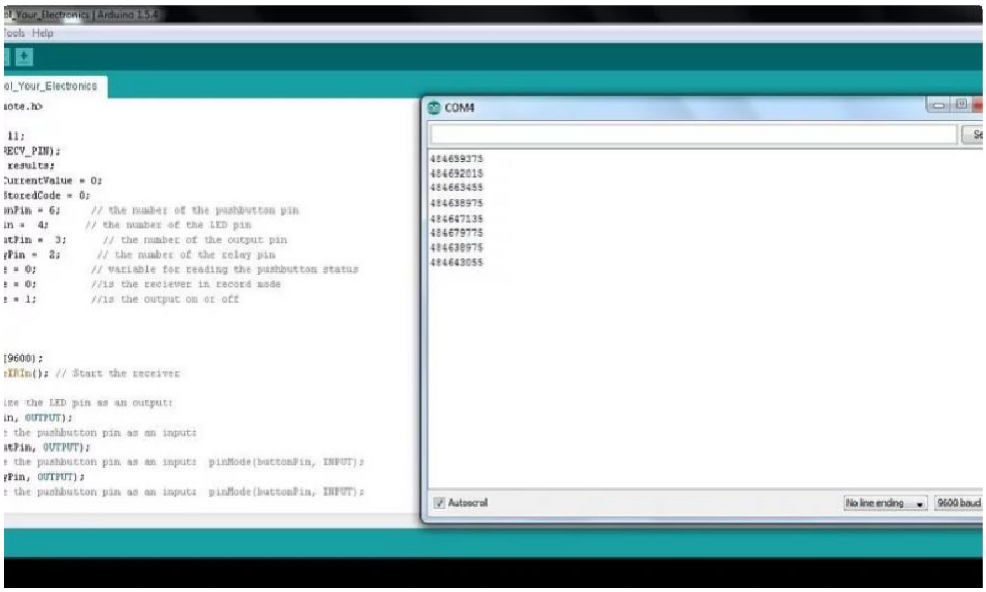


Figure 6.0. ARDUINO IDE showing the HEX number and the codes

System Performance

The remote control based home automation was employed to co-ordinates and controls the appliance and its performance was absolutely okay.

Instrument, Tools and Material (Component)

- **Instruments/ Tools**

The instruments/ tools used in construction of this work include:soldering iron

screw drivers

pliers

de-soldering pump

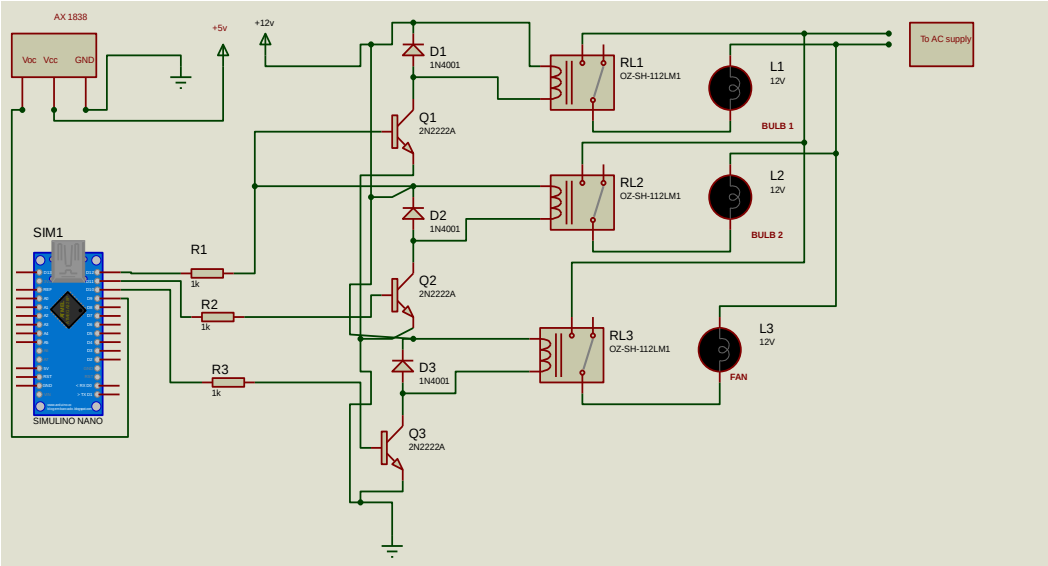
Multi-meter
Cutter etcetera.

Circuit Components

The component used includes:
Infra-Red Receiver sensor
IC 7812
IC 7805
8051 microcontroller IC
Infra-red decoder
Relays
Transistor (Switching)
ULN2003
Voltage Regulators
Transformer
Bridge Rectifier
Capacitors
Resistors
regulator (zener diode)
vero board
load jumper
integrated circuit ,etcetera

Circuit Diagram

Figure 7.0 Circuit diagram of Remote Based Home Automation



Casing of Remote control based Home Automation

In this research, plastic was employed for casing of the remote control based home automation. Figure below displays the remote control based home automation.

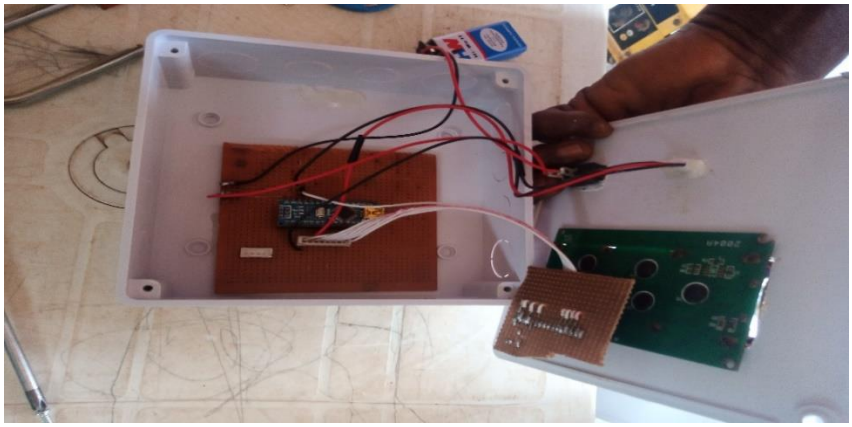


Figure 8.0. The casing of the circuit

Result

In this section the developed remote based home automation is employed to coordinate and control the security lighting system with the aid of remote control system that the user used to instruct the system. The developed electronic system responded by switching ON and OFF the entire security lighting system that was connected to it.

Discussion

The performance evaluation of this developed electronic measurement system was carried out by employing to coordinate and control the security lighting system. The coordination and control was perfectly observed in respond to instructions from the remote control system. This remote based home automation is capable of coordinating and controlling home appliances. This piece of electronic measurement is of low cost, easy to implement, occupy lesser space, easy maintenance, fast, efficiency and more reliable. This electronic system works with a great compelling result.

Conclusion

In this research, the construction and performance evaluation of temperature and humidity measurement system was properly implemented. The aim is to construct and evaluate the performance of the electronic system in order to control and coordinate home appliances. After several tests were carried out and programming with different codes, eventually the obliged outcome is put forward. It is a fast, simple, and efficient approach to control and coordinate our home appliances. This electronic system works with a great compelling result

References

Abhishek, V. and Silpa, V.M. (2012): "Embedded web server for hom appliances"at national conference on emerging trends in engineering and technology(VNCET-30).

Das, C. K., Sanaullah, M., Sarower, H.M.G and Hasse M.M,(2013):"Development of a cell phone based remote control system: an effective switching system for controlling home and office applinces", international *journal of electrical and computer sciences IJECS-IJENS.*

GILLS, K., Yang, S.H , Yao, F. and Lu, X. (2009):" A Zigbee based home automation system" IEEETransaction on consumer electronics.

Li, Rita Yi Man; Li, HerruChing Yu; Mak, Cho Kei; Tang, Tony Beiqi. (2016): "Sustainable smart home and home automation: Big data analysis approach". *Interntional journal of smart home.*

Dickey, N., Banks, D. and Sukittanon, S. (2012):

andMobiledevices".Southea stcon, proceedings of IEEE, Orlando, FL.

Rye, D. (2014): "My life at X10. Av and automation industry e-Magazine.

Xiaoneng, G., and pengtong, F. (2011): "Internet access technology based on the embedded Devices" in Multimedia technology (ICMT), international conference, Hangzhou.

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DEVELOPMENT AND PERFORMANCE EVALUATION OF AUTOMATIC TEMPERATURE AND HUMIDITY MEASUREMENT SYSTEM

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Abstract

This research reports and explains in details the development and performance evaluation of automatic temperature and humidity measurement system. The system has the potency to measure temperature and humidity of an environment and display the value in digital form. Major advantages of this automatic temperature and humidity measurement system are: low cost, easy to implement, occupy lesser space, easy maintenance, fast, efficiency and more reliable. A programmable microcontroller to control the system, DHT11 temperature-humidity sensor was employed to sense the temperature and humidity of the environment 1which serve as input to the microcontroller, some additional electronic circuit components such as light emitting diode (LED), fixed resistors, variable resistor, 9V DC battery, switch, diode, ten pin sockets, and Vero board were employed in construction of this measurement system. Studies have shown that there are difficulties in detecting variation in temperature and humidity of environments for rearing of animals, growing of crops and electricity generation. This piece of electronic system has been found to be of help. This project work focuses on the development and practical implementation of temperature and humidity measurement system to sense and measure the temperature and humidity of the environments. Users of this piece of electronic system can easily receive feedback status of the temperature and humidity under measurement via the liquid crystal display component of this system.

Keywords: Sensor DHT11, Light emitting diode, Liquid crystal display screen, Microcontroller

Introduction

The extent to which humidity and temperature plays a part in any given production processes may vary but, in many cases, it is essential that, at the very least, they are monitored and in most cases, controlled.

In electricity generation station, gas turbines are heat engines used to generate electricity. These consist of a turbine, a compressor, and a combustion chamber. During operation the turbine pulls in outside air, mixes it with gas, and ignites it in the combustion chamber. The quality of this intake air contributes to the efficiency of the electrical output, the mechanical integrity of the turbine and the amount of exhaust pollution. Unconditioned air can reduce the electrical output. Ice particles can damage the turbine blades. Hot dry air can generate more pollution. Therefore, it is important to monitor the temperature and humidity of the intake air

In crop production, the temperature of the environment is extremely important for plants as it affects multiple growing factors such as the rates of photosynthesis and respiration, germination, flowering, and ultimately, crop quality. Extreme

temperature can negatively impact plant productivity, so maintaining the temperature in a greenhouse is equally as important.

High humidity can be fatal to plants if it is not monitored, as a build up of moisture on plants promotes the germination of fungal pathogen spores such as Botrytis and powdery mildew. Therefore it is important to ensure the circulation of air through the greenhouse to reduce the water vapour around the plant (Sonawane et *al*, 2019).

Poultry farming, when birds are kept in environmental temperature above or below their comfort zone, more energy must be expended to maintain body temperature. This requires extra energy will be supply by the feed consumed. When expose to colder temperature, birds eat more feed to sustain normal body temperature. The feed is then used for warmth and cannot be converted to meat, and when they are exposing to hot temperature, the feeds are wasted as the birds attempt to keep cool (Olatayo et *al*, 2019).

These lead to the development and performance evaluation of temperature and humidity measurement system. Temperature and humidity measurement system is a piece of electronic equipment that capable of sensing and measuring temperature and humidity simultaneously and display the result of the measurement in digital format.

The aim of this research is to evaluate the performance of this developed electronic measurement system for monitoring and regulating of temperature and humidity for crop production, electricity generation, and poultry farming.

Research Methodology

Circuit Development and Procedure

This section deals with development of the electronic circuit and the block diagram of the developed electronic circuit is shown in Figure 1.0

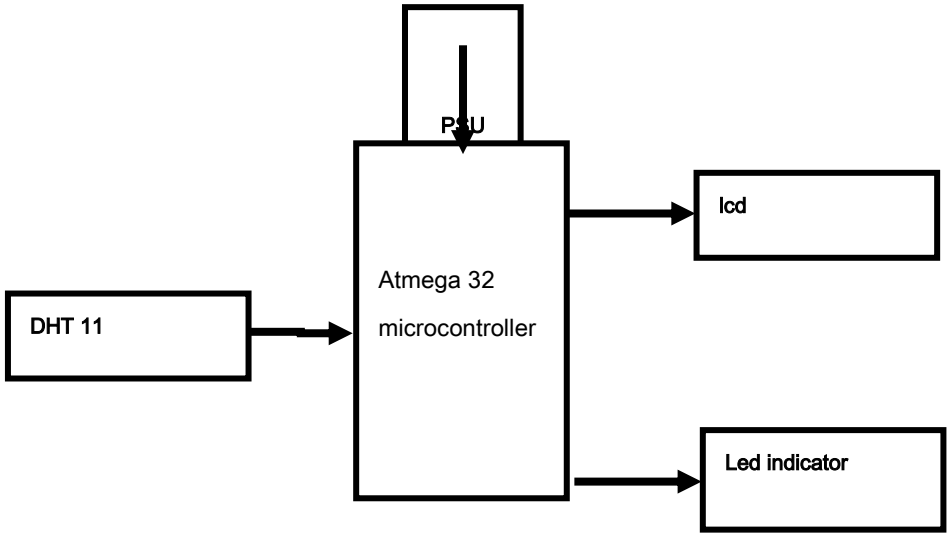

Figure 1.0 Block diagram of Temperature and Humidity Measurement System.

This block diagram shows that an Atmega 32 microcontroller is interfaced with the following electronic circuit components such as: DHT11 sensor for measuring of the users. temperature, Liquid crystal display for displaying the value of temperature and humidity measured as feedback to

Hardware Development

This electronic measurement system is made of the following units such as: power supply unit, sensing unit, control unit, and display unit.

Power Supply Unit

In this research development, the power requirements had been carried out in relation to the various components that would be used. In order to get all of these components bias at different voltage levels and types, a linear power supply was employed. A 9V battery is employed to power the electronic circuit system.

Sensing Unit

DTH11 was employed for sensing and measuring the temperature and humidity of the poultry house, green house and environment for electrical energy generation. This DTH11 has excellent quality, fast response, strong anti-jamming capability, and high cost.

Control Unit

Atmega 32 microcontroller was used in the development of electronic circuit for this electronic measurement system. It is a type of microprocessor furnished in a single integrated circuit and needing a minimum of support chips. It is the data processing element of this system and it is responsible for receiving the signal from the DTH11 temperature and humidity sensor.

A 9V battery is used to supply suitable amount of voltage to the programmable microcontroller. DTH 11 was also connected the microcontroller which in turn after processing the received signal converted them into digital signal.

Liquid Crystal Display Stage

Liquid crystal display was employed in this project work to display the result of measured values by the temperature and humidity measurement system in digital form. Liquid crystal display was interfaced with the output of the Atmega 32 microcontroller. When the temperature and humidity is detected by the DTH11, the microcontroller processed the signal and the liquid crystal display displays the measured value digitally.

Circuit Diagram of Temperature and Humidity Measurement System

The circuit diagram of temperature and humidity measurement system is depicted in Figure 2.0 below.

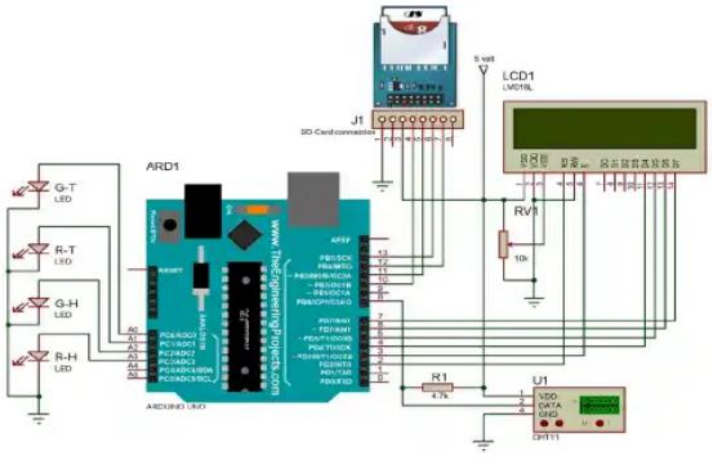

Figure 2.0. Circuit Diagram of Temperature and Humidity Measurement System; source (Sonawane *et al*., 2019)

Principle of Operation of Temperature and Humidity Measurement System

As the temperature and humidity of the environment changes, the DTH11 receives and measures the signal from the environment and transmits the measured signal to the microcontroller. The Atmega 32 microcontroller in turn converts the received signal into digital signal. The microcontroller then executes the instruction programmed in it and turns on the liquid crystal display which displays the measured value of temperature and humidity.

Construction and testing

In the cause of development of this electronic circuit, each component and material was tested before configuration to know the values of the components and to know their working conditions, so that there would not be any problems after the components had been soldered or configured on the Vero- board. Point to point continuity test was carried out; the some faults were traced and corrected.

Developed Temperature and Humidity Measurement System

Figure 2.3 shows the developed temperature and humidity measurement. This piece of electronic measurement was developed from the following electronic circuit components such as: DTH11, Atmega 32, resistor, 9V battery, LED, LCD and the other devices

.

Figure 3.0. Developed Temperature and Humid

System Performance Evaluation

The temperature and humidity measurement system was practically implemented to co-ordinates, controls and measures the temperature and humidity of the environment

Instruments and Tools

The instruments and tools used for the development of this project include:

- multimeter
- Cutter
- soldering iron
- screw drivers
- pliers

- de-soldering pump, etcetera.

Components

The component used includes:

- Atmega 32 Microcontroller
- DTH11
- Fixed Resistor
- Vero board
- Diode
- Ten Pin Socket
- Ceramic Capacitor
- Light Emitting Diode

Casing of Luminous intensity Counter System

In this research, plastic was employed for casing the temperature and humidity measurement system. Figure 2.2 displays the temperature and humidity measurement system.

Result

In this section the developed temperature and humidity measurement is employed for temperature and humidity measurement. Table 1 shows the measurement results obtained with the aid of this electronic measurement system.

Time	Place	Temperature	Humidity
8:00am	Sast Open Field	32℃	62%
	Sast complex	30℃	64%
	Sast office complex	31℃	61%
10:00am	Sast Open Field	29℃	59%
	Sast complex	30℃	56%
	Sast office complex	28℃	60%
12:00pm	Sast Open Field	32℃	63%
	Sast complex	28℃	70%
	Sast office complex	30℃	67%
2:00pm	Sast Open Field	32℃	52%
	Sast complex	30℃	77%
	Sast office complex	31℃	79%
4:00pm	Sast Open Field	33℃	50%
	Sast complex	29℃	60%
	Sast office complex	30℃	55%
6:00pm	Sast Open Field	32℃	45%
	Sast complex	30℃	50%
	Sast office complex	33℃	44%

Table 1 the results of temperature and humidity measurement by the developed Temperature and Humidity Measurement System

System Performance Evaluation

Thetemperature and humidity measurement system was practically implemented to co-ordinates, controls and measures the temperature and humidity of the environment

Casing of Luminous intensity Counter System

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Result

In this section the developed temperature and humidity measurement is employed for temperature and humidity measurement. Table 1 shows the measurement results obtained with the aid of this electronic measurement system.

Discussion

The performance evaluation of this developed electronic measurement system was carried out by employing to measure temperature and humidity of our SAST complex. The readings were taken in one day at different time intervals at SAST lecture complex, SAST open field, and SAST office complex. From the table 1, it is clearly observed that this temperature and humidity measurement system is capable of detecting and measuring temperature and humidity of any given environment in order to control and monitor the temperature and humidity simultaneously. This piece of electronic measurement is of low cost, easy to implement, occupy lesser space, easy maintenance, fast, efficiency and more reliable. This electronic system works with a great compelling result.

Conclusion

In this research, the development and performance evaluation of temperature and humidity measurement system was properly implemented. The aim is to develop and evaluate the performance of the electronic measurement system in order to control and monitor the impact of temperature and humidity on our crop productions in green house, egg productions, healthier chicken for human consumption and generation of electricity. This piece of equipment can be implemented to detect and measure the temperature and humidity and hence digitally display the quantity of measurands. After numerous tests were carried out and programming with different codes, eventually the obliged outcome is put forward. It is a fast, simple, and efficient approach to measure temperature and humidity. This electronic system works with a great compelling result.

References

Crecratft, D.I. (1993): Electronics: Analog and Digital Electronics, Open University, Delhi, India.

Gupta, J.B. (2012): Electronics and Electrical Measurement and Instrumentation,S.K. Kataria and Sons, Delhi.

James, M. (2001): Microcontroller Cook Book, 2nd Edition, Newness Butterv

Journal of Applied Science and Technology, V

London, UK.

Johnson, C.D. and Malki, H. (2001): Control Systems Technology, Pearson Education, London, UK.

Kale, V. and Kulkarni, R. (2016). Real Time Remote Temperature and Humidity Monitoring Using Arduino and Xbee S2.

International Journal of Innovative Research in Electrical, Electronics, Instrumentation and Control Engineering , 175-179.

Kissell, T.E. (1997): Industrial Electronics,Prentice Hall, New York, USA.

Manghnani, S. R. (2017). Arduino Based Wireless System for Temperature and Humidity Monitoring. *International Conference on Emerging Trends in Engineering, Science and Management* , 521- 528.

Oboh M.E, Okpanachi, B. and Abode, H.O. (2015): Basic Electronic 2nd Edition, Best Way, Auchi.

Olatayo, M.O., Mutiu, A.A., Olisaemeka, F.I., and Omodundi, B. (2018): Design and Implementation of a Temperature and Humidity Control System for a Poultry House Prototype, ATBU, *Journal of Science, Technology and Education*, 6, 1.10-114.

Scherz, P. (2000): Practical Electronics for Inventors, McGraw-Hills,USA.

Sonawane, R.N., Ghule, A.S., Bowlekar, A.P., and Zakane, A.H. (2019): Design and Development of Temperature and Humidity Monitoring System, *Agricultural Science Digest*, 39, 2,114-118.

Theraja, A.K. and Theraja, B.K. (2010): Electrical Technology, Chand and Co, Delhi, India.

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VARIATION IN THE INTERACTION BETWEEN PHASEOLUS VULGARIS L. AND MACROPHOMINA PHASEOLINA (TASSI) GOID

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ABSTRACT

Charcoal root rot caused by the soil-dwelling fungus Macrophomina phaseolina, anamorphic synonym Rhizoctonia bataticola , is more severe in regions with temperatures between 18 and 35 ° C and low humidity. Due to global warming it has become one of the most important diseases in the production of common bean in tropical countries. Its symptoms include dampping off, wilting, early defoliation and stem cancer, followed by death of plants under extreme conditions. The populations of this fungus present high pathogenic variability, which hinders its control. The objective of this study are: to investigate the variability of isolates of M. phaseolina fungus, to identify sources of horizontal and vertical resistance in bean genotypes and to investigate the differential interaction between P. vulgaris and M. phaseolina to subsidize common bean breeding programs and provide information on the disease to farmers. Twenty - four isolates of M. phaseolina were analyzed by inoculation in a common bean cultivars set. Ten isolates were classified into four pathotypes, based on the cultivars susceptibility or resistance. M. phaseolina fungus race less aggressive was the most frequent. The results show how important is knowing M. phaseolina variability and its specific interaction with P. vulgaris , to establish new strategies in beans breeding programs and incorporation of resistance genes into new cultivars.

INTRODUCTION

The common bean (*Phaseolusvulgaris* L.) is one of the most consumed legumes in the human diet, its grains are a source of proteins, lipids, carbohydrates, vitamins, minerals and fiber. A food security factor in several countries, it has greater nutritional, economic and social importance in countries in Africa, Asia and Latin America (Barbosa and Gonzaga, 2012; Yi et al., 2016). Because it is cultivated in different ecosystems, its production is affected by abiotic and biotic factors. Due to rising temperatures caused by climate change, the fungus *Macrophomina phaseolina* (Tassi) Goid has become economically important in crops such as soybeans, cotton, strawberries and sorghum in the United States, Australia, Mexico and India (Radwan et al. 2014 ; Sun et al., 2016).

*Macrophomina phaseolina*is the causal agent of gray stem rot, a disease that can cause losses of up to 60% in bean production in susceptible cultivars. This pathogen can parasitize roots, leaves and seeds throughout the bean crop cycle (Mayek et al., 2002). Symptoms include poor seed germination, seedling death, wilting, premature defoliation and stem canker followed by plant death in case of more severe infections. The disease is favored by temperatures between 18 °C and 35 °C and low humidity. It is difficult to control because it forms resistance structures that remain viable in the soil for up to 15 years (Gupta et al, 2012).

Several measures can be taken to control gray stem rot, including seed treatment, scheduling planting dates, avoiding high plant density, using proper fertilization, controlled irrigation, applying fungicides

and biological control agents to the soil, removing of crop residues at the end of the cycle and crop rotation (Kaur et al., 2012). However, these strategies have not been effective in controlling the pathogen and are often difficult to implement in developing countries due to the high costs of acquiring inputs. Therefore, the most practical option for the management of the disease is still genetic control (Mayek et al., 2001).

In order to follow this strategy, it is necessary to characterize the diversity of the *M. phaseolina population,* and provide a better understanding of the pathogen's aggressiveness and its interaction with the host. In this way, common bean breeding programs can be contributed to the development of cultivars with stable and durable resistance (Viteri & Linares, 2017). In view of the above, this work aimed to: determine the virulence of the isolates in common bean genotypes, identify cultivars that can be used as a source of vertical and horizontal resistance in breeding programs and validate the series of common bean cultivars that differentiate races physiology of the pathogen.

MATERIAL AND METHODS

CULTIVARS

Gonçalves & Wendland (2015) verified the existence of a differential interaction between *M. phaseolina* and *P. vulgaris* with the inoculation of five isolates in 59 common bean genotypes. In this study, the genotypes that make up the present study were suggested as differentiating cultivars of the physiological races.

Figure 1. Common bean cultivars evaluated in the study of differential interaction between *M. phaseolina* and *P. vulgaris*.

CONFIRMATION OF IDENTITY

Traditional methods

The 24 isolates used in the present study were analyzed to confirm and compare their taxonomic, symptomatic (Baird et al.,

2003) and cultural (Dhingra& Sinclair, 1973; Kaur et al . al., 2012) according to bibliographic reports of the fungal species *M. phaseolina*.

Molecular identification

DNA Extraction

DNA extraction was performed using the protocol of Dellaporta et al. (1983) modified. The 60 mm Petri dishes with the isolated isolates were incubated at 23 °C and photoperiod 12 h light for 5 days. When grown, the mycelium was scraped off and transferred to 2 mL eppendorf and macerated with a glass rod. 500 µl of Dellaporta buffer (1.57 g 100 mMTris-HCL; 1.86 g 50 mM EDTA; 2.92 g 500 mMNaCl; 100 ml MilliQ H_2O with pH adjusted to 8.0) was added and shaken. for 2 minutes by vortexing. Then, 40 µL of 20% SDS (2.8 g sodium dodecyl sulfate; 14 mL of $H_2 0$ MilliQ) was added, vortexed for 2 min and incubated at 65 °C for 30 minutes in a water bath; periodically shaking every 10 minutes. Immediately after the heating process, 300 µl of 5 M potassium acetate (48 g potassium acetate; 100 mL H_2O MilliQ) was added, vortexed for 2 min and centrifuged at 14,000 rpm, at 25 °C, for 10 minutes. The supernatant was transferred to a new eppendorf, to which 330 µL of ice-cold isopropanol was added, gently inverted 50 times and then centrifuged for 10 minutes at 14,000 rpm. The supernatant was discarded and 500 µL of 70% alcohol was added to the decanted material (pellet) for two consecutive times with centrifugation for 30 seconds between washes. Then 250 µL of absolute alcohol was added, centrifuged for 5 minutes and the supernatant was discarded again. The pellet was exposed for 40 minutes for drying and then 50 µL of TE pH 8.0 was added. The extracted DNA was quantified by optical density in a spectrophotometer (NanoDrop™ 2000/2000c - Thermo Fisher Scientific). The samples were diluted in TE to a concentration of 30ng DNA/ µL and stored at -20 °C.

Pathogen detection via LAMP

DNA amplification by LAMP (isothermal amplification of nucleic acids) to confirm the identity of *M. phaseolina isolates* was performed using the methodology and primers (Table 2) proposed by Rocha et al. (2017). In a 25 µL reaction containing: 1.6 µL of each MphLFIP and MphLBIP primer, 0.2 µL of each MphLF3 and MphLB3 primer, 16 U *Bst DNA Polymerase* , 1.4 mM of each dNTP, 10 mM of (NH4)2SO4 , 10 mMKCl, 20 mMTris-HCl buffer (pH 8.8), 0.8 MBetaine, 0.8 mMof (NH4)2SO4 and 25 ngof DNA and 100 mMof the neutral red dye. *M. phaseolina* DNA was replaced by *Fusariumoxysporum DNA* in the control sample and Milli-Q water for the negative control. Amplification via LAMP was performed in a T100 thermocycler (Thermal Cycler – Bio Rad) at 65 °C for 2 hours and 45 minutes. The products of the reactions were visually analyzed, samples with amplification of the target DNA and the control samples were colored reddish and yellow, respectively. To confirm the results, amplifications were performed three times.

Pathogen detection via PCR

For the PCR reaction (Polymerase Chain Reaction) oligonucleotides MphLF3 and MphLB3 specific for *M. phaseolina* designed by Rocha et al. (2017) (Table 2). In a final volume of 10 µL, the reaction was prepared with the PCR Kit (QIAGEN®) containing: 5 µL of the Master Mix; 1 µL of Q-Solution; 1 µL of MphLF3 primer (10 µM); 1 µL of MphLB3 primer (10 µM); and 2 µL of the extracted DNA. For the negative control, in place of the DNA, DNA from *Fusariumoxysporum*sp was placed, and for the blank, autoclaved MiliQ water. The reactions were placed in a thermocycler with an initial denaturation schedule (95

°C for 15 min), 40 denaturation cycles (94 °C for 1 min), 40 annealing cycles (57 °C for 1 min and 30 sec), 40 extension cycles (72 °C for 1 min and 50 sec), 40 extension cycles (60 °C for 30 sec), in addition to the was performed at a current of 5 volts/cm for one hour. The gel was developed in a 1X Tris base-boric acid-EDTA (TBE) solution for 30 minutes to visualize the RESULTS

final extension (60 °C for 30 sec). After the PCR reaction, the products were applied to a 1.5% agarose gel and stained with *blue juice*. The run

bands in a photo document. To confirm the results, amplifications were performed three tim

Result of Traditional methods

Traditionally, the identification of fungal species is done by analyzing their taxonomic characteristics. In all the isolates used in the present study, the characteristics were analyzed:

- Morphological - formation of pycnidia on the host stem (Figure 4);
- Symptomatic - hypocotyl strangulation, necrotic lesions from the root and cotyledon of seedlings (Figure 5);
- And cultural - coloring from gray to black of colonies and formation of microsclerotia in PDA medium (Figure 6).

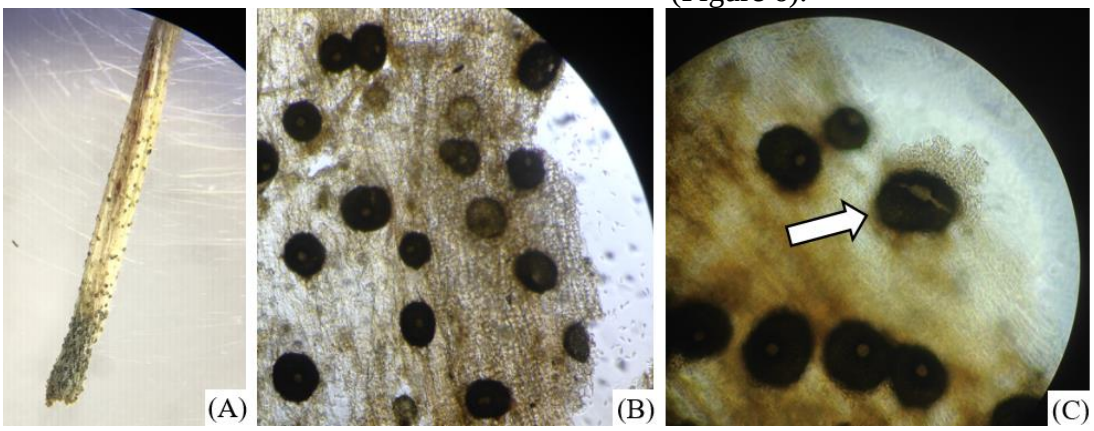

Figure 2. Photos under magnifying glass and optical microscope: (A) Presence of pycnidia on the bean stem; (B) cutting the epithelial tissue of the stem; and (C) maturepycnidia releasing conidia.

Figure 3. Pictures of gray stem rot symptoms in common bean seedlings; (A) hypocotyl strangulation; and (B) necrotic lesions from the root and cotyledons.

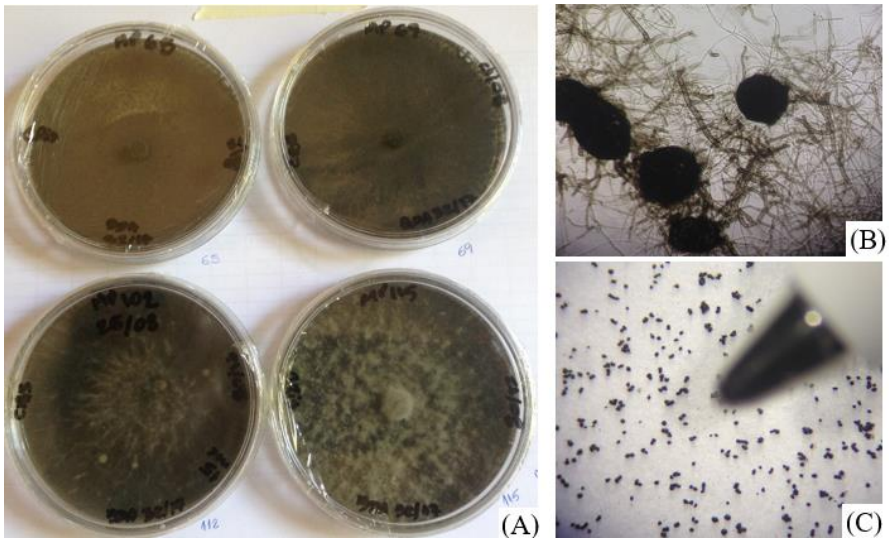

Figure 4. Characteristics of colonies in PDA medium; (A) gray to black isolates; (B) optical microscope photo of the microsclerotia; and (C) magnifying glass photo of microsclerotiaextracted from the culture medium compared to the size of a pen tip.

Molecular Identification

Molecular analyzes via PCR (Figure 7) and LAMP (Figure 8) showed that the 24 isolates tested are of the *M. phaseolina species*, as there was amplification of DNA fragments in all samples.

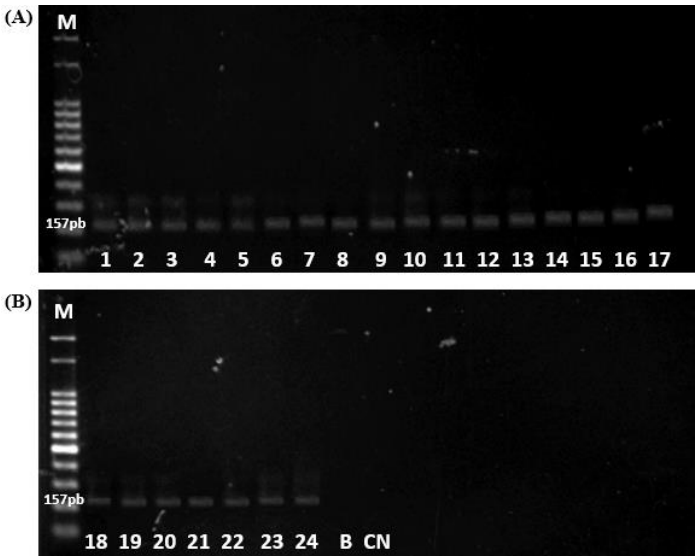

Figure 5: PCR products separated by agarose gel electrophoresis (1.5%), photo documentation. Lanes 1 to 24: amplified *M. phaseolina* DNA fragments , 157bp ; Negative control, *F. oxysporum*; White, MiliQ water; M, molecular marker.

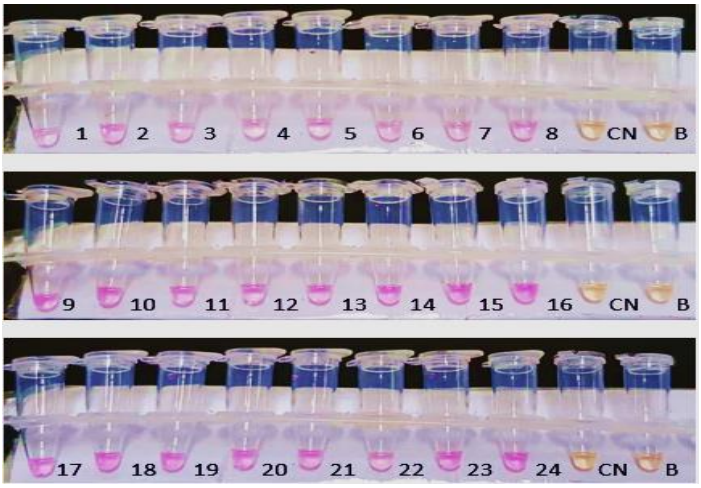

Figure 6: Visual detection of amplification of DNA fragments via LAMP. Erlenmeyer flasks numbered 1 to 24 - amplified fragments of *M. phaseolina DNA* , colored by neutral red dye; (CN) Negative control, *F. oxysporum*; White, MiliQ water.

DISCUSSION

The samples for the first trial (40.59) had the lowest value compared to the second (46.14) and third (50.55) trial. Because high temperatures and low humidity are the perfect conditions for the development of gray stem rot, such values can be highly related to temperature variations during the experiment (Islam et al. 2012). In the first, even with the average temperature (27.9 °C) favorable for the development of the disease, there were days when the minimum temperatures (12, 13 and 1.3 °C) were inadequate (Figure 6). In trials 2 and 3, temperatures remained above the minimum required for optimal disease development, 18 °C (Gupta et al., 2012). Dhingra & Chagas 1981, demonstrated that soil temperatures around 20 °C are more favorable for the colonization of common bean stems. So, such environmental conditions reflected in the severity of the disease, with higher scores which was isolated from the cultivar BRS Notável, the second most resistant of the group. The isolates 32325, 32284 and 32286 also caused susceptibility reactions in their original cultivars (Embaixador, Notável and Esplendor) and in other more resistant cultivars. Noting that the source cultivars of the isolates were always susceptible to them, which proves the specialization of the fungus *M. phaseolina*. This reaffirms the idea that isolates taken from more susceptible cultivars do not always cause disease in the most resistant ones, but isolates taken from these always show high aggressiveness to the most susceptible cultivars. Diourte et al. (1995) above 7 in thelast two trials. Higher temperatures were also related to a higher incidence of Rhizoctonia rot (Kumar & Kudada, 2018) and *danpping-off* caused by some species of the *Fusarium genus* (Gordon et al., 2015).

Differences in aggressiveness were observed between isolates collected in the same region, which frequently occurs (Dhingra & Sinclair, 1973; Mayék et al., 2001), but in general the isolates that caused the most severe symptoms originated from Rio Verde. As *M. phaseolina*is a polyphagous fungus, the high diversity of crops planted must have promoted greater selection pressure.

The isolate 32301, classified as the 5th least aggressive, only caused severity grades greater than 3 in its original cultivar, BRS Embaixador. Only BRS Pitanga was resistant to BRM 32347,

obtained the same results, isolates from resistant sorghum genotypes were more athogenic to susceptible than other isolates obtained from less resistant varieties.

Since the past, researchers have tried to relate *M. phaseolina isolates to* their place of origin and host (Pearson et al. 1986; Zazzerni&Tosi, 1989). Mihail& Taylor (1995) studying the variability of 114 *M. phaseolina isolates* from different hosts, soils and continents did not obtain evidence on the specificity of the pathogen but reported the difference in aggressiveness between isolates, concluding that this species would be too heterogeneous to be subdivided into subspecies based on virulence or taxonomic classifications. By molecular analysis, Jana et al. (2003) were able to distinguish isolates from soybeans, sesame, chickpeas, cotton, okra, common beans and 13 other hosts. By the same technique and by *cluster* analysis, Sundravadana et al. (2011) confirmed the variability between *M. phaseolina isolates* originating from different legumes according to aggressiveness and genetic characteristics. Thus, with the advancement of molecular techniques and the results of the present study, it is feasible to propose the differential interaction between *M. phaseolina isolates* and their hosts.

CONCLUSION

Gray stem rot caused by the soil-dwelling fungus *Macrophomina phaseolina*, anamorphic synonym *Rhizoctoniabataticola*, is more severe in regions with temperatures between 18 and 35°C and low humidity. Due to global warming, it has become one of the most important diseases in common bean production in tropical countries. Its symptoms include *damping off* , plant wilting, premature defoliation and stem canker, followed by plant death under severe conditions. The populations of this fungus present high pathogenic variability, which makes its control difficult. Twenty-four *M. phaseolina isolates* were analyzed by inoculation in a set of different common bean cultivars, proposed in previous studies. Ten isolates were classified into four pathotypes, based on cultivar susceptibility or resistance.

REFERENCES

Baird, R. E; Watson, C. E; Scruggs, M. Relative longevity of *Macrophominaphaseolina*and associated mycobiota on residual soybean roots in soil. Plant Disease ,vn 87, p. 563–566, 2003.

Dellaporta, SL; Wood, J.; Hicks, JB Aplant DNA minipreparation: version II. Plant Molecular Biology Reporter , Athens, v. 1, p. 19-21, 1983.

Dhingra, OD; Sinclair, JB Variation Among Isolates of *Macrophominaphaseoli*(Rhizoctoniabataticola) from Different Regions. Journal of Phytopathology v. 76, p. 200-204, 1973.

Dhingra, OD; Chagas, D. Effect of soil temperature, moisture, and nitrogen on competitive saprophytic ability of *Macrophominaphaseolina*.trans. br mycol. Soc , v. 77, no. 1 p. 15-20, 1981.

Gonçalves, FJ; Wendland, A. Differential interaction between *Macrophominaphaseolina*and *Phaseolusvulgaris* . In: Brazilian congress of phytopathology, 48., Brazilian congress of postharvest

pathology, 2., 2015, São Pedro. Anais... Brasília: Brazilian Society of Phytopathology, 2015.

Gordon, TR; Swett, CL; Winfield, MJ Management of Fusarium diseases affecting conifers. Crop Protection , v. 73, p. 28-39, 2015.

Gupta, GK; Sharma.SK; Ramteke, R. Biology, epidemiology and management of the pathogenic fungus Macrophominaphaseolina (Tassi) Goid with special reference to charcoal rot of soybean (Glycine max (L.) Merrill). Journal of Phytopathology , v.160, p.167-180, 2012.

Islam. S.; Haque, S.; Islam MM; Emdad, EM; Halim, A.; Hossen, QM; Hossain, Z.;, Ahmed, B.; Rahim, S.; Rahman, S.; Alam, M.; hou, S.;Jones, RW; Wang, HY Immunolocalization of a beta-1,4-endoglucanase from *Macrophominaphaseolina*expressed in planta. Canadian Journal of Microbiology , v. 43, p. 491–495, 1997.

Kaur, S.; Gurpeet, SD; Brar, SK; Vallad, GE; Chand, R; Chauhan, VB Emerging phytopathogen*Macrophominaphaseolina*: biology, economic importance and current diagnostic trends. Critical Reviews in Microbiology , vol. 38, p. 136–151, 2012.

Kumar, M.; Kudada, N. Effect of sowing dates and weather conditions on Rhizoctonia root rot disease incidence and green pod yield of French bean. Journal of Pharmacognosy and Phytochemistry , v. 7, no. 3, p. 893-899, 2018.

Mayek. N; Casteneada, LC; Chavira, MG; Espinosa, RG; Acosta, JG; SEE, OM; SIMPSON J. Variability of Mexican isolates of *Macrophominaphaseolina*based on pathogeny and AFLP genotype. Physiological and Molecular Plant Pathology , v.59, p. 257–264, 2001.

Mayek, N.; Garcia-Espinosa, R.; Lopez-Castaneda, C. Water relations, histopathology, and growth of common bean (*Phaseolus vulgaris L.*) during pathogeny of *Macrophominaphaseolina*under drought stress. Physiological Plant Pathology , v. 60, p. 185–195, 2002.

Radwan, O.; Rouhana, L.; Hartman, GL; Korban, SS Genetic mechanisms of host-pathogen interactions for charcoal rot in soybean. Plant MolBiol Rep v. 32, p. 617–629, 2014.

Rocha, DC; Oliveira, MB; Freitas, MA; Petrofeza, S. Rapid detection of Macrophominaphaseolina in common bean seeds using a visual loop-mediated isothermal amplification assay. Australasian Plant Pathology , v. 46, no. 2, p. 205-2012, 2017.

Sun, S.; Wang, X.; Zhendong. ZB, Men, W. Occurrence of Charcoal Rot Caused by *Macrophominaphaseolina*, an Emerging Disease of Adzuki Bean in China. Journal of phytopathology , v. 164, no. 3, p. 212-216, 2016.

Sundravadana, S.; Thirumurugan, S.; Alice, D. Exploration of molecular

variability in *Rhizoctoniabataticola*, the incitant of root rot disease of pulse crops. Journal of Plant Protection Res. , v. 51, p. 184–189, 2011.

Viteri, DM; Linares, AM Reaction of *Phaseolus*spp. genotypes to ashy stem blight caused by *Macrophominaphaseolina*.Euphyt icavp 213: 199, 2017.

Wendland, A.; Moda-Cirino, V.; Del Peloso, MJ; Costa; Oliveira, MIS; Lima, Araújo, LGD Shortobacterium wilt. In: JUNIOR, TJDP; WENDLAND, Common bean genetic improvement and disease prevention .Viçosa: EPAMIG, 2012. p. 111-124.

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AN OVERVIEW OF DIFFERENT STORAGE METHODS OF CEREALS IN ETSAKO WEST LOCAL GOVERNMENT AREA IN EDO STATE

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ABSTRACT

An appraisal of different storage techniques was carried out in Etsako West Local Government area of Edo state was undertaken. Preliminary investigation result shows that the common grain storage structures existing in this local government area are the mud rhombus, thatched rhombus, underground pit, and earthen pot and warehouse storage. The grain usually stored in unthreshed forms includes millet, sorghum, maize and cowpea. Most of these structures are not moisture proof, rodent proof and are not airtight. Structural defects occur mainly in the roof, walls and columns of the storage structures. The common types of physical defects are cracks, leakage, termite infestation and structural failure of parts. The causes of defects among others include poor strength of material, inadequate columns, low elevation and pest infestation. The result however, shows that it might be more profitable for subsistent farmers to continue the storage of unthreshed cereals and possible unshelled pulsed in rhombus and underground pits since these have relatively low storage cost per kilogram of grain. However there is need for some technical improvement on the construction materials, nature of columns, elevation and the loading/unloading facilities. The farmers' shows willingness in adopting new storage techniques provided such structures are cheap and affordable.

INTRODUCTION

The Nigerian government in recent years has provided incentives to the rural farmers, which has tremendously increased the cereal production output per hectare in the rural area. A large proportion of the population continues to remain dependent on agriculture, with production patterns being governed by food requirements for home consumption. Since the large majority of the population is dependent on agriculture, only a small proportion of the total food production finds its way out into the market. The large parts of the food grains produced are being retained on the farm for home consumption (Olumeko, 1991). The increase in yield through improved cropping systems and the introduction of high yielding varieties has re-emphasized the need for more resources to prevent post harvest losses.

Storage is an important activity, which enhances marketing efficiency by providing utility. Storage is particularly important in agriculture because agricultural production is seasonal while the demands for agricultural commodities are more evenly spread throughout the year. In this circumstance, there is need to meet average demand by storing excess supply during the harvesting season for gradual release to the market during off-season periods. In the process, seasonal prices are stabilized. Post harvest facilities or appropriate storage technology has been the major problem of Nigerian agriculture for a long time. This has resulted in considerable waste of agricultural output and hence considerable loss to the economy. Nigeria is losing about 2.4 billion tonnes of food yearly to poor harvest and storage facilities (Olumeko, 1999). The losses were mainly in maize, rice, sorghum, millet, cowpea, groundnut, soya beans, yam, cassava, plantain and fruits. In monetary term, the country is

losing a total of N48billion annually on post harvest losses. It has been observed that different localities in Nigeria have peculiar storage methods depending on the types of crop grown (Adesida, 1988). It has been noted that farmers achieve varying degree of success in applying the basic principles involved in the safe storage of food (Birewar, 1989).

In Nigeria the average proportion of food production retained by the farmers for their own (non sale) is usually assumed to be 70% (Talabi, 1989). However, there is a high degree of variation reflecting among other factors the size of operational holding, the interaction of consumption pattern with cropping patterns, level of indebtedness and the form of labour payment. Grains kept in farmer's structures are mainly for household consumption; any surplus grain to consumption requirement may be sold within two or three months of harvest. After harvest, grains may be stored temporarily in bulk or in bags for a month or two before being transferred to a structure.

The traditional cereal storage structures in different part of Nigeria are made of varying locally available materials. Usually, the type of locally available materials indicates the type of structures. The structures are made of paddy straw, split or whole bamboo poles, planks, reeds, robes, mud brick e. t. c. Most of the structures are constructed at the beginning of harvesting season. The time of harvesting varies slightly throughout the agroclimatological zones usually between the months August and January. The grains are stored either in threshed or unthreshed forms. The different types of on-farm storage structure found in the three different climatic zones of Nigeria had been appraised. (Igbeka and Olumeko, 1996; Olumeko, 1991). The prominent structure found in the Sudan savannah includes mud rhombus, thatched rhombus and underground pit. The structure found in the guinea savannah zone includes mud and thatched rhombus, platforms, cribs and earthen pots; while in the southern rain forest zones, the structures includes mud rhombus, maize crib, platforms, domestic or indoor storage such as plastic containers, gourds, earthen pots and metal container. Other storage structures includes bags, could be made of jute, Hessian, polyethylene or plant fiber. The bag storage has been found to be convenient for bulk storage of shelled or threshed grains as the bags is very convenient in transporting the grains.

Postharvest loss includes the food loss across the food supply chain from harvesting of crop until its consumption. The losses can broadly be categorized as weight loss due to spoilage, quality loss, nutritional loss, seed viability loss, and commercial loss. Magnitude of postharvest losses in the food supply chain vary greatly among different crops, areas, and economies. In developing countries, people try to make the best use of the food produced, however, a significant amount of produce is lost in postharvest operations due to a lack of knowledge, inadequate

technology and/or poor storage infrastructure. On the contrary, in developed countries, food loss in the middle stages of the supply chain is relatively low due to availability of advanced technologies and efficient crop handling and storage systems. However, a large portion of food is lost at the end of the supply chain, known as food waste. "Food waste" can be defined as food discarded or alternatively the intentional non-food use of the food or due to spoilage/expiration of food. In 2010, estimates suggested that about 133 billion pounds of food (31% of the total available food) was wasted at retail and consumer level in the United States. Among different agricultural commodities, the studies estimated that on a weight basis, cereal crops, roots crops, and fruit and vegetables account for about 19%, 20%, and 44% losses respectively. On a calorific content basis, losses in cereal crops hold the largest share (53%). Cereal grains, such as wheat, rice, and maize are the most popular food crops in the world, and are the basis of staple food in most of the developing countries. Minimizing cereal losses in the supply chain could be one resource-efficient way that can help in strengthening food security, sustainably combating hunger, reducing the agricultural land needed for production, rural development, and improving farmers' livelihoods (Adekoya and Ajayi, 2000).

Cereal Storage on a subsistence farm is primarily based on minimizing grain loss. In modern agricultural practices there are methods of managing under 1% grain loss, but small subsistence farms can see 20% - 100% of grain loss. This causes starvation and an unstable food supply. Grain loss can be caused by mold growth, bugs, birds, or any other contamination. One method of preventing loss is hermetic grain storage. Hermetic grain storage strives to eliminate all exchange of gases within the storage system. This mitigates bacterial activity and prevents rodents and bugs from being able to breathe inside the storage systems.

In Nigeria, about 70% of farm produce is stored by farmers for their own consumption. Farmers store cereal crops in bulk, using different types of storage structures made from locally available materials. The pre-treatment necessary for better storage life is cleaning and drying of the grain, but storage structure design material and its construction also play a vital role in reducing or increasing the losses during storage. Storage losses constitute a major share of food grain loss in postproduction operations.

Due to unavailability of proper storage structures farmers are scared of producing grains at commercial level, low cost storage structures are not readily affordable by farmers and the technical knowhow for preparing grains for storage and ventilating them properly is also lacking largely amongst puissant farmers. Ramesh (1999) reported that high wastage and value loss are due to lack of storage infrastructure at the farm level. The losses during storage are quantity losses and quality losses. Quantity losses occur when insects, rodents, mites, birds and microorganisms, consume the grain. Infestation causes reduced seed germination, increase in moisture, free fatty acid levels, and decrease in pH and protein contents etc. resulting in total quality loss. Quality losses affect the economic value of the food grains fetching low prices to farmers (Ipsita et al., 2013).The objective of

this paper is to carry out a review on the appraisal the different storage techniques for cereal crops in Etsako West Local government area.

RESEARCH METHODOLOGY

Description of the Study area

Etsako West Local government area of Edo State is located between latitude 6^0 70 ”00” to 7^0 18 ”00” North of the Equator and longitude 6^o 49 ”00” East of the Greenwich Meridian. Auchi is the headquarters of Etsako-West Local Government Area of Edo State, Nigeria (Figures 3.1 & 3.2). The area covers a total land area of 94,562 km^2. Auchi is underlain by sedimentary formation of **the Miocene-Pleistocene age (Odemerho** and Onokherhoraye, 1985). The area is found in the South Central (lower Niger sedimentary rock areas). The sedimentary rocks in the area are easily broken down by the incidence of water which results in the removal of particles of the rock. Auchi area is generally sloppy and as a result, the topography allows the easy movement of soil particles such that with little rainfall, rapid movement of materials is encouraged. Auchi area is referred to as the “Oshibujie” plain and it is surrounded by the Kuruku hills up North and Samorika hills with a height of about 672 meters above mean sea level.

The area has a sub-humid climate with temperature ran ranges from 20^0C to 35^0C and annual rainfall which ranges betweenits diurnal range of temperature is about 12.4^{o}C.. 1000mm-1500mm. It has a two distinctAuchi soil is mai only lateritic and it is a product seasons in a year: the rainy season and the dryof tropical weathering. It is red or at times reddish season. The rainy season occurs between Aprilbrown in col colour with or without concentration and October with a short break in August and theand is generally found below hardened dry season lasts from November to April with coldferruginous crusts or hard pan harmattan dust in December. The annual average (Odemerho and Onokherhoraye, 1985).

Figure 1: Google map of Auchi, Edo state

Figure 2: Map of Edo State, showing Auchi

Methodology

Preliminary investigations were carried out on the types of cereal storage structures existing in Etsako West, Edo State. The structures were technically surveyed with the aid of personal interviews with the farmers and Visual observation. Ten villages in the local government area were randomly selected for the survey, these villages are: Auchi, Jattu, Agbede, Aviele, Jagbe, Awain, Otukue, Afowa, Iyamho and Ibienafe.

1.The efficiencies of the construction materials with particular reference to the types of construction materials, strength of the materials used, shape, size, types, causes and location of defects annual maintenance methods and the age of structure.

2. An estimation of the percentage grain losses and losses in economic value with reference to the types of grains stored, form which grains are stored, types of grain damage, causes of damages and percentage loss in market value.

1. The cost efficiency of the various types of structure with reference to the capacity cost of construction, duration of storage, loss in economic value and annual maintenance cost.
2. The willingness of adopting other storage techniques will also be investigated.

Types of Grains Storage Structures found in the study area

The prominent types of grain storage structures found in the study area include mud rhombus, thatched rhombus, in-hut storage such as earthen pot and warehouse storage.

Mud rhombus

A mud rhombus is a specially built structure made from a mixture of dry grass and clay. It consists of a bin resting on large stones and covered with a thatched roof plate 1.

A mud rhombus consists basically the following

a. Foundation – floor assembly

b. Shell or wall

c. Roof

Plate 1: Mud Rhombus with stone grillage foundation floor assembly and thatched roof at Ibienafe

The shape and sizes of the mudrhombus depend on the tradition of the area and the availability of the materials. The shape could be cylindrical, spherical, or circular shaped. The height ranges from 7 – 10 meters while the diameter ranges between 3 and 7 meters. The capacity of mud rhombus ranges from 1000kg – 8,000kg of unthreshed cereals and legumes. They are generally not moisture proof, rodent proof or airtight. The cost of construction ranges between N6000 – N10, 000, and it basically depends on the capacity, location and availability of materials. 4 or 8 men manually load grains into the storage structure through the roof. Mud rhombus generally does not have any external support or reinforcement.

(i) Types of physical defects

The physical defects of mud rhombus are usually on the roof and wall of the structure. The physical defect includes leakages of roof, cracks on the wall. The defects are usually as a result of poor strength of materials, change in climate condition and structural failure. The maintenance methods include the repair and replacement of structural parts. Annual maintenance cost depends on the extent of defects, locality, age and regularity of maintenance. The average annual maintenance cost according to the man inteviewed ranges between N2, 000 – N6, 000

(ii) Storage practices

Loading of grains into mud rhombus is done by the removal of the roof, since no appropriate design for loading and unloading is included. The major crops found to be stored in the areas investigated are unthreshed millet and sorghum. It was learnt that the crop is stored for a minimum of two years and maximum of between seven and 10 years. The unthreshed crops are brought from the

farm in bundles tied with ropes. The with four bundles giving approximately 100kg of threshed grain, where the millet is to be stored for a duration of one year or less, the bundles are not loosened, but stacked in the rhombus where however, the storage is to be for a longer duration, the bundles are loosened and put into the bin by special arrangement carried out by 4 to 8 people depending on the size and the height of the rhombus.

The first person stays on the ground, looses the bundles and passes it to the 2nd person who is on top of the bin or on the ladder. The 3rd person who is inside the rhombus or on another ladder inside the rhombus collects the millet heads and together with the 4th person known as the 'good hand' arranges the millet heads in the structure. The man referred to as the good hand is a person believed to be naturally disposed to storage, such that when he stores a product, minimum deterioration occurs compared to other people. In arranging the millet heads, the 'good hand arrange them in a concentrically over locking pattern, such that the millet are self-supporting in a way. After about 30 to 40cm depth of storage 3 to 5 other men climb into the structure and together with the 2 men already inside, the stored product is compressed. This procedure is continued until the bin is filled to the brim; the roof is then lifted and put in place. After about 3 to 4 weeks, the rhombus bin is inspected for settlement. Settlement is the settling of the stored millet mass by virtue of its self weight resulting in both the reduction of depth of storage height and width. When about 5cm reduction in depth with a corresponding gap of about one to two centimeter between stored millet and

(i) Foundation Floor Assembly

The foundation – floor assembly is usually made of irregular stones and tree stems or tree poles stems penetrating the ground, bundles are of same size

rhombus shell is observed, the storage is considered good, the mouth of the bin is then sealed with a mud disc and the roof put in place.

Unloading of the stored crop could sometimes become difficult and cumbersome because of the above loading procedure. In large capacity rhombus bin part of the shell may have to be broken to create an opening for easy off – loading, the created opening is later sealed after complete evaluation of stored products.

(iii) Storage losses/damage

The types of grain damage/losses in crops stored in mud rhumbu include change in taste, colour and odors, pest infestation. The damage often results from pest/insect infestation, structural failure, variation in climatic conditions, micro – organisms among others. The losses in grain and economic values are between 10 – 20% during a storage period of 6 months to 3 years the end use of the stored grains are for home consumption and sales.

Thatched Rhombus

The thatched rhombus is made of woven grass stems resting on irregular stores and or tree stems. They are usually cylindrical or circular in share with various capacities. The capacity ranges from 500kg – 8,000kgs depending on the size. The grains are usually stored in unthreshed forms. They are generally not airtight, moisture and rodent proof. Construction cost is between N2, 000 and N 8,000. They usually have external support ranging from 6 – 16 units depending on the size of the rhomb

and crossed with other tree stems. The elevation is generally low. It ranges between 100mm and 600mm, no rodent guard provided only very few rhombus has thorn used as rodent guard

(ii) Wall

The wall of the thatched rhombus made of woven grass stems has two layers, being reinforced with two or three tension rings. In, Aviele, they use cow/animal dung in between the two layers to prevent farm animals from eating – up the wall of the rhombus. 6 – 16 tree stems depending on the size of the rhombus support the walls externally. The support usually does not penetrate the ground

(iii) Roof

The roof of the thatched rhombus is usually conical in shape. The materials usually used for the roofs are straw/thatched, tree stem, polyethylene sheets, and robes. They are usually of 2 – 3 layers to prevent water seepage.

Plate 2: Thatched Rhombus with tree stem columns, showing tree stem external support at Agbede

Plate 3: Failed Thatched Rhombus after 8 years of use at Jattu

(iv) Types of Defects

The physical defects are usually on the wall, foundation and roof. The defects includes inadequate support, low elevation, termite infestation which are as a result of poor strength of material structural failure, inadequate design, of the foundation and age of the structures maintenance is usually done by the repair and or replacement of structural parts and cleaning.

The maintenance cost range between N500 and N3, 000 depending on the locality, capacity availability of materials and extent of damage.

(v) Storage Losses or Damage in Thatched Rhombus

The loading is usually done through the roof and is the same as that of the loading of the mud rhombus. Grains are usually stored in unthreshed form and they include millet, sorghum and maize. Fumigants are usually applied, but are usually not effective because the structure is not airtight. The type of grain loss includes change taste, colour, and odour, pest sorghum and cowpea; they are stored in threshed form. The capacity ranges from 1000kg – 6000kg and above. After loading infestation. The extent of loss depends on the duration of storage. The main causes of losses include pest infestation, poor strength of material, and structural failure among others. The percentage loss in quantity and economic value ranges between 10 % and 20% and above respectively, which depends on the duration of storage and other factors of deterioration.

Thatched/Sorghum Stalk Rhombus

This type of rhombus has the stone – grillage foundation – floor assembly, the wall has 2 layers of woven grass stem and a wall of well arranged sorghum stalk held in place by local robes.

4.1.3 Underground Pit

This is commonly found in low water table areas like Auchi and Jagbe. The pit, which may be round or square in cross section, is 1 – 3m deep and 1 – 3 m in diameter or square. The underground pit is usually lined with straw. The pit and the straw mat are padded with 40 – 60cm of corn husk. Also a layer of husk padding or insulation is provided at the bottom of the pit. The common types of grain stored are millet,

the grains into the pit, tree stems are placed across the pit then covered with polyethylene or metal sheet.

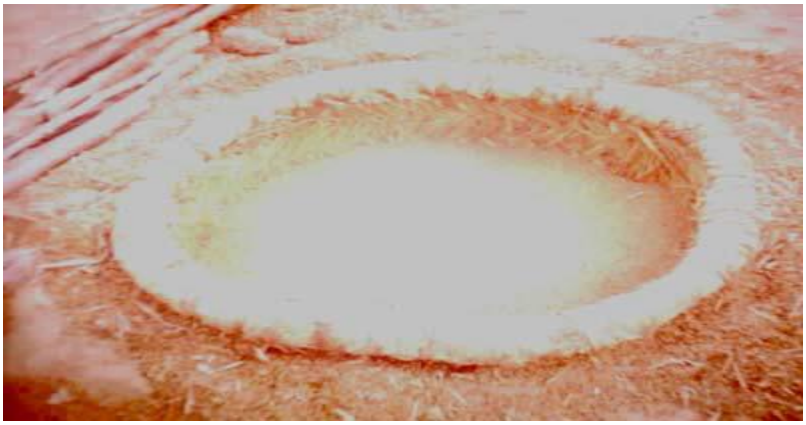

Plate 5: Underground pit, showing woven grass stem lining used for storing millet in Jagbe.

Then a layer of husk before finally layers of sand or laterite is used to cover it. The duration of storage could be between 1 and 5 years without opening and usually, once

opened; all the content must be emptied. The same site can be used for up to 12 years with annual re-digging. The location of the defect is usually in the wall lining, which may be eaten up by termite, and the structure is not rodent proof. Maintenance is usually done by cleaning and replacement of the wall lining. Maintenance cost is dependent on the locality and availability of material.

The damages or loses in stored grains includes charge in colour, odour and taste. It is believed that these grains have low viability. Grains stored in this structure are protected against insect attack because of reduced oxygen level. Causes of grain damage/loss include microbial organisms, structural failure and changes in the chemical composition of grains. Approximate percentage loss of quantity and economic values are 10 – 20% and 5 – 10 % respectively. The underground pit is easy and cheap to construct and requires minimum materials, but however great difficulty is experienced in emptying and cleaning the structure.

Earthen Pot

The earthen pot storage was found in very few villages like Ibienafe and Iyakpi. It is made of burnt clay. The shape and sizes differ with the locality. They have capacity of between 5 – 20kg of threshed or shelled cereals. The grains stored in this pot are used mainly for seedlings. The grain crops stored includes cowpea, maize and sorghum. The main defects are that, it is fragile, small in capacity and absorbs moisture from the ground where it is place. Damages on seed include mould growth, and spouting, change in colour, odour and taste

Local Warehouse Storage

The store is constructed using clay brick with aluminum roofing sheet. The grains usually in unthreshed or unshelled forms are tied in bundles and placed on the bare ground of the storehouse. There is a small entrance into the store with no window. The grain stored in this form, is usually attacked by insect pest e.g. weevils and termite and, rodent from the ground. The crops also absorb moisture from the ground. The loss in these types of storage method is usually above 20% during a period of 2 years of storage plate showing local warehouse storage.

Conclusion

A technical survey of the village level cereal storage structures existing in Etsako West Local Government area of Edo state was undertaken. Preliminary investigation result shows that the common grain storage structures existing in this local government area are the mud rhombus, thatched rhombus, underground pit, and earthen pot and warehouse storage. The grain usually stored in unthreshed forms includes millet, sorghum, maize and cowpea. Most of these structures are not moisture proof, rodent proof and are not airtight. Structural defects occur mainly in the roof, walls and columns of the storage structures. The common types of physical defects are cracks, leakage, termite infestation and structural failure of parts. The causes of defects among others include poor strength of material, inadequate columns, low elevation and pest infestation. The result however, shows that it might be more profitable for subsistent farmers to continue the storage of unthreshed cereal grain and possible unshelled pulsed in rhombus and underground pits since these have relatively low storage cost per kilogram of grain. However there is need for some technical improvement on the construction materials, nature of columns, elevation and the loading/unloading facilities. The farmers' shows willingness in adopting new storage techniques provided such structures are cheap and affordable.

The use of silos and modern technologies for processing and storing food grains will go a long way to aid food security at the village level. But the cost of procurement, operational know how, maintenance and availability of spare parts are usually the limitation of such modern methods. Therefore this process should take into consideration the technologies of the people. The farmers will readily accept a concept or technology that builds up on or improves that which they are used to rather than one which imposes a totally new idea.

Recommendations

On the basis of the finding of this study, the following recommendations are offered:

1. The thatched rhombuses were found to be infested with termites, and farm animals usually eat up walls; the elevations are low and external support inadequate. The use of chemical to prevent termite infestation by the farmers, apart from the cost had been ineffective probably due to improper applications or fake or adulterated chemicals; hence the use of thatched rhombus should
2. be discouraged.
3. The mud rhombus has columns made of irregular stones, the floor foundation assembly is made of clay and tree stems. These were discovered to be infested by termites, and are not water resistant. The loading and unloading is usually done through the roof, which is inadequate. It should be redesigned, in terms of foundation floor assembly and should be made of concrete and water resistant materials.
4. The underground pit is observed to be infested with termite, and water seeps into the pit due to inappropriate cover. An improvement on the wall lining by the use of water and pest resistant material are suggested; the cover or roof should be made more secured.
5. The earthen pot is discovered to be of small capacity, fragile and it absorbs water from the floor where it is placed. Small containers of plastic or metals placed on wooden floor should be used instead.
6. The local warehouse is observed to be open to pilfering, it is neither moisture proof nor rodent proof and the grains are stacked on the bare floor. An improved cheap warehouse that is rodent proof and more secured should be adopted.
7. More grain reserves should be established whereby farmers would be able to bring their grains for storage. A standard quality control unit for grading grains should be established to assist in checking the grain quality before

REFERENCES

Adekoya, A.E and Ajayi, M.A. (2000). An assessment of farmers' awareness and practices of land Management techniques in Iddo Local Government Area of Oyo State. Journal of Environmental Extension.1. 2:98-104.

Adesida, A. (1988). On farm storage structure in Nigeria. A paper presented at processing and storage unit seminar (April 12-18 1988) in Nigeria.

Birewar, B. R. (1990) Existing On-farm Grain Storage facilities in Nigeria and suggested improvements.Journal of Agricultural Mechanization in Asia, Africa and Latin America. Vol 21, No 2.pp 58-60.

Food and Agricultural Organisation. (2005). The state of Food Insecurity in the World. Published in 2005 by the

Food and Agriculture Organization of the United Nations. Vialedelle Terme di Caracalla, 00100 Rome, Italyhttp://www.fao.org/docrep/008/a0200e/a0200e00.htm. Accessed 25/4/2011.

Ipsita, D., Girish, K., and Narendra, G.S. 2013.Microwave Heating as an Alternative Quarantine Method for Disinfestation of Stored Food Grains.International Journal of Food Science, 13.

Olumeko, D. O. (1998).The Role of the Crop Storage Unit in the National Food Security Programme. Proceedings of National Workshop on Post-harvest Food Loss Prevention, organized by Crop Storage Unit of Federal Department of Agriculture, June 1998.Pp 29-39.

Olumeko, D. O. (1999) Technical Survey, Evaluation and Economic Review of Storage Structures in Nigeria. Unpublished M. Phil Thesis submitted to the Department of Agricultural Engineering, University of Ibadan. Nigeria.

Olumeko, D. O. (1999).Simulation and Performance Evaluation of Metal and Brick On-farm Grain Storage Structure in Southwestern Nigeria. Unpublished PhD Thesis Submitted to the Department of Agricultural Engineering, University of Ibadan, Nigeria.

Ramesh, A.1999. Priorities and Constraints of Post harvest Technology in India, In: Y. Nawa, Post harvest Technology in Asia. Japan International Research Centre for Agricultural Sciences, Tokyo, 37p.

Talabi, A. E. (1989). A review of the roles of the three tiers of Government on project implementation. A Paper Presented at the N.A.D.C. Meeting, Port Harcourt, Nigeria. April 8-12.

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THE TOURISM INDUSTRY AND ITS NEGATIVE IMPACT ON THE ENVIRONMENT: A REVIEW

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ABSTRACT

The paper focuses on the effect of tourism on the surroundings environment as tourism activities have outstanding potentials to contribute positively to the surroundings and social development. But there are many harmful results on the environment due to tourist's activities. Some are the emission of chlorofloro carbon, carbon dioxide, amplify temperature, strain on herbal resources and land degradation based totally on the ozone layer depletion materials that are launched from hotels, resort centers and visitor vehicles. The paper observed that there is emission of ozone layer depletion substances, which causes skin cancer, excessive temperature and extend in sea level and there are stress on the use of natural resources. The study encouraged that tips for setting up environmental administration device be put in place, insisting that accommodations and tourism establishments should use renewable strength and chorofloro carbon free equipment for the hospitality industry. The lookup concluded that international warming has consequences on tourism development, even though tourism and local weather exchange has reversible phenomenon.

Keyword: **Tourism, Tourist Activities, Environment, ozone layer Depletion, Tourist Health.**

INTRODUCTION

The travel and tourism industry is the world's largest economic system as it creates quite number types of jobs in international, national and regional economies in retail, construction, manufacturing and telecommunications. It is one of the important parts of existence that is without delay related to all leaving things. According to current reports, tour and tourism without delay and circuitously generated 12% of GDP and nearly 200 million jobs in the world financial system in 2000. For 2015, these numbers are forecast to complete 12% or three hundred million.

Contribution to Sustainable Development: The United Nations Conference on Environment and Development (UNCED), the "Rio Earth Summit" (1992) recognized that travel and tourism is one of the key sectors of the economy that can make a wonderful contribution to accomplishing sustainable improvement should make a contribution to development. Tourism need to be based totally on enjoying and appreciating the local culture, built heritage and natural environment. Tourism can play a effective role in increasing purchaser dedication to sustainable development, as properly as imparting an economic incentive to preserve natural environments and habitats that contribute to biodiversity conservation. However, journey and tourism cr

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financial and social development. It has many negative effects on the environment and destroys neighborhood cultures as it is poorly managed. One of them is global temperature upward push and the direct/indirect effect on ozone depletion. In the remaining 100 years, the average temperature of the air at the Earth's floor has

elevated via ~1°C, making the Earth now hotter i.e. international warming. Today, many governments and agencies have begun to pay interest to the influence of the hospitality enterprise on neighborhood people, as well as the nearby and international environment. As a result, a new quarter of hospitality has emerged regarded as ecotourism or green travel. About the ozone layer: Ozone (O3) is a naturally happening fuel and ninety percent of all ozone is determined in the higher surroundings or stratosphere (12-50 km) above the earth's surface, regarded as the "ozone layer". It absorbs the sun's dangerous UV rays and prevents them from achieving the earth's surface.

The effects of ozone depletion: Although stratospheric ozone protects the earth from the sun's dangerous radiation, ground-level ozone causes environmental pollution and is hazardous to human health. As oftentimes found as urban smog, it can motive respiratory problems.

In present day times, one of the major motivations for travel is to keep away from the familiar surroundings and are looking for someplace with a relaxed vicinity to relieve psychological stress and stress (Ritchie and Crouch, 2003). Therefore, environmental satisfactory need to be regarded as an vital component in the decision-making system of viable tourists, as it can have a significant impact on the competitiveness of tourism destinations (Zhang et al., 2015; Becken et al., 2017). However, the environment in some main tourism vacation spot international locations such as Egypt, China and India is deteriorating as urbanization and industrialization progress. Beyond the "traditional" environmental problems such as rubbish disposal and water pollution, the haze air pollution problem has risen in current years, and air exceptional (AQ) has grow to be a frequent difficulty (Chen et al., 2017) that is now included in the broader class the environmental quality.

Air fantastic must appeal to the physical surroundings as air first-rate is closely linked to health dangers (Costa et al., 2014). However, the existing literature will pay enormously little interest to impact on air high-quality tourism demand and the competitiveness of tourism destinations. Overall, the influence of environmental and climate alternate troubles on tourism has been mentioned in various studies, however few of them have centered directly on the Effect of air air pollution on the competitiveness of a tourism vacation spot (Saenz-de-Miera and Rosselló, 2013; Rossello- Nadal, 2014; Sajjad et al., 2014). Air first-class also influences bodily comfort, which is quintessential for traveler experiences and has grow to be a serious trouble for human health. Medical proof suggests that short- and long-term publicity to air pollution can purpose a variety of acute and continual health issues (Seaton et al., 1995; WHO, 2017). In addition, atmospheric emission additionally impacts aesthetic enjoyment, due to the fact people's perception is affected with the aid of the presence of particles and haze, which is increasingly more being researched (Rizzi et al., 2014).

DEPLETION OF NATURAL RESOURCES

Tourism and Hospitality development can put pressure on natural resources when it increases consum

resources are already scarce.

Local Resources

Tourism can create great pressure on local resources like energy, food, and other raw

materials that may already be in short supply. Greater extraction and transport of these resources exacerbates physical impacts associated with their exploitation. Because of the seasonal character of the industry, many destinations have ten times more inhabitants in the high season than in the low season. High demand is placed upon these resources to meet the high expectations tourists often have (proper heating, hot water, etc.).

Land Degradation

Important land resources include minerals, fossil fuels, fertile soil, forests, wetland and wildlife. Increased construction of tourism and recreational facilities has increased pressure on these resources and on scenic landscapes. Direct impact on natural resources, both renewable and non-renewable, in the provision of tourist facilities can be caused by the use of land for accommodation and other infrastructure provision, and the use of building materials. Forests often suffer negative impacts of tourism in the form of deforestation caused by fuel wood collection and land clearing. For example, one trekking tourist in Nepal and area already suffering the effects of deforestation can use four to five kilograms of wood a day (UNEP, 1999).

Water Resources

Water, and especially fresh water, is one of the most critical natural resources. The tourism industry generally overuses water resources for hotels, swimming pools, golf courses and personal use of water by tourists. This can result in water shortages and degradation of water supplies, as well as generating a greater volume of waste water. In dry and hot regions like the Mediterranean, the issue of water scarcity is of particular concern. Because of the hot climate and the tendency of tourists to consume more water when on holiday than they do at home, the amount used can run up to 440 liters a day. This is almost double what the inhabitants of an average Spanish city use (UNEP, 1999).

Pollution

Tourism can cause the same forms of pollution as any other industry: air emissions, noise, solid waste and littering, releases of sewage, oil and chemicals, even architectural/visual pollution.

Air Pollution and Noise: Transport by air, road, and rail is continuously increasing in response to the rising number of tourists and their greater mobility. The International Civil Aviation Organization reported that the number of international air passengers worldwide rose from 88 million in 1972 to 344 million in 1994. One consequence of this increase in air transport is that tourism now accounts for more than 60% of air travel and is therefore responsible for an important share of air emissions. One study estimated that a single transatlantic return flight emits almost half the CO emissions produced by all other sources (lighting, heating, car use, etc.) consumed by an average person per year (ICAO, 2001). Transport emissions and emissions from energy production and use are linked to acid rain, global warming and photochemical pollution. Air pollution from tourist transportation has impacts on global level, especially from carbon dioxide (CO) emissions related to transportation energy use. And it can contribute to severe local air pollution. Some of these impacts are quite specific to tourist activities. For example, especially in very hot or cold countries, tour buses often leave their motors running for hours while the tourists go out for an excursion because they want to return to a comfortably air-conditioned bus. Noise pollution from airplanes, cars, and buses, as well as recreational vehicles such as snowmobiles and jet skis, is a problem of

modern life. In addition to causing annoyance, stress, and even hearing loss for humans, it causes distress to wildlife, especially in sensitive areas.

Solid Waste and Littering In areas with high concentrations of tourist activities and appealing natural attractions, waste disposal is a serious problem and improper disposal can be a major despoiler of the natural environment, rivers, scenic areas, and roadsides. For example, cruise ships in the Caribbean are estimated to produce more than 70,000 tons of waste each year. Solid waste and littering can degrade the physical appearance of the water and shoreline and cause the death of marine animals (UNEP, 1999). Tourists on expedition leave behind their garbage, oxygen cylinders and even camping equipment. Such practices degrade the environment with all the detritus typical of the developed world, in remote areas that have few garbage collection or disposal facilities.

Sewage Construction of hotels, recreation and other facilities often leads to increased sewage pollution. Wastewater pollutes seas and lakes surrounding tourist attractions, damaging the flora and fauna. Sewage runoff causes serious damage to coral reefs because it contains lots of nutrients and it stimulates the growth of algae, which cover the filter-feeding corals, hindering their ability to survive. Changes in salinity and transparency can have wide-ranging impacts on coastal environments. And sewage pollution can threaten the health of humans and animals.

EFFECT OF TOURISM ON OZONE DEPLETION:

Over the past 20-30 years, sufficient Ozone Depletion Substances (ODS) like CFC (ChloroFluoroCarbons: CCl_3F, CCl_2F_2, CCl_4, $CHCl_3$, $CHFBr_2$, CHF_2Br etc.) have been released into the atmosphere which causes serious damage to the ozone layer. The tourism and hotel industry uses Ozone Depletion Substances in variety of applications, and must therefore be a part of the process to halt the depletion of the ozone layer. Tourism operations have a direct impact on the environment. This starts with the construction of new developments and continues during daily management and operations. Tourism facilities are resource intensive: they are large consumers of water and energy, and generate significant volumes of waste, emissions and effluent. What is not often realized is that they also consume and emit ozone depletion substances. Refrigeration, air conditioning, fire-fighting equipment, foam insulation, solvents and aerosol propellants all contain Ozone Depletion Substances and are widely used in the hotel and tourism industry. The tourism industry has a greater vested interest than most in protecting the global environment. Tourism destinations rely on a clean and healthy environment for the long-term quality and viability of the product. Without such environmental quality, much tourism would disappear. Unless all tourism operators, large and small, take action to maintain and enhance environmental quality, the future of the industry is in threat. Tourists, especially those who set out to enjoy the pleasures of the open air, will be some of the first to be concerned about the impacts of excessive UV radiation. By taking early action to phase out Ozone Depletion Substances, you demonstrate to your customers-guests or tour operators that you are a responsible operator.

SOURCE OF OZONE DEPLETION SUBSTANCES IN THE HOSPITALITY AND TOURISM INDUSTRY

As Travels and Tourism are mainly concerns about hospitality; it is most important consumers of (ODCs) ozone layer depletion chemicals mainly used in refrigeration, food and drinks, cold storage, display cabinets, freezers, ice machines and mini-bars etc

(i) Foams food packaging, trays and containers, pipe insulation, seat and back cushions, head rests, bedding and other upholstery uses, carpet underlay, car and bus interiors, protective packaging for a wide variety of goods
(ii) Dry cleaning and degreasing solvents used in dry cleaning and special cleaning applications (iii) Aerosols spot cleaning, bathroom and other surface cleaners, small area paints, adhesives, insecticides and pesticides
(iv) Air conditioning in buildings general amenity areas, conference and meeting facilities, individual room units in vehicles, mobile units in cars and buses
(v) Fire extinguishers are used in fixed and portable fire extinguishers

WAYS TO CONTROL PROPER USE OF OZONE LAYER DEPLETION SUBSTANCES

The World Tourism Organization and the Earth Council in 1996 combined together to launch an action plan entitled "Agenda 21 for the Travel & Tourism Industry: Towards Environmentally Sustainable Development. They have put forward some action plan for environment protection that includes:

a.WTTC have also launched "ECoNETT", a website containing advice and data on good practice and sources of help and advice for environmental information, good practice, new techniques and technologies.
b. Raised environmental awareness and developed programs through joint workshops to develop their own environmental awareness programs in the hotel industry
c. Published advice including practical publications
d. Guidelines for setting up environmental management systems
e. Use of energy efficient and CFC free equipment's for good hospitality
f. Insists Hotel and organizations to use renewable energy sources and
g. Action plan regarding the waste water management
i. introduced the codes of conduct and also try to influence consumer behavior

REDUCTION OF OZONE LAYER DEPLETION SUBSTANCES

1. Good housekeeping measures to avoid leakage or loss of existing ozone depletion substances during equipment operation and maintenance
2. Recovery and re-use of original refrigerant after equipment dismantling for servicing
3. Converting existing equipment to utilize low ozone layer depletion potential (ODP) this generally requires replacement of existing equipment parts and/or a change of lubricating oil; in some instances, it may be possible to use a 'drop in' replacement instead of a complete retrofit (a drop-in replacement, as it name suggests, entails replacing Ozone Depletion Substances with low- or non-ODP (ozone layer depletion potential) substitutes without modifying the equipment)
4. Purchasing new equipment that uses zero-ODP chemicals when the service life of the original equipment is over or the original chemicals are no longer available and retrofitting is not possible

FINDINGS AND OBSERVATIONS

This paper observed that there is emission of ozone layer depletion substances which leads to ozone layer depletion, which causes pores and skin cancer, variation in local weather and weather, extremely excessive temperature and increase in sea level. The

findings also revealed that there are pressures on the use of herbal resource

CONCLUSION

the launch of ozone-depleting substances.

Travel and tourism has a range of advantages over other industrial sectors. However, its activities have a direct impact on our environmental ecology. Ozone depletion and tourism have a negative however reversible have an effect oneach other. A poorly managed tourism endeavor has a bad effect on the ozone layer. Likewise, the tourism enterprise faces the challenges of ozone depletion and world warming.

In order to remedy these problems, the industry must make a concerted effort to set up packages for sustainable development. The widely wide-spread conclusion is that tourism improvement is strongly influenced through world warming - a final result of the depletion of the ozone layer

RECOMMENDATIONS

It is encouraged to adopt regulatory measures
that control the movement and things to do of vacationers within the protected areas. When this is done, the have an impact on of these things to do will be reduced and the vitality and integrity of the web sites will be maintained. Limits ought to be imposed on Loss or leakage of current ozone-depleting materials all
through equipment use and preservation have
to be managed by excellent housekeeping practices. Hotels are here by counseled to preserve a zero ozone depletion workable in their variety of units in order to limit the launch of

the activities of these tourists; this should be decided after a thorough evaluation of
the maximum sustainable ability of the visitors Maintained. Limits ought to be imposed on the activities of these tourists. This should be

ozone-depleting materials. There ought to be true budgetary measures to include the leakage or loss of current ozone-depleting elements throughout operation
and maintenance of equipment, and accommodations need to utilize zero ozone-depleting achievable in their quite a number gadgets to minimize

REFRENCES

Bukur, F. (2006): Global Economy in Global Tourism Third Edition London, Elsevier Inc.

Becken, S., Jin, X., Zhang, C. and Gao, J. (2017), "Urban air pollution in China: destination image and risk perceptions", Journal of Sustainable Tourism, Vol. 25 No. 1, pp. 130-147. Urban air pollution in China: destination image and risk perceptions", Journal of Sustainable Tourism, Vol. 25(1), 130-147.

Chen, C.-M., Lin, Y.-L. and Hsu, C.-L. (2017), "Does air pollution drive away tourists? A case study of the sun moon lake national scenic area, Taiwan", Transportation Research

Part D:Transport and Environment, Vol.53, 398-402.

Costa, S., Ferreira, J., Silveira, C., Costa, C., Lopes, D., Relvas, H., Borrego, C., Ro ebeling, P., Miranda, A.I. and Paulo Teixeira, J. (2014), "Integrating health on air quality assessment – review report on health risks of two major European outdoor air pollutants: PM and NO_2", Journal of Toxicology and Environmental Health Part B Critical Review, Vol. 17 (6), 307-340.

Goni U,(1997): Ozone hole over Buenos Aires'. Time Magazine, 5, 17.International Civil Aviation Organization ICAO, (2001) Summit.

Ritchie, J.R.B. and Crouch, G.I. (2003), The Competitive Destination? A Sustainable Tourism Perspective, CABI Publishing,

Rizzi,L.I.,DeMaza, C., Cifuentes, L.A. and Gómez, J. (2014), "Valuing air quality impacts using stated choice analysis: trading off visibility against morbidity effects", Journal of Environmental Management, Vol. 146,. 470-480

Rossello-Nadal, J. (2014), "How to evaluate the effects of climate change on tourism",TourismManagement, Vol. 42, 334-340.

Saenz-de-Miera, O. and Rosselló, J. (2013), "Tropospheric ozone, air pollution and tourism: a case study of Mallorca", Journal of Sustainable Tourism, Vol. 21(8), 1232-1243.

Sajjad, F., Noreen, U. and Zaman, K. (2014) , "Climate change and air pollution jointly creatin nightmare for tourism industry", Environmental Science and Pollution Research, Vol. 21 (21) 12403-12418.

Seaton, A., Godden, D., MacNee, W. an d Donaldson, K. (1995), "Particulate air pollution and acute health effects", Vol. 345(8943) 176-178

UNEP; (1999) How the Hotel and Tourism Industry Can Protect the Ozone Layer, ISBN: 92- 807-1668-9

United Nations Conference on Environment and Development (UNCED)', the 'Rio Earth Summit' (1992)

Venkatesh M., P. Stanley Joseph, (2016) *International Journal* of Scientific Engineering and Applied Science (IJSEAS), 2, 167. 6.

World Health Organization [WHO] (2017), World Health Statistics 2018: monitoring Health for the SDGs, Sustainable Development Goals, World Health Organization, Geneva.

Zhang, et al., (2015), "Tourists' perception of haze pollution and the potential impacts on travel:reshaping the features of tourism seasonality in Beijing, China", Sustainability,. Vol. 7(3), 2397-2414

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ATTITUDE OF FOOD SERVICE PROVIDERS TO SANITATION AND HYGIENE IN NIGERIA: A REVIEW

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ABSTRACT

The paper focused on the attitude of food service providers to sanitation and hygiene in Nigeria in relation to food safety and consumption. Food borne related diseases and illnesses have increased over the years and have negatively affected the health and economic wellbeing of citizens and society. The study discusses food service; food safety and service providers; food sanitation and hygiene and the attitude of food providers to sanitation and hygiene. The paper observed that, unsafe food or contaminated food increases economic losses, trade rejections, food and agricultural waste, proper knowledge of food safety and hygiene enhance improved attitude and better food handling practices and personal hygiene of food handlers is an impetus for safe food and disease free environment. Some of the recommendations include, food providers should develop a strong positive and professional attitude to food preparation, service and after sale to reduce food toxic and waste that contaminate the environment and promote climate change, personal hygiene and sanitation of food handlers should be compulsorily enforced by the regulating authorities to promote cleanliness and healthy society and food sanitation and hygiene training should be regularly organized for food providers to update their knowledge in food handling and service. The paper concluded that food providers should create a sustainable and secure food supply for everyone with safe farm to fork ideology, promoting the enjoyable experience of food consumed while being sure that the food they get is safe.

Keywords: Food Safety, Sanitation and Hygiene, Food Providers, Safer Food, Food Service.

Introduction

Access to safe and nutritious food is key to sustaining life and promoting good health. Man is made up of what he eats. The constituents of food which are proteins, carbohydrates, fats, vitamins, minerals and water nourishes the body and make man healthy and good looking (WHO, 2023 and Samphina, 2023). The production and consumption of safe food have immediate and long benefits for people, planet and the economy.

Food is anything which when taken into the body serves to nourish or build up the body tissues or to supply body health. However, unsafe food contains harmful bacteria, viruses, parasites or chemical substances that cause different diseases ranging from diarrhea to cancers. Unsafe food creates vicious cycle of diseases and malnutrition particularly affecting infants, young children, elderly and the sick. The presence of these harmful contaminants not originally present in the food is believed to be introduced by human although some do occur naturally.

It has been estimated that 600 million people almost 1 in 10 people fall ill after eating contaminated food each year, resulting in 420,000 deaths and the loss of 33 million healthy life (WHO, 2023). Food borne diseases impede social economic development, by straining health care system and harming national economics, tourism and trade; food safety, food

sanitation and hygiene and food security are closely linked.

Food safety is the practice that is observed during the handling, processing and distribution of food to ensure that contaminants that cause food borne illnesses or diseases are not present (Hanson, 2021). Food sanitation is the operations that help create a clean working environment by keeping any equipment, contact surface and the whole facility clean and free from common food hazards. Therefore, food service providers or handlers are to ensure that the health and safety of the consumers are protected from any food related diseases or pathogens.

Food service is a process or system where food is served to a guest or customer in an establishment (Unilever Food Solutions, 2023). It is concerns taking food and beverage from their source to its consumer. Food service is centered on the customer experience; servers, cooks and hosts all play a role in creating a friendly atmosphere that welcome guests. The food service industry encompasses any establishment that serves food to people outside their homes. This include restaurants, cafeterias, catering and vending companies, hotels, hospital cafeterias, university dining halls, inns, rehab and retirement centers and so on.

Food service is a large industry which deals with the preparation and service of food and beverage. The global food service market size is growing and is forecast to reach a whopping $4.1 Trillion USD by the year 2026 with a growth rate of 5.4% over the analysis period (EHL, 2022 and Mahon, 2023). The industry is a major contributor to economic development worldwide. The food service outlets are part of the daily lives of billions of customers as a place for them to get food or simply spend some quality time with their friends and family.

New types of consumers are created; conscious and well informed about what they buy. The decision to consume a produce will be increasingly based on how it is made, and what process were used to produce it, because every produce and service needs to meet the expectation of the ethical buyers. And so, businesses that want to stay relevant in this space will need to develop skills in search engine optimization and delivery applications as main tools for organizational success.

Types of Food and Beverage Service

According to Unilever Food Solutions (2023), these include:

1. **Waiter Service -** more commonly known as sit-down service. This type of service requires a waiter to take care of everything from taking orders to serving food and payment.
2. **Chinese Banquet Service -** this is another form of waiter service where each waiter takes care of a table or group of tables with 10-12 guests. Dishes are usually communal and shared.
3. **Buffet Service -** in a buffet service, guests can choose their food from a wider selection made available on buffet line. There are 4 four common types of buffet services.
   (a) **Simple Buffet -** guests get food from a buffet line and waiter move around to clear tables and help guests with minor requests.
   (b) **Station-Buffet line -** waiter serve the beverages while guests get the dishes from the buffet line.
   (c) **Modified Deluxe Buffet -** tables are set with cuttering; waiters serve the beverages, coffee and dessert while guests get the dishes from the buffet line.
   (d) **Deluxe Buffet -** waiters serve appetizers, soup and/or salads

and beverages. Guests take the other dishes from the buffet line.

4. **Self Service -** in this type of food service, dinner place, pay and pick up their orders at a counter similar to fast, casual and fast-food establishment.
5. **Semi-Self Service -** similar to self-service, this type of service requires dinners to order and pay for their orders at the counter but waiters serve the food when ready.

In all, is for the food service establishment to expand their customers' base, increase revenue and give customers varieties of service options and offers unparalled conveniences to their guests.

Food Safety and Service Providers

Food being an organic substance grows bacterial very easily and also contaminate easily. Food hygiene is the conditions and measures necessary to ensure the safety of food from production to consumption. According to WHO (2023), food safety is the conditions and measures that are necessary during the production, processing, storage, distribution and preparation of food to ensure that it is safe, sound and wholesome and fit for human consumption. Food safety is essential to prevent food borne illnesses and diseases and enhance the wellbeing of humans (Alemayehu, et al., 2021).

Food borne related illnesses have increased over the years and negatively affect the health and economic wellbeing of many developing and developed countries. There is growing evidence that food safety have been neglected in developing countries. In Africa, food borne diseases may be an important contributor to gastrointestinal disease and poor hygienic practices during food preparation, handling and storage are one of the commonest causes of morbidity. Consuming unsafe food can affect economic progress, productivity loss and huge medical expenses as a result of food borne disease burden.

Food safety is negatively affected by contaminations along food supply chain (Hanson, 2022). Controlling food safety requires knowledge about what could endanger its integrity and stability by food service providers. Food provider is a person or establishment that provides or sells prepared food or raw food or beverage to the general public to be consumed on the premise or for take away consumption (Law, 2023).

Without proper management and control, food safety issues such as food borne diseases and food-related injuries can occur. Food service providers are to ensure that cleanliness and hygienic standards are followed and the require temperature for optimum storage maintained. The food service providers control and oversee the handling, preparation and storage of food in a manner to prevent food contamination and poisoning.

Reasons for Keeping Food Safe

1. Protection from food borne illnesses and other food related injuries. These effects are mainly caused by food borne pathogens.
2. Reduced cost from food-safety issue. Unsafe food with proven safety issues may be deemed unfit for consumption. Proper food safety practices can reduce costly health care expenses or law suits concerning damages from less risk of disease occurrence.
3. Reduced waste. Through the use of proper food safety practices, a food business can cut waste as a result of a better food production process.
4. A better way of living. Any food borne illness affects productivity in consumers. If food safety practices applied both in a food service establishment and at home, the

cases of food borne diseases can be prevented and everyone enjoys the delight that food bring and the healthy life.

5. Sustainable food production. Proper food safety practice includes food manufacturing processes that not only protect the consumer but also take into account the environment.
6. Safer food globalization. With proper food safety management systems, routes to a broader distribution channel become open for your food business (Hanson, 2021).

Keys to Safer Food

According to WHO (2023), the keys to safer food were developed to educate safe food handling behaviours to all consumers and food handlers. They are:

1. Keep clean
2. Separate raw and cooked
3. Cook thoroughly
4. Keep food at safe temperature
5. Use safe water and raw materials.

Because food safety is a shared responsibility, the consumers and food handlers play a huge role in preventing food borne diseases.

Food Sanitation and Hygiene

Safe food is food which is free of contaminants and will not cause illness or harm (Bhavya, 2023). Food sanitation and hygiene play a vital role in promoting and protecting the health and well being of hundreds of people. The foods, materials and equipment are subject to constant handling by people at every stage of food production and service. It is the duty of every caterer to ensure that personal hygiene and sanitation become a habit of all food handlers.

Safe food is essential to human health and well-being. The adoption of positive attitude and practices towards food hygiene and sanitation by food providers is crucial in enhancing healthy population. There are poor washing facilities for some of the food vendors and food were contaminated through unwashed hands, unsafe materials and reusable polyethylene bags. Some of the challenges of food hygiene and sanitation had to do with inadequate refuse disposal and waste bins, poor water supply and drainage system, overcrowding, poor handling and storage of products and unhygienic surroundings (Mohammed, 2023).

Food sanitation and hygiene operations include proper hygiene practices and cleaning and sanitizing operations such as wiping, sweeping and application of approved sanitizers. It is necessary to create and maintain hygienic and sanitary conditions to safe guard our food. This involves the prevention of objectionable matter(s) from getting into food, including harmful bacteria, poisons and foreign bodies. Food sanitation reduces the growth of microorganism on the equipment and dirt on the food; that causes food borne diseases and food spoilage. Sanitary principles also apply to waste disposal and reduce pollution and improve ecological balance.

Hygiene Tips for Food Service

Improper food handling can be a major cause of food contamination. Practicing good personal hygiene is essential to food safety. Below are some of the hygienic practices as suggested by State Food Safety Resources (2023).

1. Have a clean uniform - the goal is simple; bring as few pathogens as possible to work. Your uniform could contain a variety of

pathogens especially when it is dirty.
2. Wash your hands - your hands contain millions of bacteria and viruses that can easily spread to food. To help the pathogens from spreading to food, you should wash your hands often.
3. Wear gloves (and change them regularly) - before putting on a pair of gloves, you should wash your hands. Once you put on your gloves, do not touch your hair, face or other surfaces that could contaminate them.
4. Wear a hair covering - hair can create both a physical and biological hazard. Wearing a hair covering such as a hair net and cap will help keep hairs from falling into food. If customers find hair(s) in food, they are less likely to return to your establishment.
5. Keep finger nails clean and trimmed - fingers nails can easily trap dirt and pathogens. Finger nails should be kept short and clean.
6. Beware of Jewelry - pathogens can get trapped under jewelry which creates a biological hazard. Jewelry such as bracelets, watches and rings could fall into food, creating a physical hazard.
7. No eating, drinking and smoking - when you eat, drink or smoke your hands spend a lot of time near your mouth. The pathogens in our mouth could contaminate the food you are preparing if you do these activities around the food.

Attitude of Food Providers to Sanitation and Hygiene

Food is one of the major necessities for human being to survive. Food safety and sanitation is a collective effort from all the members of the food supply chain. All members of food supply chain must comply with established standard and legislation to maintain food sanitation and hygiene; whether you are a food supplier, a food business owner, a manufacturer or a customer. Food sanitation and hygiene practices are performed to reduce the risk of causing foodborne illness to customers. A food vendor or establishment is always expected to work in a clean and conducive atmosphere.

Food providers or handlers are typically involved in the packing, preparing, storing or serving of food items that are sold to customers. They are food vendors or employed by food manufacturing or packing companies, restaurants, hotels, hospitals, cafeterias, where they follow proper food handling procedures to ensure that food items are safe for human consumption (Betterteam, 2023). Food handlers should be knowledgeable in food health, safety regulation and display a positive attitude and practice for proper food handling tactics. On the contrary, some of the food handlers lack the knowledge of food safety and hygiene due to lack of training and poor inspection and monitoring by food inspectors on safe handling of food.

Food handlers with poor personal hygiene and sanitation and lack of awareness are of important issues in preventing food borne diseases; working in food establishment could be potential sources of infection of many intestinal helminthes of protozoa and estrogenic pathogens (Lema, et al., 2022). Food borne diseases (FBD) is of public health significance to many nations. The occurrence of food diseases is more common in developing nations because of poor hygiene and inappropriate absence of drinking water and contaminated food safety education.

Findings/Observations

1. Proper knowledge of food safety and hygiene enhance improved

attitude and better food handling practices.
2. Poor food sanitation and hygiene behavior promote food poisoning and food contamination.
3. Food pathogens or contaminants cause food borne diseases and huge health care losses even death.
4. Personal hygiene of food handlers is an impetus for safe food and disease free environment.
5. Unsafe food or contaminated food increase economic losses, trade rejections, food and agricultural waste.

Conclusion

Food sanitation and hygiene are essential matter of public health for protection and prevention of diseases caused by unsafe food due to lack of food quality and control from production to consumption. Food handlers are expected to have excellent sanitation and hygiene practices to reduce the risk of food contamination and poisoning to protect consumers from food borne diseases.

Safe food allows for the uptake of nourishing nutrients and promotes human development. Safe food support national economics, trade and tourism by stimulating cultural advancement in form of food culture and productive opportunities. Unsafe food breeds food borne illnesses or diseases, loss of healthy society and people, increases public health burden and retard economic sustainability.

Therefore, it is important for food handlers to have adequate knowledge regarding personal hygiene and sanitation, cross contamination and pathogens infections and sanitation and food temperature to be able to prevent food poisoning and protect the consumers.

Food sanitation and hygiene practices in conjunction with the positive attitude of the food providers help to detect and control food safety risks and contribute to economic prosperity, food security and sustainable development. And so, the ultimate goal of the food providers is to create a sustainable and secure food supply for everyone with safe farm to fork ideology, promoting the enjoyable experience of food consumed while being sure that the food they get is safe.

Recommendations

1. Food providers should develop a strong positive and professional attitude to food preparation, service and after sale to reduce toxic and food waste that contaminate the environment and promote climate change.
2. Personal hygiene and sanitation of food handlers should be compulsorily enforced by the regulating authorities to promote cleanliness and healthy society
3. Foods handlers should be compel to strictly follow food regulations and control to avoid the incident of food contamination and poison.
4. Food sanitation and hygiene training should be regularly organized for food providers to update their knowledge in food handling and service.
5. Hygiene enablers such as portable water, infrastructures and disposable equipment should be put in place to create a conducive and disease free environment to reduce the risk of food borne illnesses.

References

Alemayehu, T., Aderaw, Z., Giza, M. & Dires, G., (2021), Food safety knowledge, handling practices and associated factors among food

handlers in food establishment in Debre Markos Town, Northwest Ethiopia: Institutional-Based cross ectionalstudy.www.dovepress.com

Betterteam (2023), Food handler job description. www.betterteam.com

Bhavya (2023), Hygiene and sanitation in food sector. www.hmhub.in

EHL, (2023), What is the food service industry? All your questions answered.www.hospitalityinsights.ehl.edu

Hanson, E. M., (2021), Food safety definition and why is food safety important. www.fooddocs.com

Hanson, E. M., (2022), What is food solution? Tips for effective sanitation. www.fooddocs.com

Law insider (2023), Food provider definition. www.lawinsider.com

Lema, K., Abuhay, N., Kindie, W., Dagne, H. & Guadu, T. (2022), Food hygiene practice and its determinants among food handlers at University of Gondar, Northwest Ethiopia. Online Publication, Nov. 23, 2022.

McMahon, M., (2023), What is food servicewww.practicaladultinsights.com

Mohammed, R., (2023), Examming food safety knowledge, attitude and practice of street food vendors in Tamale Metropolis. www.afribary.com

Samphina Academy, (2023), Knowledge and practice of food hygiene among food vendors. www.samphina.com.ng

State Food Safety Resources (2023), February Carton: 7 hygiene tips for foodservice.www.statefoodsafety.com

Unilever Food Solutions, (2023), Types of food and beverage service. www.unileverfoodsolutions.com.ph

WHO, (2023), Food safety. www.who.net

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THE IMPACTS OF TOURISM ON CULTURE: A REVIEW

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ABSTRACT

This paper examined the impact of tourism on culture, it assume that the creation and development of the cultural attractions of a place is to attract tourists and also have a positive impact on the life condition of the local community, not only for increased money availability but also for a change of inhabitants' mind-set. The study observed that tourism has helped in the cultural preservation of any community and also some negative effect, such as Commodification of culture, moraldecadence. I therefore recommend that tourism and tourist activities should be properly managed to curb the negative effects of tourism on culture.

Keyword: Culture, Tourism Activities Tourist Behaviour and the Community.

INTRODUCTION

The tourism industry is one of the most important economic sectors and also one of the most impactful in today society. It represents 10 % of world GDP (WTTC, 2020), with increasing weight on the total value produced, and employs millions of people worldwide (World Tourism Organization, 2019). The possible impacts on society coming from tourism industry are very diversified and may affect people's lives in more than one aspects, both in positive and negative ways.Tourism is a broad concept, and there are near-infinite combinations of the composing factors, leading to infinite different combinations of possible consequences, meaning the behavior of each part of the tourism supply chain may have profound results on the whole community. Among the existing types of tourism, the cultural tourism is the object of our investigation, since recent literature has highlighted this kind of concept and may help in reducing some of the negative downsides and improving the impact on the local community.

We can state that cultural tourism is a very complex segment of the 'tourism industry,' its supply is diverse and versatile. The future positions of the discipline will probably be strengthened both directly and indirectly as with the change of the recreational needs.

The purpose of this paper is to examine the impact of culture on tourism demand in Nigeria. City tourism has been cited as one of the fastest growing travel segments globally. In many state of the country. Cities are attractive destinations for various segments of the tourist market. Young people are attracted to the nightlife and entertainment as well as sporting events held in the city. Older and more educated tourists are attracted to the cultural heritage of the city. The options available to travellers in a city surpass those of other destination types due to the density of cultural offerings available.

The role of culture in attracting tourists to cities has not been overlooked by the tourism industry. Since the 1980s, many destinations have focused on cultural tourism as a source of economic development (OECD, 2009). Some states in Nigeria are increasingly targeting tourism as a key sector for local development and are investing in cultural attractions and infrastructure to secure a

niche position in the tourist market. The contribution of culture to tourism has received extensive consideration in the academic literature. There are, however, two notable shortcomings in the literature. Firstly, the range of cultural amenities considered in the literature is limited. Many studies focus on a narrow range of cultural amenities with many of the cultural amenities listed by the UNWTO (2019) being overlooked. Secondly, studies are narrow in geographical scope, with many studies focusing on a specific location or multiple locations within a specific country. Location-specific case studies are useful as they allow for in-depth analyses on the contribution of culture to tourism demand.

Culture is that complex whole which includes knowledge, belief, art, morals, law, custom, and any other capabilities and habits acquired by man as a member of society. Culture refers to the cumulative deposit of knowledge, experience, beliefs, values, attitudes, meanings, hierarchies, religion, notions of time, roles, spatial relations, concepts of the universe, and material objects and possessions acquired by a group of people in the course of generations through individual and group striving.

According to HOFSTEDE (1997) the core of a culture is formed by the values which in terms of tourism will be the basics for the attraction of a given destinations well. The different levels of culture will be the rituals, the heroes and the symbols of the given culture which again would serve as a basis for tourism purpose travels.

Culture refers to the following Ways of Life, including but not limited to:

a. Language: the oldest human institution and the most sophisticated medium of expression.

b. Arts & Sciences: the most advanced and refined forms of human expression.

c. Thought: the ways in which people perceive, interpret, and understand the world around them.

d. Spirituality: the value system transmitted through generations for the inner well-being of human beings, expressed through language and actions.

e. Social activity: the shared pursuits within a cultural community, demonstrated in a variety of festivities and life-celebrating events.

f. Interaction: the social aspects of human contact, including the give-and-take of socialization, negotiation, protocol, and conventions". (http://www.roshaninstitute.org/474552)

Based on these we can state that culture is part of the lifestyle which a multitude of people are sharing. The similarities in spoken and written language, behaviour, lifestyle, customs, heritage, ideology and even technology connect the individuals to groups of people in a certain culture.

CULTURAL TOURISM

Cultural tourism defines the phenomenon of people travelling specifically for the sake of either experiencing another culture or the cultural attractions of a place. Cultural tourism is defined by attendance by inbound visitors at one or more of thefollowing cultural attractions during their visit to Australia: festivals or fairs (music, dance, comedy, visual arts, multi-arts and heritage); performing arts or concerts (theatre, opera, ballet and classical and contemporary music); museums or art galleries; historic or heritage. Cultural tourism is not enclosed in a single and straightforward definition, and it is not merely a shade of the definition of tourism. The United Nations World Tourism Organization gives the following description:

cultural tourism: "the movement of persons to cultural attractions in cities in countries other than their normal place of residence, with the intention to gather new information and experiences to satisfy

their cultural needs, and all movements of persons to specific cultural attractions, such as heritage sites, artistic and cultural manifestations, arts and drama to cities outside their normal country of residence" (Greg, 1996).

TYPES OF CULTURA TOURISM

•Natural and cultural heritage (Nature-based or ecotourism)

Built heritage
Architectural sites,
World heritage sites,
National and historical memorials
Literature,
Arts,

• Cultural heritage sites (historical)

Museums, collections,
Libraries,
Theatres,
Event locations

Other types are Local cultures' traditions, Ethnic diversity, Cultural festivals and events

TOURISM

The United Nations World Tourism Organization (UNWTO) defines tourism as people "traveling to and staying in places outside their usual environment" for leisure, business, or other purposes. For travel to qualify as tourism under this definition, it must last more than 24 hours and not last more than one year. Moreover, tourism can be divided into domestic tourism, which occurs within one country, or international tourism, where a person travels outside their country and there are other forms of tourism. Tourism is a major contributor to many countries' economies, and popular tourist destinations will often include a wide range of businesses specifically designed to cater to travelers from other locations.

The concept of traveling to other locations for pleasure existed in Ancient Egyptian, Greek, and Roman civilizations, meaning tourism, as a concept, has existed for thousands of years. Of course, in early times, travel primarily occurred on horseback or using boats, which meant long-distance travel, was difficult. Initially, tourism was also a pursuit for the most wealthy and powerful in society. This gradually started to change as the centuries passed. However, modern tourism is often traced back to the Industrial Revolution, with British society being among the first to truly embrace the idea of ordinary people traveling for leisure.

Today, the concept of tourism is firmly established within many cultures. It is common for countries to promote this idea, with tourism meaning more visitors and money spent on local businesses

NEGATIVE IMPACTS OF TOURISM ON CULTURE

Acculturation effects

Acculturation is a process of adaptation and adjustment (Ebin et al., 2001). When local people entertain tourists, they adapt tourists' needs, attitudes and values and ultimately start following them. This often happens in a less sophisticated society where, stronger culture dominates the weaker (Burns, 1999). But the diffusion of innovation of cultural components in a social framework depends on compatibility, advantage, and complexity of the cultural objects. Particularly, the youth are vulnerable to this impact and women are ahead of their male counterpart in this regard (Ghuman, 1997). Following tourists' lifestyle young people bring changes in the material goods they use and in their gestures and postures, which is defined as demonstration effects. For

example, young people are putting on western modern dress instead of traditional. Now they prefer western in love-marriage instead of arrange-marriage, drink with friends or organise a dinner party outside home, and ride bicycles instead of the carts. The elders are totally against and worry about this cultural change. However, by carefully analysing the case, it is found that not only tourism, there are some other factors like easy access to Indian movies, dress, and communication vehicles also cause this impact.

Tourism should be acclaimed here because tourists gave idea, education, and modern facilities, which brought youngsters, out of

Commodification of culture

Tourism can lead to the commodification of culture and is the process whereby cultural elements is represented for the mass consumption of tourist, In most cases the reinterpretation of culture is done by outsider, such as tourist or tour operation. These outsider takes the liberty of choosing which part of local is to

POSITIVE IMPACTS OF TOURISM ON CULTURE

Cultural preservation

Tourism provides funding to preserve and conserve cultural heritage, gives back cultural pride, revitalize customs & traditions as well as opens door for cultural sharing and learning (Smith, 2009). Most of the common positive impacts of tourism on culture include increasing cross cultural interaction; understanding, maintaining and keeping local culture, arts, crafts and traditions; empowering host communities; and strengthening cultural values. Tourism

Building institutional infrastructure

Tourism certainly increases awareness among local people those are not involved

hairstyles, disobey their elders' Kinship title, involve

superstition to freedom in work and men and women relationships.

Hybrid culture

In Nigeria, the western and native culture together has created a new culture, which is called the hybrid culture. The examples include borrowing artificial elements and adding them to traditional dance, music, events and activities; serving hybrid cuisine instead of traditional ones and Cultural commodification results in the transformation of value-from sacred to profane and from real to the unauthentic.

showcase, biases their biases, understanding and aesthetic sense.

Moral Decadence

The predisposition to vices is not limited to the tourist, but also the residents. And tourism sometime leads to degradation of morality and sexual permissiveness.

has opened the door to establish the cultural identities like houses, clothes, and megaliths dress as their group identity and recognition of political power through photographing and marketing these identities in promotional materials at provincial level. People believe tourism has facilitated them in manipulating economic and political capital to gain power of church and position in state administration. On the other hand, due to the social recognition and power, conflict and crime might be increased in the major tourist destinations (Tsoudis, 2001).

in their traditional arts & crafts business. By building some institutional

infrastructure, tourism keeps these cultural components alive. Modern advertising materials and broadcasted around the world, and organised trade fair to attract tourists.

Motivating collectivism and Derived demand

Through social contact people sometimes come out of self-centric position to collectivism. This social contact helped to show morality, honesty and hospitality to strangers.

MANAGING THE IMPACTS OF TOURISM ON CULTURE

Democratic management

A democratic management group in the destinations is important to empower community people and manage their socio-cultural issues. In Nigeria group can be formed by the members of every clan for raising voice against corruption, collecting and distributing funds and deciding on cultural performances, which is now protecting the culture and harvesting benefits of tourism.

Managing the process and strengthening the local cultural value

Priority should be given to the process, not only the product and Strengthening local cultural value is a vital point. The local people agreed to stage dance and music, but disagreed to stage rituals because they didn't want to commodify their core value. A structured link between formal and informal sectors in the tourism industry is valuable to reduce tourists' hassle and conflict between stakeholders.

Considering the health impact

Tour operators, hotels, and even local community need to analyse health impacts and to work with international health organisations. Wall and Matheison (2006) think workers, community people, and guests everybody has direct and indirect health impacts on others. To reduce the HIV impacts, the International HIV/Aids Alliance is successfully promoting guidelines for leaders, guides, community people and tourists on sex related issues in the country. To manage the negative impact of gambling like a casino, the development should not take place at the introductory level of the tourist destination life cycle.

RECOMMENDATIONS

Based on the findings, it is therefore recommended that tourism and tourism activities should be well mange in order not to impact on culture of the people negatively and there should be public awareness about cultural preservation through tourism.

CONCLUSION

Tourism and tourism activities has a great impact on the cultural heritage the country both positive and negative, but the positive impact of tourism on culture is of great importance as it has helped in the perseveration of our cultural heritage in the country.

In the study of a tourism product we have to be aware not only the positive effects but the negative aspects of tourism development as well. The ever growing and rapidly increasing

Cultural tourism in the recent years has raised the question whether it really serves the needs sustainable tourism especially in small communities. Cultural tourism started as a form of alternative tourism and nowadays it can be considered in certain tourism destinations as a dominant part of mass tourism. Local communities have to face with the degradation of their 'original' culture so "there are a growing number of places in search of new forms of articulation between culture and tourism which can help to strengthen rather than water down local culture.

REFERENCE

Burns, P. (1999) An Introduction to Tourism and Anthropology. London: Routledge.

Ebin, V. Sneed, C. Morisky, D. Rotheram–Borus, M. Magnuson, A. and Malotte, C. (2001). Acculturation and interrelationships between problem and health promoting behaviours among Latino adolescents. Journal of Adolescent Health, 28,62–72.

Greg, R. (1996). Cultural Tourism in Europe. Cultural Tourism in Europe. Wallingford. Retrieved fromhttp://www.tram-research.com/cultural_tourism_in_europe.PDF

Ghuman, P. (1997) Assimilation or integration? A study of Asian adolescents. Educational Review, 39, 23–35.

Hofstede, G. (1997). Cultures and Organizations: Software of the mind. New York: McGraw

OECD (2009). The Impact of Culture on Tourism Paris: OECD 159 p.

http://www.em.gov.lv/images/modules/items/OECD_Tourism_Culture.pdf

Office of National Tourism (1997). Fact Sheet No 10 Cultural Tourism www.Dcita.gov.au/swg/publication sculttour.html

.

Tsoudis, O. (2001) The portrayal of crime in the Cypriot media: An exploratory study. Western Criminology Review, 3(1), 1-46.

United Nations World Tourism Organization (UNWTO). (2019)

Wall, G. and Matheison, A. (2006) Tourism: change, impacts and opportunities, Essex: pearson Education Limited

World Tourism Organization. (2019). International Tourism Highlights. Madrid. https://doi.org/https://doi.org/10.18111/9789284421152

WTTC. (2020). Share of GDP generated by the travel and tourism industry worldwide from 2000 to 2019. Retrieved May 20, 2020, from https://www-statista com.ezproxy.biblio.polito.it/statistics/1099933/travel-and-tourism-share-of-gdp/

http://www.roshan-institute.org/474552

Antimicrobial Activities of Citrus Zest against Some Microbial Isolates

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ABSTRACT

The antimicrobial effect of water and ethanol extracts of citrus zests (Orange, Lemon, Lime) against pathogenic *Escherichia coli, Staphylococcus aureus,* and food spoilage fungi *Aspergillus niger, Aspergillus flavus* and *Fusarum* spp was determined. This work aimed to discover the effect of citrus zests (Orange, Lemon and Lime) extracts on selected pathogenic bacteria and food spoilage fungi. The method used for the preparation of the zest was Soxhlet extraction method with two different Solvent; Water and Ethanol, Agar well diffusion method was used for the zone of inhibition. Two different concentrations (200mg/ml and 100mg/ml) of each extract were used against the test organisms. The test isolates demonstrated susceptibility to the varying concentrations of the crude extracts; this was evident in the different diameters of zones of inhibition displayed by the organisms. For the bacterial isolates *Escherichia coli* had the highest susceptibility (1.00) while *Staphylococcus aureus* had the least susceptible (0.8) to the various extracts at different concentrations while for the fungal isolates, *Aspergillus niger* had the highest susceptibility (1.50) while *Fusarum* spp had the least susceptible (0.40). The result of this research work showed that the *Citrus* Zest (Orange, Lemon and Lime) can be used as natural food preservatives.

Keywords: antimicrobial effect, bacterial isolates, Citrus zest, fungal isolates

INTRODUCTION

Natural antimicrobials derived from plants have been recognized for centuries, but only scientifically confirmed in the last 30 years (Al-Ani *et al*., 2009). Nowadays natural antimicrobials receive a compact awareness from series of issues. The presence of both antioxidant and antimicrobial properties in a single molecule makes them more effective and better suited as micro-organism control and pharmaceutical active compounds (Tajkarimi *et al*., 2010). Citrus species such as orange (*Citrus sinensis*), lemon (*C. limon*) and lime (*C. aurantifolia*) peels and oil contain bioactive substances that can act as natural antimicrobial against food spoilage pathogens (Shuping and Eloff, 2017). Natural antimicrobials are safe, less toxic, readily biodegradable, ecosystem friendly, readily available and cheap. Natural antimicrobials can be adopted for sustainable control and management of pathogens of crop diseases and food spoilage (Effiom *et al*., 2019).

Antimicrobial activity of the peel extract of citrus is as a result of their antimicrobial components which include Limonoids from *C. limon*, with antibacterial and antifungal activity. Extracts of citrus fruit (lemon, lime, orange and grape fruit) are among the most studied natural antimicrobials for food applications (Hindi and Chabuck, 2013).

Essential oils extracted from citrus peels have been added to food to inhibit the activities of *Salmonella typhi*, *Bacillus cereus* and *Staphylococcus aureus* (Al-Dalahi *et al*., 2019). Extracted biophytochemicals from citrus have been added to food during packaging to prolong

their shelf life (Irkin and Esmer, 2015). The addition of Edible Films and Coatings (EFC) is a new preservation method that can be used to improve the quality of food products by adding natural antimicrobials to food to inhibit food spoilage microorganisms and growth of pathogenic microbes (Al-Dalahi *et al*., 2019).

The health benefits generated from *Citrus* fruits are as a result of vitamins, especially vitamin C (Choi, 2002), phytochemical compounds like synephrine, hesperidin flavonoid, polyphenols, pectin. A single orange, lemon or lime is said to have about 170 phytonutrients and over 60 flavonoids (which are antimicrobial agents) that helps fight pathogenic diseases (Oikeh *et al*., 2013).

The potential antimicrobial components in citrus peel are D-limonene, terpenes, sesquiterpene, oxygenated monoterpene, linalool, acid esters, aliphatic hydrocarbons and other unidentified hydrocarbons. The antimicrobial activity of citrus peel extract can be assessed on various food borne pathogens (Shalu *et al.* 2015).

The overall aim of this study was to evaluate the antimicrobial activities of three *Citrus* fruit (Orange, Lemon and Lime) zest on selected pathogenic bacteria and food spoilage fungi.

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MATERIALS AND METHODS

The study was conducted at the Microbiology laboratory of Food Technology, Auchi Polytechnic, Auchi, Edo- State, Nigeria.

PLANT MATERIALS

Oranges (*Citrus sinensis*), Lemon (*Citrus limon*) and Lime (*Citrus aurantifolia*) were obtained from Uchi market, Auchi, Edo-State, Nigeria.

TEST MICROOGANISMS

Microbial strains used in this study include *Staphylococcus aureus, Escherichia coli, Aspergillus niger, Aspergillus flavus* and *Fusarum* spp. The bacterial cultures were obtained from Auchi Polytechnic Cottage hospital laboratory, Auchi, Edo-State while the Fungi were isolated from spoilt baked food at the Microbiology Laboratory of Food Technology, Auchi Polytechnic, Auchi, Edo-State. Bacterial cultures were grown and maintained on Nutrient Broth while fungi cultures were maintained using Potato Dextrose broth.

EXPERIMENTAL METHOD

Preparation of Zest

The Zest was removed from the skin of each citrus (Orange, Lemon and Lime) as described by Hasija *et al*. (2015) using a sterile knife, after they have been washed thoroughly in sterile distilled water. The peels or zest were dried at 40^{o}C followed by grinding using mixer for 3min to make fine particles, each samples were kept in air tight containers and labeled prior to use.

Preparation of Extract.

Extraction of the citrus zest was carried using soxhlet extraction method as described by Li *et al*. (2006) with two different solvents, ethanol and water respectively. 35.5g of orange zest which is now in fine powdered form was weighed into a beaker of 200ml of distilled water and also into a beaker of 200ml of ethanol, 27.50g of lemon zest in powdered form was weighed into a beaker containing 200ml of distilled water and a beaker containing 200ml of ethanol, 9.44g of lime zest in powdered form was weighed into 100ml of distilled water and also into a beaker conyaining100ml of ethanol. The mixture of each was left standing for 72 hours with shaking at regular intervals 5 hourly. At the end of this period, the zests were filtered using Whatman No.1 filter paper. Each filtrate (2g) was dissolved in 5ml of Dimethyl Sulfoxide ($DMSO_4$) which was used as stock solution

SCREENING FOR ANTIMICROBIAL ACTIVITY

The water extract and ethanol extract of the *Citrus* zest (Orange, Lemon, and Lime) were used for the antimicrobial screening using the agar well diffusion method as described by Henderson *et al*. (2018) with slight modification. Potatoes Dextrose Agar (PDA) and Nutrient Agar(NA) were sterilized at 121^{o}C for 15mins and poured asceptically on sterile petri dishes and allowed to solidify. The solidified PDA and NA were punched with sterile borer to make 7mm diameter wells. The wells were filled with two concentrations of the extracts 100mg/ml and 200mg/ml respectively. All assays were carried out in triplicates. The plates were then incubated at 37^{o}C for 24 hours. Results were recorded by measuring the diameter of zone of inhibition. Clear inhibition zones around the isolates indicated the presence of antimicrobial activity.

MINIMUM INHIBITORY CONCENTRATION

The minimum inhibitory concentration was determined using the tube dilution method as described by Henderson *et al*. (2018) in which 9ml of sterile nutrient broth for bacterial and potato dextrose broth were dispensed into test tubes and 1ml of the extract of varying concentration was added into the different test tubes and 0.1ml of standardized organism the test tubes containing bacterial isolates were incubated at 37^{o}C for 24hours while the test tube containing fungal isolates were incubated at 28^{0}C for 72hours. The test tube with least concentration of extract that show no turbidity was taken as the minimum inhibitory concentration.

MINIMUM BACTERICIDAL CONCENTRATION

Minumum bactericidal concentration was carried out as described by Henderson *et* al. (2018) with slight modification. 9ml of sterile nutrient broth and 1ml of the citrus zest extract were added into test tubes that containing 0.1ml of the test organism and incubated at 37^{o}C for 24 hours for bacterial isolates while 0.1ml of the fungi isolate were inoculated into 9ml sterile potato dextrose broth with 1ml of the citrus zest extract at different concentration and incubated at 28^{0}C for 72hours. After the incubation time, the inhibition zones were determined; the plates that contained the lowest concentration of extract that showed no growth were taken as the minimum bactericidal concentration.

RESULTS AND DISCUSSION

Antimicrobial activity of citrus (orange, lemon and lime) zests were assessed against pathogenic bacteria (*Staphylococcus aureus* and *Escherichia coli*) and food borne pathogens (*Aspergillus niger, Aspergillus flavus* and *Fusarum* spp.) using disc diffusion method. Tables 1- 5 showed diameter zones of inhibition (mm) produced against the test organisms by the ethanol and water extracts at 200mg/ml and 100mg/ml concentrations which indicate potency of the extracts against the organisms. Table 6 shows the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of the extracts against the test isolates

.Table1. Mean diameter of zone of inhibition (mm) of Ethanol Extract of Orange peel (zest)

Con (mg/ml)	*E. coli*	*S. aureus*	*Asp. niger*	*Asp. flavus*	*Fusarum* spp
200	1.00	NA	0.50	NA	NA
100	0.10	NA	NA	0.70	NA

KEY:NA=No Activity

Table 2. Mean diameter of zone of inhibition (mm) of Water Extract of Orange peel (zest)

Conc. (mg/ml)	*E. coli*	*S. aureus*	*Asp. niger*	*Asp. Flavus*	*Fusarum* spp
200	0.60	0.90	NA	NA	NA
100	NA	NA	NA	NA	NA

KEY: NA = No Activity

Table 3. Mean diameter of zone of inhibition (mm) of Ethanol Extract of Lemon peel (zest)

Conc. (mg/ml)	*E. coli*	*S. aureus*	*Asp. niger*	*Asp. Flavus*	*Fusarum* spp
200	0.80	NA	NA	NA	NA
100	NA	NA	NA	NA	NA

KEY: NA = No Activity

Table 4. Mean diameter of zone of inhibition (mm) of Water Extract of Lemon peel (zest)

Conc. mg/ml	*E. coli*	*S. aureus*	*Asp. niger*	*Asp. Flavus*	*Fusarum* spp
100	0.60	0.80	1.50	NA	0.40
200	0.50	NA	0.50	NA	NA

KEY: NA = No Activity

Table 5. Mean diameter of zone of inhibition (mm) of Water Extract of Lime peel (zest)

Conc. mg/ml	*E. coli*	*S. aureus*	*Asp. niger*	*Asp. Flavus*	*Fusarum*
200	NA	0.50	NA	NA	NA
100	NA	NA	1.10	NA	NA

KEY: NA = No Activity

Table 6. Comparing the Susceptibility of the test isolates to the extracts minimum inhibitory concentration and the minimum bactericidal concentration.

Isolates	Conc. mg/ml	Water	Ethanol
E. coli	200	MBC	MBC
	100	MIC	MIC
S. aureus	200	MBC	NA
	100	NA	NA
Asp. niger	200	MBC	MBC
	100	MBC	MBC
Asp. flavus	200	NA	NA
	100	NA	NA
Fusarum	200	NA	NA
	100	NA	NA

KEY: NA = No Activity, MBC= Minimum Bactericidal concentration, MIC= Minimum inhibitory concentration.

DISCUSSION

The results obtained from the antimicrobial analysis showed characteristic zones of inhibition around the test organisms. These organisms which include both bacteria (*Escherichia coli* and *Staphylococcus aureus*) as well as fungi (*Aspergillus niger, Aspergillus flavus* and *Fusarum* spp.). The ethanol extract exhibit a significant activity against *Escherichia coli, Aspergillus niger* and *Aspergillus flavus* for orange peel extract. The Orange peels (zest) extracted with ethanol showed no activity against *Staphylococcus aureus* and *Fusarum* spp but was active against *Escherichia coli* at a concentration of 200mg/ml with a zone of inhibition of 1.00mm and 0.10 at a concentration of 100mg/ml. *Aspergillus niger* showed a zone of inhibition of 0.50mm at 200mg/ml. *Aspergillus flavus* showed a zone of inhibition of 0.70mm at 100mg/ml.

The Ethanol Extract of Lemon peels showed no activity against *Staphylococcus aureus, Aspergillus niger, Aspergillus flavus* and *Fusarum* spp but was active against *Escherichia coli* at a concentration of 200mg/ml with a zone of inhibition of 0.80mm and was not active at 100mg/ml.

The Water Extract showed no activity against *Aspergillus niger, Aspergilllus flavus* and *Fusarum* spp for Orange, showed no activity for *Aspergillus flavus* for Lemon and showed no activity for *Escherichia coli, Aspergillus flavus* and *Fusarum* spp for Lime. The Water Extract of Orange peel (zest) was active against *Escherichia coli* with a zone of inhibition of 0.60mm at a concentration of 200mg/ml. *Staphylococcus aureus* shows the highest zone of inhibition of 0.90mm at a concentration of 200mg/ml. The Water Extract of Lemon peel (zest) was active against *Escherichia coli* with a zone of inhibition of 0.60mm at a concentration of 200mg/ml and 0.50mm at a concentration of 100mg/ml, *Staphylococcus aureus* 0.80mm at 200mg/ml, *Fusarum* 0.40mm at 200mg/ml while *Aspergillus niger* 0.50 at 100mg/ml, showing the highest activity zone of inhibition 1.50mm at 200mg/ml.

The Water Extract of Lime peel (zest) was active against *Staphylococcus aureus* with a zone of inhibition of 0.50mm at a concentration of 200mg/ml and *Aspergillus*

niger having the highest zone of inhibition of 1.10mm at 100mg/ml.

The result showed that the potency of Citrus peel extracts on the organisms that causes diseases and spoilage have different hierarchy of susceptibility among the organisms.

Generally, 200mg/ml concentration of the extract showed a greater zone of inhibition against the isolated bacteria and fungi, this result was in agreement with the report of (Hindi and Chabuck, 2013) which stated that the higher the concentration of antibacterial substance, the higher the zone of inhibition. Israa, (2015) in their studies on the antibacterial activities of plant extracts on *Staphylococcus aureus* and *Escherichia coli* reported that the extracts from alumina had profound activities on the test organisms. Li *et al*. (2006) studied the therapeutic usefulness of an Indian medical plant (*Terminalia chebula* Retz) and some of its isolated compounds, along with their safety evaluation and clearly demonstrated its activities against the similar test organisms just as in the present study. Studies of the activities of *Eucalyptus chapmaniana* leaves extracts on *Escherichia coli* were carried out by Suleiman *et al*. (2013) and concluded that the plant extracts is useful against the diseases caused by the organism. From the results it is clear that *Staphylococcus aureus* is the least susceptible to the different fractions of the extract for the bacterial while for the fungi, *Fusarum* is the least susceptible.

Rahman *et al*. (2011) documented that, during the detection of microbial susceptibility to different plant extracts, the size of inhibition zone to indicate relative antibacterial activity is not adequate. The zone must be affected by the solubility and rate of diffusion in agar medium or its volatilization, and thus the results could be affected. The new trend is the use of natural antimicrobial compounds to extend the shelf life of food (Lucera *et al*., 2012). These natural antimicrobial compounds can be extracted from citrus, whether from peel, seed, pulp or as essential oils and they can be applied to food products as preservatives (Al-Dalahi *et al*., 2019).

CONCLUSION

Citrus extracts have an important role as antimicrobial agents against microorganisms. Pathogenic bacteria and fungi are developing resistance to common conventional antibiotics and this is becoming alarming, therefore it is heartwarming to note that we could find aids in abundantly available local remedy like Citrus peels (Orange, Lemon, and Lime) which are natural, cheap, and safe for the treatment of diseases and to prevent food spoilage.

RECOMMENDATIONS

The antimicrobial agents present in *Citrus* peels (zest) are natural and safe due to increase in antibiotic resistance among bacterial. I hereby recommend that awareness should be created and Nigerians should be enlightened about Citrus peels (zest) and its uses. I also recommend that therapeutics should be developed from *Citrus* zest (peels) by the medical industries for the prevention of food spoilage pathogen of post-harvest crops

REFERENCES

Al-Ani, W.W., Al-Haliem, S.M. and Tawfik, N.O.(2009). Evaluation of the antibacterial Activity of Citrus juices: An invitro study. *Al-Rafidan Dent Journal*, 10 (2): 376-382.

Al-Dalahi, S., Zheng, F. and Al-Farga, A. (2019). Prolonged the shelf-life of different foods using citrus by-products as antimicrobials: A Review, *Annals of Agricultural and Crop Sciences*, 4(1): 1039-1047

Choi M .H.,Kim G.H., Lee H.S.(2002). Effects of ascorbic acid retention on juice color and Pigment Stability in blood Orange (*Citrus sinensis*) Juice during refrigerated Storage. *Food Research International*, 35 (8): 753-759.

Effiom, O. E., Ubi, P.O. and Olushola, T. O. (2019). Growth inhibitory activities of extract from peels of five species of citrus fruit on bacterial isolates obtained from rotten tomatoes fruits. *Journal of Agricultural Science and Botany*, 3(1):12-16.

Hasija, S. Ibrahim, G. and Wadia, A. (2015). Antimicrobial activity of *Citrus sinensis* (orange), *Citrus limentta* (sweet lime) and *Citrus limon* (lemon) peel oil on selected foodborne pathogens, *International Journal of Life Sciences Research*, 3(3):35-39.

Henserson, A. H., Fachiral, E. and Lister, I. N. E. (2018). Antimicrobial activity of lemon (*Citrus limon*) peel extract against *Escherichia coli*. *American Scientific Journal of Engineering, Technology and Science (ASRJETS)*, 39(1):268-273.

Hindi, N.K.K. and Chabuck, Z. A. G. (2013). Antimicrobial activity of different aqueous lemon extracts, *Journal of Applied Pharmaceutical Science*, 3(6):074-078. Doi.10.7324/JAPS.2013.3611.

Irkin, R. and Esmer, O. K. (2015). Novel food packaging systems with natural antimicrobial agents. *Journal of Food Science and Technology*, 52:6095-6111.

Israa, A. J. I. (2015). The Activit 117 Medical plant Extracts with A1203 Nanoparticles on the vitality of Bacteria and their Genomes. *Journal of Chemical and Pharmaceutical Research*, 7 (9): 645-652.

Lucera, A., Costa, C., Conte, A. and Del-Nobile, M. A. (2012). Food application of natural antimicrobial compounds. *Frontiers in Microbiology*, 1-13.

Li, S., Lo, C.Y. and Ho, C.T. (2006). Hydroxylated polymethoxy- flavones and methylated flavonoids in sweet Orange (*Citrus sinensis*) peel. *Journal of Agricultural Food Chemistry*, 54: 4176-4185.

Oikeh, E.I, Oriakh, K. and Omoregie E.S, (2013).Proximate Analysis and phytochemical screening of *Citrus sinensis* Fruit Wastes. *The Bioscientist*: 1(2): 164-170.

Rahman, S., Parvez, A.k., Islam, R. and Khan, M.H. (2011). Antibacterial activity of natural species on multiple drug resistant *Escherichia coli* isolated from drinking water, Bangladesh. *Annals of Clinical Microbiology*, 10:1-10.

Shalu, H., Geeta, I. and Ashok, W. (2015). Antimicrobial activity of *Citrus sinensis* (orange), *Citrus limetta* (sweet lime), *Citrus limon* (lemon) peel oil on selected Food borne pathogens. *International Journal of Life Science Research*3(3): 35-39.

Shuping, D.S.S. and Eloff, J. N. (2017). The use of plant extract to protect plant and food against fungal pathogens: A Review. *African journal of Traditional Complementary and*

*Alternative Medicine*14(4):120-127.

Suleiman, G.M., Mohammed, W.H., Marzoog, T.R., Al-Amiery, A.A.A., Kadhum, A.A.H., Mohammed, A.B. (2013). Green Synthesis, antimicrobial and Cytotoxic effects of silver nanoparticles using *Eucalyptus chapmaniana* leaves extract. *Asian pacific Journal of Tropical Biomedicine*, 3(1): 58-63.

Tajkarimi, M., Ibrahim, S., Cliver, D. (2010). Antimicrobial herb and spice compounds in food. *Food control*, 21: 1187-1199.

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EFFECT OF S O M E COMMONLY USED HERBICIDES ON PLANT SUCCESSION AND SOIL CHEMISTRY IN NIGERIA

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Abstract

The growing concerns of the high concentration of metals in soils as a result of agricultural activities are becoming alarming. This study investigated some physiochemical properties of soil such as pH, conductivity, organic matter, organic carbon, nitrogen, phosphorus, sodium, potassium, calcium, and magnesium. Soil samples were collected from nine different points at three different sites, sprayed with different herbicides. Three control samples were used. The physiochemical characteristics of the various soil samples were as follow; Herbicide A (delsate): pH (5.317± 0.26), conductivity (264 ± 77.67), organic matter (1.20 ± 0.07), organic carbon (0.71 ± 0.04), nitrogen (0.50 ± 0.05), phosphorus (4.04 ± 0.29), sodium (146 ± 3.33), potassium (113.3 ± 4.67), calcium (37.26 ± 10.27), and magnesium (19.7 ±1.33). Herbicide B(force-up): pH (4.97 ± 0.17) conductivity (73.17 ± 22.07) organic matter (1.08 ± 0.15) organic carbon (0.64 ± 0.09) nitrogen (0.53 ± 0.04) phosphorus (4.05 ± 0.59) sodium (111.3 ± 2.67), potassium (100 ± 12), calcium(44.8 ± 5.61), and magnesium (23.3±4.67) Herbicide C(maxiquat), pH (5.3 ± 0.26) conductivity, (150.10 ± 24.53), organic matter (1.22±0.03), organic carbon (0.7±0.02), nitrogen (0.52 ± 0.01), phosphorus (4.75 ± 0.62), sodium (116.7 ± 11.33), potassium (103.3 ± 6.67), calcium (33.22 ± 3.57), and magnesium (14 ± 2.67).These findings reveal that medicinal herbs, are lost as new plant species are replaced in the soil under investigation. The metal level was found to be elevated. This is attributed to the effect of the herbicide on the soil.

Key words: Herbs, Succession, Herbicides, Soil Chemistry, Physiochemical properties.

INTRODUCTION

Herbicides also commonly known as weed killers and are used to kill unwanted plants or weeds. Selective herbicides kill specific targets, while leaving the desired plants relatively unharmed.

Herbicides used to clear waste ground, industrial sites, railways and railway embankments are not selective, but kill all plant material with which they come into contact. Smaller quantities are used in forestry, pasture systems, and management of areas set aside as wildlife habitat. Some plants produce natural herbicides such as the genus juglans (walnuts) or the tree of heaven and such action of natural herbicides and other related chemical interactions is called allelopathy (Peterson, 1967).

Herbicides have widely variable toxicity. In addition to acute toxicity from high exposure levels; there is concern of possible carcinogenicity as well as other long term problems, such as contributing to Parkinson's disease. Some herbicides cause a number by health effects rangi from skin rashes to death. The pathway of attack can arise from intentional or unintentional direct consumption, improper application resulting in herbicide coming into direct contact with people or wildlife, inhalation of aerial

sprays or food consumption prior to the labeled pre- harvest interval. Under extreme conditions, herbicides can also be transported via surface run-off to contaminate distant water sources. Most herbicides decompose via soil microbial decomposition, hydrolysis or photolysis (Reuber, 1981). Herbicides can be grouped by activity, chemical family, mode of action, or type of vegetation controlled. Crop safety for selective Herbicides is the relative absence of damage or stress to the crop (Kolberg, 2002).

Fertilizers, pesticides, herbicides, and some other materials applied to soil often contribute to water and air pollution. Therefore, soil is a key component of environment chemical cycles. Dissolved mineral matter in soil is largely present as ions. Prominent among the cations are H^+, Ca^{2+}, Mg^{2+}, K^+, Na^+ and usually very low levels of Fe^{2+}, Mn^{2+} and Al^{3+} . Cultivation of land and agricultural practices (use of herbicides) can influence both the atmosphere and hydrosphere. Pesticides, particularly insecticides and herbicides are an integral part of modern agricultural production as soil remains the fundamental requirement for agriculture. To humans and most terrestrial organisms, soil is the most important part of the geosphere. In addition to being bound to hydrogen ions in species such as carbonate, anions may be complexed with metal ions such as AIF_2^+. Multivalent cations and anions form ion pairs with each other in soil, solutions (Maynard, 2000).

The effectiveness and safe use of the numerous brand name and generic herbicides registered for forest vegetation management in Florida. (Osiecka *et al*. 2009) requires developing site-specific herbicide prescriptions made with careful consideration of site factors and knowledge of herbicide characteristics. Familiarity with chemical and physical properties of herbicides and their effects on biological systems enables the selection of appropriate herbicide products, application methods, rates, and timing. In addition to understanding herbicide characteristics, it is crucial to understand terminology used on herbicide labels. These labels are legally binding documents for herbicide use. The single most important rule in employing herbicides is to always read and follow the label instructions which are provided on the herbicide container. Herbicide characteristics, such as: Mode of action, Selectivity, Activity, Mobility in soil, Volatility, Persistence in the environment, Toxicity are determined by the active ingredients as well as by the formulations of the herbicide products in which the active ingredients are packaged.

HERBICIDE PRODUCTS OR FORMULATIONS

They may contain one or more active ingredients, adjuvants, and a carrier/solvent. Formulated herbicide products are registered and marketed under various trade names intended for uses specified on the product label. Because of the multitude of existing herbicide formulations, one has to make sure that the selected formulation is appropriate for the desired application. The important determining factors include: application method, target weed species, crop tree species and environmental considerations. Knowing the formulation helps the applicator properly dispense herbicides in an appropriate carrier, add appropriate adjuvants if necessary, and select the appropriate application method. Forestry herbicides are manufactured as liquid or solid formulations (Osiecka and Monigue,2009)

LIQUID FORMULATIONS

They may be used undiluted, as in the case of "ready-to-use (RTU)" herbicide products, mixed with water to form a solution, mixed with oil, or mixed with water plus oil carriers to form an emulsion. In a solution, herbicide product is dissolved in water and is dispersed uniformly in the spray preparation. In an emulsion, the herbicide is suspended in minute globules of oil (micelles) in a predominately water carrier through the use of an emulsifier. The water and oil phases will separate over time without agitation. Invert emulsions are a suspension of water droplets containing herbicide in a predominately oil carrier, are very thick in nature, and provide some drift mitigation because they produce a larger droplet size. Mode of Action is the mechanism by which an active ingredient interferes with the metabolism of a plant in order to kill or suppress it. It largely determines the effectiveness of a herbicide in controlling a particular species, and thus the selectivity of the herbicide product. A herbicide's mode of action includes the nature by which it is absorbed by plants (activity), the pattern of movement within the plant (translocation) and the physiological processes that are affected by the herbicide (site of action).The mode of action is generally determined by the chemical structure and properties of an active ingredient. Herbicide active ingredients that are chemically similar belong to the same "herbicide family" and tend to have the same mode of action.

However, active ingredients belonging to different families may also exhibit the same mode of action. For example, triclopyr and clopyralid which are in the pyridine family, and 2, 4-D within the phenoxy family, are all auxin analogs and mimic this plant hormone physiologically.

Alternating between herbicides with different modes of action helps prevent creating herbicide- resistant weed populations. The most common modes of action among forestry herbicides are:

- Auxin analogs (e.g. 2, 4-D, clopyralid, and triclopyr) mimic the plant growth hormone auxin, resulting in disorganized growth.
- Mitosis inhibitors (e.g. fosamine, pendimethalin) affect cell division, preventing new growth.
- Photosynthesis inhibitors (e.g. hexazinone, atrazine) interfere with photosynthesis.
- Amino acid synthesis inhibitors (e.g. glyphosate, imazapyr, and sulfometuron methyl) prevent the synthesis of amino acids (the building blocks of proteins).
- Lipid biosynthesis inhibitors (e.g. fluazifop-p-butyl, triclopyr and sethoxydim) prevent the synthesis of lipids, crucial elements of cell membranes (Osiecka and Minogue, 2009).

Selectivity refers to a herbicide's capability to affect different categories of plants to different extents due to physiological or morphological differences between species, and is largely dictated by the herbicide active ingredient's mode of action. Ideally, differences between crop plants and weed species are exploited. Selective herbicides have a specific range of susceptible species and often target a particular class of plants, for example broadleaf plants (e.g. fluroxypyr and 2, 4-D) or grasses (e.g., fluazifop-P-butyl and clethodim). Non-selective or broad-spectrum herbicides (like glyphosate) negatively affect most species by impacting metabolic processes common to manyplant groups. Non-selective herbicides therefore are normally not used over-the-top of crop trees, especially during periods of active growth. Selectivity may also be achieved by manipulating application method, rate, and timing. For example, one can control undesirable trees by applying a non-selective herbicide directly to their stems or foliage without damaging crop trees. Also, some

herbicides applied at lower rates may selectively control unwanted vegetation without affecting crop trees. Non-selective herbicides can sometimes be applied with selectivity over-the- top of crop trees during their dormancy (Osiecka and Monigue, 2009)

MATERIALS AND METHODS

MATERIALS

The soil samples were collected from three different sites that were sprayed with chosen herbicides (delsate, force-up, and maxiquat) within Auchi Polytechnic, Auchi, Edo State. In each site, herbicides, three locations were selected at a row and it was tagged A, B, and C respectively.

RESEARCH DESIGN

In each sampling point, a stainless steel auger was used to collect the samples from the sub- surface layer at a depth of 13cm. The collected soil samples were then poured together to form a composite sample. The composite samples were mixed thoroughly and stored in polyethylene bags. The soil samples were then air-dried for one week, passed through 2.0mm sieve, and further pulverized to a fine powder. Three different samples collected 10meters from the sprayed sample served as control samples. The control samples were tagged as CD (Delsate), CF (Force-up), and CM (Maxiquat). Designations used to represent the selected herbicides are DA (Delsate in site A), FB (Force-up in site B), and MC (Maxiquat in site C).

0.5g of the powdered and sieved soil sample was digested in a mixture of perchloric acid (70%), nitric acid (70%) and Hydrofluoric acid (40%). 5ml of $HNO_3/HCLO_4$ mixture (2:1) and 5ml HF were added to the sample in the digestion crucible using an automatic dispenser. The acid-soil mixture was heated for about 30 minutes at 80ºC - 90ºC on a hot plate until there was no more evolution of brown fumes. The temperature was raised to 150ºC for 20minutes until the content was close to dryness. It was then allowed to cool. About 10ml of distilled water was added and the mixture filtered into a 50ml volumetric flask. More distilled water was added to make up to mark. The resulting solution was used for the analysis.

DETERMINATION OF PHYSIOCHEMICAL PARAMETERS

Physiochemical parameters of soil samples such as were determined according to the method of Vernma (2012), while organic matter, exchangeable sodium and potassium were determined according to the method proposed by Udo and ogunwale (1978). Total phosphorus and exchangeable calcium and magnesium were determined according to the method of Head (2012). Carbon was determined according to the method of Jackson and Vansyke (

Table 1: **Physio-chemical properties and trace metals of content soil from Delsate treated site.**

Parameter	Value	Control
Ph	5.317 ± 0.26	2.20 ± 0.03
Conductivity	264 ± 77.67	150 ± 0.66
Organic matter	1.20 ± 0.077	1.00 ±.0.04

Phosphorus	4.04 ± 0.29	2.90 ± 0.01
Sodium	146 ± 3.33	115 ± 0.67
Potassium	113.3 ± 4.67	102 ± 0.67
Calcium	37.26 ± 10.27	30.40 ±0.05
Magnesium	19.67 ± 1.33	17.24 ±0.01

Results displayed are triplicate determination (mean ± SEM)

Table 2: **Physiochemical properties and trace metals content of soil from Force-up treated site.**

Parameter	Value	Control
pH	4.97 ± 0.17	2.42 ± 0.01
Conductivity	73.17 ± 22.07	70.55± 0.01
Organic matter	1.08 ± 0.15	0.78 ± 0.01
Organic carbon	0.64 ± 0.08	0.37 ±0.01
Nitrogen	0.53 ± 0.04	0.38 ±0.01
Phosphorus	4.05 ± 0.59	2.20 ± 0.01
Sodium	111.33 ± 2.67	100 ± 0.17
Potassium	100 ± 0.12	89 ± 0.10
Calcium	44.77 ± 5.61	21.62 ±0.01
Magnesium	23.33 ± 4.67	18.14 ±0.01

Results displayed are triplicate determination (mean ± SEM)

Table 3: **Physiochemical properties and trace metals content of soil from Maxiquat treated site**.

Parameter	Value	Control
pH	5.32 ± 0.26	2.86 ±0.01
Conductivity	150.10 ± 24.53	139 ± 0.50

Organic matter	1.22 ± 0.03	1.09 ± 0.67
Organic carbon	0.70 ± 0.02	0.50 ±0.01
Nitrogen	0.52 ± 0.01	0.28 ± 0.01
Phosphorus	4.75 ± 0.62	2.60 ± 0.01
Sodium	116.67 ± 11.33	112± 0.33
Potassium	103.33 ± 6.67	89 ± 0.83
Calcium	33.22 ± 3.57	28.19 ±0.01
Magnesium	14.00 ± 2.67	10.75 ± 0.01

Results displayed are triplicate determination (mean ± SEM)

DISCUSSION

The metal content of the soil samples taken varies from one herbicide to another as presented in tables 1, 2 and 3. The investigation of the metal content in the tested soil was restricted to the top (0 – 13cm) because detailed studies have shown that surface soils are better indicators of metallic burdens (Nyangababo and Hamya, 1986).The soils pH range from 4.9 – 5.3, indicating that the tested soils are acidic in nature. The textural class of the tested soil is sandy loam with mean sand proportion of 85.25%, clay of 6.28%, and silt of 8.48% respectively. The sandy nature of the soil makes it highly permeable and this will allow large quantities of leachate to pass through the soil thereby polluting the underground water around these areas. (Nyangababo and Hamya, 1986).The physiochemical parameters assayed for phosphorus, conductivity, organic matter, and organic carbon range from 4.04 – 4.75, 73.2 – 264, 1.07 – 1.2, and 0.6 – 0.7 mg/kg 4.67) in the herbicide, delsate when compared to the control (80) and other herbicides force-up and maxiquat. Calcium was highest (44.77 ± 5.61) in the herbicide, respectively. The presence of the organic matter is due to the deposition of carbon containing compounds in the test site. This acts as a reservoir for essential and non-essential mineral elements for plant growth and development (Odoemelam and Ajunwa, 2008). The detected amount sodium, Potassium, calcium and magnesium are (111.3 – 146, 100 - 113.3, 33.32 – 44.8 and 14-23.3) mg/kg respectively. The total nitrogen content was low in all the study area, 0.5. Conductivity was found to have the highest value (264 ± 77.67) with the herbicide, delsate when compared to the control and with other herbicides force-up and maxiquat. Total phosphorus was highest (4.75 ± 0.623) with maxiquat than the control (2.72) and other herbicides force-up and delsate. Sodium was highest (146 ±3.33) with the herbicide, delsate when compared to the control (118) and other herbicides force-up and maxiquat. Potassium was highest (113.3 ± force-up but was equal with the control but higher in the values obtained for the herbicide

CONCLUSION

The level of metals obtained from the selected herbicide is an indication of contamination in the treated soil. However, with years of anthropogenic (man-made herbicides) usage, these metals could rise to a level that could be hazardous to the ecosystem and subsequently affect humans and animals living in the environment through food chains. As a result of the effect of herbicides on soil, the plants are also affected and in most cases, new species of plant emerges through succession.

After critical study has been made, it was discovered that usage of some of the selected herbicides increases the level of metals in the soil to toxicity level and they find their way into the human system through food chains. Hence herbicides should not be sprayed on fields containing medicinal plants, alternative method of weed control should be advocated and vivid knowledge about herbicides before application should be made.

Further studies should be carried out to ascertain the effect of these chemicals on the crops, stimulate teratogenic effect of the chemicals and the effect of the herbicides on animals that feeds on the affected plants.

REFERENCES

Head, K.H (2012) Manual of soil laboratory testing.

Jackson, M. and Van Syke, R. (1982) Procedures in sed. Petrology. 214- 242.

Kolberg, R., Robert, I. and Lori, J. (2002). Effect of steam application on cropland weeds. *Weed technology*. Vol 16(1) 43 – 49

Maynard, E. (2000). Heavy metal contamination in soils of urban highways, Cincinati, Ohio. *Journal of water and soil pollution*.133:293 -

Nyangababo, J. and Hamya, J. W. (1986). The decomposition of lead, cadmium,zinc, and copper from motor traffic on Brachiaria enimi and soil along a major Bombo road in Kampala city.J.Environ Studies (27) 115 – 118.

Osiecka, A., Minogue,P,. Long, A., Nowak , J. and Mossler, M.(2009). Herbicides Registered for Pine Management in Florida – 2008. CIR 1475. Florida Cooperative Extension Service, IFAS, Universityof Florida, Gainesville, Fl. 14pp.,

Osiecka, A. and Monigue, P. J. (2009). School of Forest Resources and Conservation, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

Peterson, G.E. (1967). The discovery and development of 2, 4- D. Agricultural History 41(3):243 – 254.

Reuber, M . D (1981). Carcinogenicity of pocloram. *Journal of Toxicology and Environmental health.* 7(2) 207 – 222.

Udo, E.J. and Ogunwale, E.J.(1978). Laboratory manual for the analysis of soil, plant and water sample. Dept of Agronomy, University of Ibadan.

Vernma,R.M.(2012).Analytical Chemistry, theory and practice, third edition.

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ACUTE AND SUB-ACUTE TOXICTY OF OIL FROM THE PULP OF *Dacroydes edulis*

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ABSTRACT

Acute toxicity studies was carried out on twenty five(25) male Albino rats shared into five groups with the oil concentrations of 100mg/kg, 250mg/kg, 5,000mg/kg, 10,000mg/kg ,and 0mg/kg respectively. Sub-acute studies was performed on twenty five (25) male Albino rats shared into five groups pre-treated with 1000mg/kg, 1500mg/kg, 2000mg/kg, 5000mg/kg, and 0mg/kg of the oil respectively. The study aimed at investigating the toxicological, biochemical, hematological, and histopathological evaluation of Albino rats pre-treated with *D.edulis pulp* oil using petroleum ether as extracting solvent. The scope of this study include; histopathology of liver ,kidney, and heart tissues, evaluation of biochemical parameters in plasma, liver, kidney, and heart tissues, *in vivo*enzymatic antioxidant studies (Thiobarbituric acid reactivesubstance (TBARS), Superoxide dismutase (SOD), and Catalase), electrolyte profile, liver enzyme assays, and lipid profile. The acute toxicity test reveals that the extract was tolerated with no observable adverse effects or mortality up to a dose of 10,000mg/kg body weight. For sub-acute toxicity test, the extract was tolerated by Albino rats with no observable adverse effects. The extract did not compromise the activities of the liver, kidney, and heart tissues as the plasma levels of Gamma glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were not significantly elevated ($P > 0.05$).Plasma cholesterol, triglycerides, High density lipoprotein (HDL) and Low density lipoprotein (LDL) were not elevated .There were no hypernatremia and hyperkalemia. urea, creatinine and electrolytes were not significantly elevated in the plasma ($P > 0.05$ .Antioxidant enzymes were not elevated in the liver, kidney, and heart tissues but TBARS activities were elevated. Haematological parameters were not adversely affected. Total bilirubin, conjugated bilirubin, total protein and albumin were not significantly elevated ($P > 0.05$). Histopathological examination did not show significant biochemical lesions in the kidney and heart tissues but in the liver, there were mild biochemical lesions observed. *D. edulis* pulp oil is safe for domestic and industrial uses.

Key words; pulp oil , toxicological, biochemical, hematological, histopathological,*D.edulis*

INTRODUCTION

African pear (*D. edulis*) belong to the family of Buseraceae. It is known as Safou in French,ube in Igbo, elemi in Yoruba, eben in Efik and orumu in Benin(Kengue and Nyangatou,

1990).There are two varieties of Dacryodes edulis in Nigeria viz; *Devar edulis* and *Devar parvicarpa* (Isaac and Ekpa,2009). The implication of high total cholesterol, Low-Density-Lipoprotein (LDL)-cholesterol, triacylglycerol and low High-Density Lipoprotein (HDL)-cholesterol in the development of cardiovascular disorders such as hypertension, arteriosclerosis, stroke and heart failure can never be over emphasized (Ghasi *et al*., 2000). There has been tremendous increase in the use of functional foods and, or nutraceuticals due to their beneficial effects on human health. Oil extracted from prickly pear seeds oil has been found to exhibit hypoglycemic and hypocholesterolemic effects (Ennouri *et al*., 2007). Changes in the lipoprotein composition of the plasma or serum could be attributed to the type of fat ingested in the diet. Bush pear oil is one of the most important rated versatile vegetable oil. It has been advocated that the Safou oil should take its place in the food industry, the pharmaceutical and the cosmetics industry (soap, perfume, creams) as well as in other branches of industry where raw fat materials are needed.

Years back, it was observed that plasma levels of potassium significantly decreased in the albino rats treated with oils extracted from *Xylopia aethiopica, Piper guineense* and *Tetrapleura tetrapter*a plants. Hypoalaemic effect was evident in albino rat(Nwaichi and Igbinogbaro,2012) Also two of the animals fed with *Tetrapleura tetraptera* diet died in the third week which could be attributable to possible hypernatremia evident in sodium observed levels. These problems mentioned above have influence the toxicological evaluation of albino rat fed on the oil from the pulp of *Dacryodesedulis*.

Till date there exist paucity of information in relation to toxicity of oil from the pulp and its possible pharmacological potentials and there is a growing interest in the possibility of using *D. edulis* oil as a source of edible oil. This has necessitated the need for a reasonable toxicological evaluation of this oil. So this study is therefore designed to evaluate the acute and sub-acute toxicity evaluation of the oil from the pulp of *D. edulis* and to ascertain the toxicity and the nutritional status of the oil.

MATERIALS AND METHODS

Plant

The collection of African pear were obtained from Auchi market, Etsako West, Edo Nigeria, and was identified by Prof M. Idu (Taxonomist),Botany department, University of Benin, Edo State Nigeria a sample each was deposited at the departmental Hebarium. Pre-extraction activities were carried out such as washing, and removal of foreign element.

The ripe fruits were washed thoroughly with distilled water and split open with a sharp knife to remove the seed from the pulp. The pulp samples were dried in a Gallenkamp hot air oven model OV 160 for 48hours. The dried samples were milled with corona traditional grain mill REF 121 (100 µm mesh size

).

Animal

A total of fifty (50) healthy male albino rats of the Wister strain purchased at the animal house of Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, were used for the experiments. These were used for the *in vivo* experiments to meet the research objectives. They were housed in metal cages with plastic base. They were

housed in the animal house of the department of pharmacy, Department of Pharmacology and Toxicology, University of Benin at room temperature. They were fed with commercially available standard pelleted feed and water *ad libitum*. The animal was acclimatized for fourteen days. All animals were handled in strict accordance with the approved guidelines.

Chemicals

Analytical chemicals were used and all were products of British Drug House (BDH), Poole England. Triacylglycerol total cholesterol, LDL-cholesterol and HDL-cholesterol diagnostic Kits were obtained from Randox Laboratories Ltd., Ardmore, U.K.

Experimentation and Design

The animals were divided into two groups of eight rats each in accordance with their weights and housed in separate cages.

Acute studies was carried out on fifty (50) male Albino rats shared into five groups with concentrations of 100mg/kg ,250mg/kg, 5,000mg/kg, 10,000mg/kg ,and 0mg/kg . Sub-acute studies was performed on fifty (50) male Albino rats shared into five groups pre-treated with 1,000mg/kg, 1,500mg/kg, 2,000mg/kg, 5,000mg/kg, and 0mg/kg respectively.

BLOOD SAMPLE COLLECTION AND ANALYSIS

Blood sample were collected by cardiac puncture into heparinized bottles. They were centrifuged at 3,000rpm for 15mins. The plasma obtained was separated into new plain sample bottles and stored frozen until required for biochemical assays, all of which were carried out within a few days. Samples were placed on ice following 2-3min at room temperature.

ASSAY METHODS

Malondialdehyde level was estimated by the method of Buege and Aust (1978).

The method of Cohen *et al.*, (1970) was adopted for catalase. Superoxide dismutase (SOD) activity was determined according to the method of Misra and Fridovich (1989).

Liver enzymes (ALT, AST, ALP, and GGT) were estimated using Selectra Pros machine

Total protein was determined by means of the Biuret method as described by Okutucu *et al.*, (2007).

Plasma albumin was determined by Bromocresol Green Method (Doumas *et al.*, 1987)

Creatinine assay was determined by Jaffes method.

Urea was determined by the method of Berthelot (1967).

Serum total cholesterol, LDL-cholesterol, HDL-cholesterol and Triacylglycerol levels were determined using diagnostic kits from Randox Laboratories, U.K.

Electrolyte**s** concentration were determined using ion selective electrode (ise) method.

Haematological evaluation were determined using an automated Haematolizer.

Histopathological analyses for liver, heart and kidney tissues were determined according to Awvioro, (2002).

Statistical analysis: Mean and standard deviation were calculated and all the data obtained were analyzed statistically using GraphpadPrism version 6.All results represented were Mean+ Standard deviation of five (5) determinations.

RESULTS AND DISCUSSIONS

ACUTE AND SUB-ACUTE TOXICITY RESULT

Acute and sub-acute toxicity the behavioral changes observed during the study include, restlessness, hype-respiration, jerking, over-reactive (hyperactive) and itching. At acute toxicity study, no animal death was observed even at a dose of 10,000mg/kg of the oil . Similarly, at sub-acute toxicity study no death was observed even at a dose of 5,000mg/kg which indicates that the pulp oil is safe orally. *D. edulis* is known to be non- toxic Ajibesin *et al.*, 2002; Obasi and Okolie, 1993) supported these findings when they reported lack of toxic principles in the seed of the plant. However, there is yet to be report on the toxicity study of the plant in experimental animals (Ajibesin, 2011). As treatment periods of 24-48 hours are well suited to the development of rapid toxicity tests and can be used in the early assessment of hepatotoxicity and xenobiotic metabolism (Hewitt *et al.*, 2002; Li *et al.*, 1999a).

Changes in *in vivo* Antioxidant status of pulp oil of *D. edulis.*

Findings reveal the antioxidant changes in liver, heart, and kidney TBARS, SOD, and CATALASE as presented in tables 1, 2 and 3 below.

TABLE 1: ANTIOXIDANT ASSAY RESULTS FOR PULP OIL (LIVER)

GROUP	TBARS MEAN VALUE(µmol/L)	SOD MEAN VALUE(µmol/L)	CAT MEAN VALUE(µmol/L)
CONTROL	5.63 ±2.07[a]	O.019± 0.004 [a]	0.37±0.002 [a]
GROUP 1	14.6 ±3.47 [b]	0.018 ± 0.004 [a]	0.29±0.04 [a]
GROUP 2	14.25±2.58 [c]	0.015 ± 0.005 [a]	0.29±0.02 [a]
GROUP 3	12.93 ± 0.53 [d]	0.016 ± 0.003 [a]	0.287±0.03 [a]
GROUP 4	20. 25±0.90 [e]	0.011 ± 0.001 [a]	0.31± 0.07 [a]

Values are expressed as Mean ± SD (n = 5) Values with the same letters on the same column are not significantly different

TABLE 2: ANTIOXIDANT ASSAY RESULTS FOR PULP OIL (HEART)

GROUP	TBARS MEAN VALUE(µmol/L)	SOD MEAN VALUE(µmol/L)	CAT MEAN VALUE(µmol/L)
CONTROL	1.87± 0.33 [a]	0.066± 0.019 [a]	2.00± 0.45[a]
GROUP 1	1.96± 0.54 [a]	0.052 ±0.015 [a]	0.81 ±0.13 [b]
GROUP 2	2.43 ±0.18 [a]	0.057 ± 0.027 [a]	0.62 ±0.12 [c]
GROUP 3	2.21 ± 0.71 [a]	0.065 ± 0.006 [a]	0.50 ±0.03 [d]
GROUP 4	1.92±0.50 [a]	0.052 ±0.006 [a]	0.58 ±0.08 [e]

Values are expressed as Mean ± SD (n = 5) Values with the same letters on the same column are not significantly different.

TABLE 3: ANTIOXIDANT ASSAY RESULTS FOR PULP OIL (KIDNEY)

GROUP	TBARS MEAN VALUE(µmol/L)	SOD MEAN VALUE(µmol/L)	CAT MEAN VALUE(µmol/L)
CONTROL	12.20±0.71[a]	0.158± 0.034 [a]	0.37±0.04[a]
GROUP 1	12.84 ± 1.96 [a]	0.039± 0.007[a]	0.32 ±0.04[a]
GROUP 2	15.70 ±1.05 [a]	0.052± 0.021[a]	0.35 ±0.02[a]
GROUP 3	18.35±1.48 [b]	0.075 ± 0.015[a]	0.41 ± 0.01[a]
GROUP 4	20.20 ±3.77 [c]	0.065 ±0.024[a]	0.50±0.16[a]

Values are expressed as Mean ± SD (n = 5) .Values with the same letters on the same column are not significantly different.

TBARS activities (liver, heart, and kidney tissues) was observed to increase significantly in all groups (1000mg/kg, 1500mg/kg, 2000mg/kg, and 5000mg/kg) when compared to

control ($P < 0.05$) as shown in tables 1, 2and 3 above.This result could be attributed to the fact that the extract from the pulp undergo lipid peroxidation which could trigger oxidative stress in the tissues. Lipid peroxidation of polyunsaturated fatty acids form products such as malondialdehyde (MDA) which is regarded as a carcinogen (Sehrawat and Sultana, 2006). So the lipid peroxidation report in this study is due to elevated levels of MDA, a marker for lipid peroxidation. The result suggests that pre-treatment of Albino rats with *D. edulis* pulp oil for 28 days significantly increased MDA levels in the respective tissues.

Superoxide dismutase (SOD) and catalase activities (liver, heart, and kidney tissues), it was observed not to increase significantly in all groups (1,000mg/kg, 1,500mg/kg, 2,000mg/kg, and 5,000mg/kg) when compared to control($P > 0.05$).The most important enzymes for removal of ROS in the cell are superoxide dismutase (SOD) and catalase (CAT). SOD catalyzes the dismutation of superoxide anion to hydrogen peroxide and molecular oxygen (Fridovich, 1986), whereas CAT mediates the cleavage of hydrogen peroxide evolving molecular oxygen (Scandalios, 1993). Increased SOD and/or CAT synthesis is correlated with increased tolerance to oxidative stress in bacteria (Ma and Eaton 1992), yeast and plants (Arisi *et al.*, 1998, Mittler, 2002). This clearly indicates that SOD and/or CAT protect eucaryotic metabolic enzymes against damage by ROS. This buttress the finding of this work that *D.edulis* pulp oil does not cause oxidative stress in the liver of male Albino rats of Wistar strain.

TABLE 4: CHANGES IN PLASMA UREA, CREATININE AND ELECTROLYTE

GROUP	UREA(mg/ml)	CREATININE (mg/ml)	Na^+(mEq/L)	K^+ (mEq/L)	HCO_3^-(mmol/L)	Cl^- (mEq/L)
Control	22.00±6.38[a]	0.55±0.06[a]	144.60±4.51[a]	5.08±0.62 [a]	23.40±2.70 [a]	103.80±6.02 [a]
Group 1	28.25±3.77[a]					
Group 2	28.75±4.00[a]	0.63±0.05[a]	143.80±2.95 [a]	5.60±0.93 [a]	23.80±2.77 [a]	107.60±6.19 [a]
Group 3	26.50±4.93[a]	0.63±0.05[a]	143.40±3.51 [a]	4.90±0.69 [a]	21.80±2.05 [a]	
Group 4	25.00±2.83[a]	0.65±0.10[a]	144.00±4.00 [a]	5.42±1.03 [a]	23.00±2.35 [a]	109.00±3.32 [a]
		0.60±0.14[a]	141.60±1.52 [a]	5.42±1.79 [a]	23.60±1.52 [a]	105.60±3.29 [a]
						106.20±5.40 [a]

Values are expressed as Mean ± SD (n = 5).Values with the same letters on the same column are not significantly different

Table 4 shown above indicates changes in plasma urea, creatinine, and electrolyte. The respective changes are as discussed below;

Electrolyte of Na^+, K^+, HCO_3^- and Cl^- shows no significant difference in all treatment groups (i.e.1,000mg/kg, 1,500mg/kg, 2,000mg/kg, and 5,000mg/kg) respectively ($P>0.05$).

Urea concentration reveals no significant difference in all treatment group i.e. (1000mg/kg, 1500mg/kg, 2000mg/kg, 5000mg/kg) respectively when compared to the control (P >0.05).

Creatinine level also shows no significant difference in all treatment group (i.e. 1000mg/kg, 1500mg/kg, 2000mg/kg, 5000mg/kg) respectively at (P >0.05). Creatinine clearance calculated from creatinine concentrations in urine and plasma samples, and the urine flow rate, as well as urea clearance, is used to determine the glomerular filtration rate of the kidneys. Although not commonly done anymore, they remain useful tests for renal function. Thus, plasma concentrations of creatinine and urea could be used as indicators of nephrotoxicity (Saka *et al*., 2012). Low clearance of creatinine or/and urea indicates a diminished impaired ability of the kidneys to filter these waste products from the blood and excrete them in urine. As their clearance values decrease, their blood levels increase. Hence, an abnormally elevated blood creatinine is diagnostic of impaired renal function. This study revealed that pulp oil causes a non-significant increase in both serum urea and creatinine levels. This study suggests that pulp oil could not be ascertained to promote nephrotoxicity or cause impaired renal function as there was no increase in serum urea, creatinine and electrolyte which would have given credence to this claim.

TABLE 5: CHANGES IN PLASMA ALT, AST, ALP AND GGT

GROUP	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	GGT (IU/L)
Control	87.40±6.31 [a]	113.60±8.73 [a]	29 20±7.63 [a]	103.25 ±13.85 [a]
Group 1	74.80±7.63 [a]	112.60±9.66 [a]	25.50±3.57 [a]	97.75 ± 5.12 [a]
Group 2	78.40±9.44 [a]	117.40±18.94 [a]	28.00±1,87 [a]	103.20 ± 12. 92 [a]
Group 3	74.00±13.31 [a]	104.20±10.40 [a]	24.80±5.49 [a]	104.00±22.99 [a]
Group 4	66.40±10.89 [a]	106.60± 9,24 [a]	23.00±1.85 [a]	94.60±6.28 [a]

Values are expressed as Mean ± SD (n = 5) Values with the same letters on the same column are not significantly different.

Table 5 shown above indicates changes in plasma ALT, AST, ALP, and GGT respectively. Their resultant effects are interpreted below.

Gamma glutamyl transferase (**GGT)** shows no significant difference in all treatment groups (i.e. 1000mg/kg, 1500mg/kg, 2000mg/kg, 5000mg/kg) respectively for each of the treatment (P > 0.05). Other liver enzymes i.e. alanine aminotransferase **(ALT),** aspartate aminotransferase **(AST),** alkaline phosphatase **(ALP)** reveal the same trend or pattern. This appears to be the first study to be investigated and documented. The non-elevated plasma levels of these enzymes observed in this study suggests non-impaired liver function as increased activities of plasma ALP is due to increased synthesis in the presence of increasing biliary pressure. Plasma alkaline phosphatase is known to increase when there is biliary obstruction as seen in cholestasis disease of the liver. GGT is a membrane-bound enzyme and an elevated level in plasma is an indicator of cell or tissue damage (Vasudha *et al.*,2006). These statements above justify the claims of the non-toxic nature of the pulp oil of *D. edulis* as marker enzymes were not elevated

ABLE 6: CHANGES IN PLASMA LIPID PROFILE

GROUP	TG/TRIG	CHOL	LDL	HDL
Control	105.00±13.40 [a]	112.00±6.89 [a]	52. 40±9.75 [a]	46.20±4.17 [a]
Group 1	98.50± 19.16[a]	115.50±4.39 [a]	50.00±11.66 [a]	48.40±3.20 [a]
Group 2	90.25±8.47 [a]	123.00±10.95 [a]	52.40±8.73 [a]	47.00±7.29 [a]
Group 3	93.50±16.71 [a]	110.75±10.94 [a]	68.80±15.64 [a]	45.00±5.83 [a]
Group 4	72.40± 10.31 [a]	104.50±6.87 [a]	51.80±12.45 [a]	43.80±6.55 [a]

Values are expressed as Mean ± SD (n = 5) Values with the same letters on the same column are not significantly different.

Findings in table 6 above reveals that TG, CHOL, HDL and LDL reveals the same pattern as they were not significantly different From the control group ($p > 0.05$) . Also supporting the above inferences is the fact that supplementation of the diet with *Dacryodes edulis* fruit pulp oil (DFPO) did not produce any remarkable alterations in the serum total-cholesterol, LDL-cholesterol, HDL-cholesterol,VLDL-cholesterol and triacylglycerol levels of the Albino rat (Alonso *et al*., 2001; Gaiva *et al*., 2003). The implication of high total cholesterol, Low-Density-Lipoprotein (LDL)-cholesterol, triacylglycerol and low High-Density Lipoprotein (HDL)-cholesterol in the development of cardiovascular disorders such as hypertension, arteriosclerosis, stroke and heart failure can never be over emphasized (Ghasi *et al*., 2000

TABLE 7: CHANGES IN PLASMA TOTAL BILIRUBIN, CONJUGATED BILIRUBIN, TOTAL PROTEIN , AND ALBUMIN

GROUP	T.B (mg/dl)	C.B (mg/dl)	T.P (mg/dl)	ALB (mg/dl)
Control	0.66± 0.11[a]	0.34±0.09 [a]	7.56±0.47 [a]	3.72±0.41 [a]
Group 1	0.46±0.09 [a]	0.24±0.09 [a]	7.64±0.46 [a]	3.60± 0.34 [a]
Group 2	0.53±0.13 [a]	0.25±0.13 [a]	7.43±0.67 [a]	3.53± 0.34 [a]
Group 3	0.52±0.13 [a]	0.28±0.08 [a]	8.22±0.38 [a]	3.74±0.34 [a]
Group 4	0.64±0.21 [a]	0.32±0.11 [a]	7.56±0.56 [a]	3.88±0.38 [a]

Values are expressed as Mean ± SD (n = 5) Values with the same letters on the same column are not significantly different.

The major plasma /serum proteins include albumin, globulin, conjugated proteins such as glycoprotein and mucoprotein, protein polysaccharide compounds, lipoproteins and fibrinogen in the plasma (Chawla, 1999). Albumin contributes to the amino acid pool and plasma colloid osmotic pressure counteracting the effects of capillary blood pressure which tends to force water into tissue spaces. Albumin also helps in transport of a wide range of naturally occurring substances such as bilirubin, fatty acids, urate, calcium, magnesium and drugs.

Table 7 above shows changes in plasma total bilirubin, conjugated bilirubin, total protein, and albumin respectively. The detailed effects of each parameter are as discussed below.

Total protein (TP) levels: The serum total protein value was not significant in all the

treatment groups when compared with the control (P > 0.05) as shown above. Generally, this study inferred that *D. edulis* pulp oil did not impair protein metabolism.

Albumin (Alb): The serum level of albumin fractions decreased non-significantly in treatment group 1 and 2 (1000mg/kg and 1500mg/kg) respectively while it increases non-significantly in group 3 and 4 treatment (2000mg/kg and 5000mg/kg) respectively (P > 0.05). This implies that albumin metabolism by the liver was not affected by the administration of pulp oil of *D. edulis* at lower concentrations.

In this study, Total bilirubin values decreased non-significantly for Albino rats in all the treatment groups: (1000mg/kg, 1500mg/kg, 2000mg/kg and 5000mg/kg) respectively when compared with the control(P > 0.05).

Conjugated Bilirubin**:** Total (unconjugated) bilirubin is not water soluble, as such; albumin binding aids its transportation across the watery plasma. Direct (conjugated) bilirubin is water soluble and when present in the blood can be filtered through glomerulus appearing in the urine. Secretion of conjugated bilirubin in bile across the biliary canalicular membrane is a rate limiting process and is sensitive to liver damage. Evident from this study reveal that there was no significant difference in the bilirubin (total and conjugated) concentrations (p > 0.05) between the control and the treated rat groups. This implied that pulp oil of *D. edulis* had little or no effects on the excretion of bilirubin.

TABLE 8: CHANGES IN RED CELL PARAMETERS

GROUP	Hb(g/dl)	RBC(/µl)	MCV(Fl)	MCH(pg)	MCHC(g/dl)	HCT%	RDW(Fl)
Control	15.22± 1.70 [a]	66.00 ±7.10 [a]	64.40± 2.70 [a]	21.20± 0.70 [a]	33.04± 1.80 [a]	42.30± 4.50 [a]	41.02±3 .00 [a]
Group1	16.44± 0.50 [a]	73.00 ±2.00 [a]	67.50± 2.40 [a]	22.30± 0.60 [a]	33.04± 1.20 [a]	49.34± 1.70 [a]	48.20±2 .70 [b]
Group2	15.60± 0.60 [a]	70.00 ±2.00 [a]	68.32± 1.90 [a]	22.10± 0.70 [a]	32.34± 0.70 [a]	48.14± 1.70 [a]	48.34±1 .20 [c]
Group3	16.71± 0.80 [a]	73.00 ±3.70 [a]	69.48± 3.80 [a]	22.70± 1.20 [a]	32.68± 1.20 [a]	50.46± 1.90 [a]	50.10±1 .90 [d]
Group4	15.44± 0.90 [a]	70.00±3.40 [a]	69.06± 3.70 [a]	22.06± 0.90 [a]	32.00± 1.70 [a]	54.12± 5.60 [a]	54.12±5 .60 [e]

Values are expressed as Mean ± SD (n =5) Values with the same letters on the same column are not significantly different.

TABLE 9: CHANGES IN WHITE CELL PARAMETERS

	TOTAL WBC (/µl) $x10^3$	**LY (/µl)** $x10^3$	**MO(/µl)** $x10^3$	**GR(/µl)** $x10^3$	**LY%**	**MO%**	**GR%**	**PLT(/µl)** $x10^3$	**PCT%**	**MPV (Fl)**	**PDW(Fl)**
Control	6.0± 1.2 [a]	3.8 ±8.2 [a]	0.50± 1.9 [a]	1.6± 5.7 [a]	63.48± 7.7	7.96± 1.6	28.60± 8.2	610.0± 1.7 [a]	0.424± 0.135	6.82± 0.6 [a]	9.62 ±1.3 [a]
Group 1	9.6± 3.1 [a]	6.8± 2.1 [a]	0.76± 3.8 [a]	2.0± 7.9 [a]	71.10± 4.2	7.70± 1.6	21.20± 3.3	650.0± 2.0 [a]	0.438±0.125	6.80± 0.2 [a]	10.40±0.6 [a]
Group 2	5.2± 3.0 [a]	5.2± 2.7 [a]	0.54± 2.8 [a]	1.9± 5.6 [a]	67.44± 5.3	7.32± 1.2	25.22± 6.1	680.0± 1.6 [a]	0.460± 0.104	6.78± 0.4 [a]	10.14±0.7 [a]
Group 3	6.0± 2.3 [a]	3.8± 1.6 [a]	0.50± 2.6 [a]	1.7± 5.1 [a]	62.78± 6.1	7.94± 1.1	29.28± 6.1	590.0± 1.1 [a]	0.417±0.1	7.08± 0.3 [a]	10.26± 0.6 [a]
Group 4	9.8± 3.8 [a]	6.4± 2.6 [a]	0.90± 4.6 [a]	2.6± 9.2 [a]	64.50± 5.9	8.58± 2.1	26.92± 4.8	692.4± 1.1 [a]	0.547±0.063	7.86± 0.8 [a]	13.86±3.0 [a]

Values are expressed as Mean ± SD (n =5) Values with the same letters on the same column are not significantly different.

Tables 8 and 9 shows changes in red cell and white cell parameters. Detailed specific parameters are as discussed below.

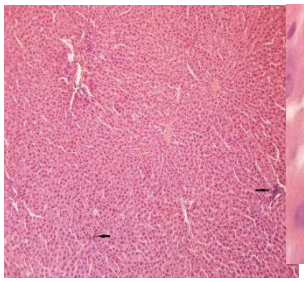
Assessment of haematological parameters can be used to explain haematological functions of a chemical compound or plant extracts in an organism (Yakubu *et al.,* 2007). Generally, during the 28 days of the experimental study, the level of red blood cell parameters **RBC,Hgb,HCT,MCV,MCH, MCHC** for pulp oil did not change significantly among the normal rat model in the control group except for **RDW** which was significantly different at all group of treatment. As shown in table 8 above, at administration of the extract at dose level (1,000mg/kg, 1,500mg/kg, 2,000mg/kg, 5,000mg/kg) respectively. This is an indication that *Dacryodes edulis* pulp oil does not contain factors that are deleterious to normal blood formation. The red blood count (RBC), packed cell volume PCV), platelets and haemoglobin (Hb) were observed not to be significantly different from the control (P > 0.05). This finding in table 8 above reflect that the pulp oil possess erythropoietin stimulating activity that can improve hematopoietic activity of the cell and hence can play a vital role in management and or prevention of anaemia. This appears to be the first study to investigate and documented changes in red cell parameters of male albino rat fed on *D. edulis* pulp oil.

Administration of *D.edulis* petroleum ether pulp oil extracts did not induce changes in total and differential WBC count of the extracts at the different dose level of (1,000mg/kg, 1,500mg/kg, 2,000mg/kg, 5,000mg/kg) it cause no significant difference in the levels of **WBC, LY, MO, GR, PLT, MPV, PDW** when compared to the control group (P >0.05) as shown in table 9 above The introduction of foreign body by a toxicant increased the WBC values. If WBC are elevated it is due to the stimulation of immune defence system (Kashinath, 1990). Similarly literatures have shown that increased concentration of antigen in the body results in high values of WBC (Schalm *et al.,* 1975). This findings shows that the pulp oil does not stimulate changes in total and differential WBC count. However, this

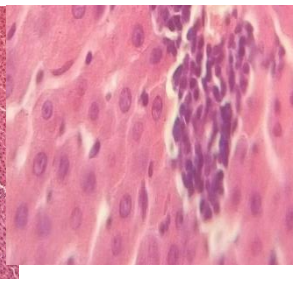
appears to be the first study to investigate and documented changes in white cell parameters of male albino rat fed on *D. edulis* pulp oil.

HISTOPATHOLOGICAL EXAMINATION

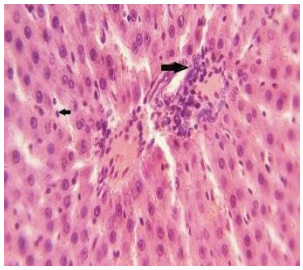
PULP OIL (LIVER) CONTROL **PULP OIL (LIVER) TEST**



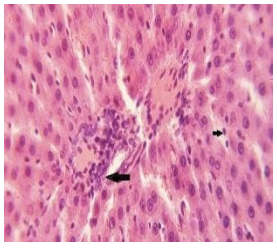
(Plate.1A) Liver showing mild periportal inflammation with lymphocytes aggregating aroundthe portal tracts (arrows) *(Haematoxylin and eosin X 100).*



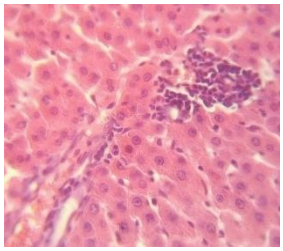
(Plate. 2) Liver showing mild periportal and lobular inflammation *(Haematoxylin and eosin X 400).*



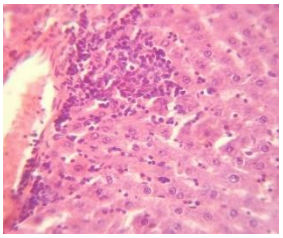
(Plate 3)Liver showing mild periportal (big arrow) and lobular (small arrow) inflammation *(Haematoxylin and eosin X 400).*



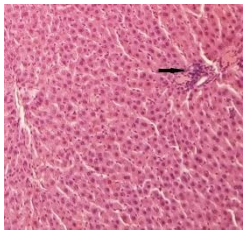
(Plate. 1B) Liver showing periportal (big arrow) and lobular (small arrow) lymphocytic inflammation *(Haematoxylin and eosin X 100).*



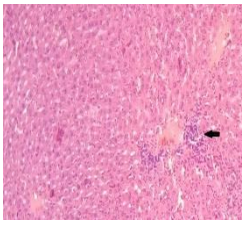
(Plate. 5). Liver showing mild periportal and lobular inflammation *(Haematoxylin and eosin X 100).*



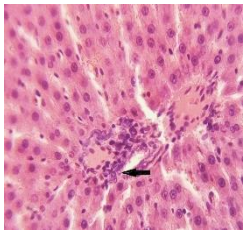
(Plate 6)Liver showing relatively more severe periportal and lobular inflammation *(Haematoxylin and eosin X 100).*



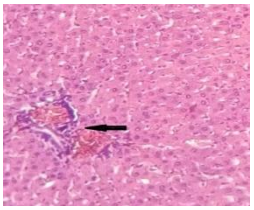
(Plate 7) Liver with mild periportal *E X 100).*lobular inflammation



(Plate 8) (Plate 9) Liver with mild periportal and periportal and lobular inflammation *(H & E X 100).*

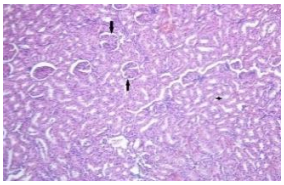


(Plate 10) Liver with mild and lobular inflammation *(H & E X 100).*

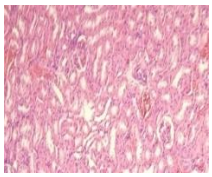


Liver with mild periporinflammation *(H & E X 100).*

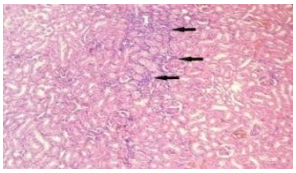
PULP OIL (KIDNEY) CONTROL **PULP OIL (KIDNEY) TESTPULP OIL (KIDNEY) TEST**



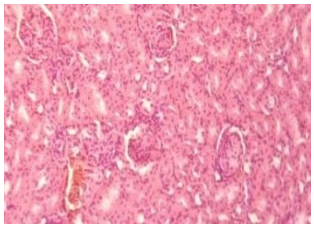
(Plate 2A)Normal kidney with normal glomeruli (arrows) and tubules (star) *(Haematoxylin and eosin X 100).*



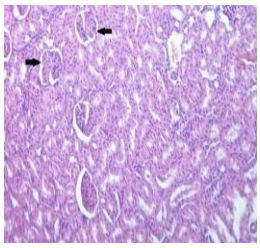
(Plate 11) Normal kidney, but the slide was labeled "H" (*H and EX 100).*



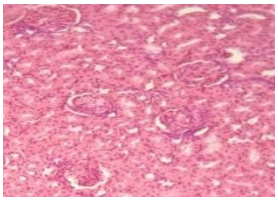
(Plate 12)Kidney showingfocal tubulointerstitial nephritis, withlymphocytes focally infiltrating the renal tubules and interstitium (arrows). Other areas of the field show normal kidney architecture *(Haematoxyli and eosin X 100).*



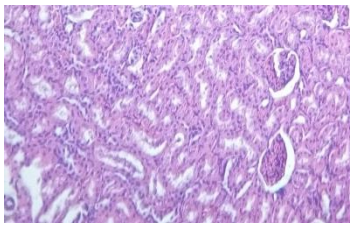
(Plate 2B) Normal kidney with normal glomeruli and tubules *(Haematoxylin and eosin X 100).*



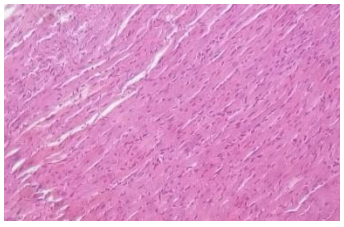
(Plate 13) Normal kidney with normal glomeruli (arrows) on a background of normal renal tubules *(Haematoxylin and eosin X 100).*



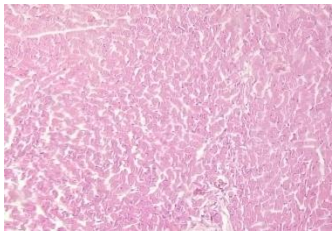
(Plate 14) Normal kidney with normal glomeruli on a background of normal renal tubules *(Haematoxylin and eosin X 100).*



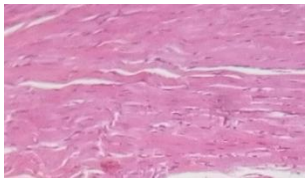
(Plate 15)Normal kidney: the left half of the field shows mild and focal tubulointerstitial nephritis with lymphocytes, while the right half contains normal glomeruli and tubules *(Haematoxylin and eosin X 100).***PULP OIL (HEART) CONTROL PULP OIL (HEART) TEST**



(Plate. 3A) Normal heart. Normal myocardial fibres are seen here in longitudinal section *(Haematoxylin and eosin X 100).*

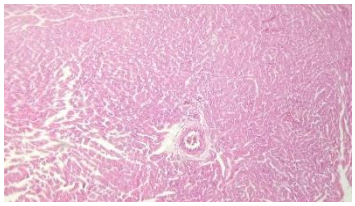


(Plate 16) Normal heart with the myocardial fibres displayed in transverse section with peripherally placed small nuclei *(Haematoxylin*

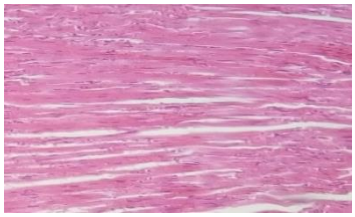


(Plate 17) Normal Heart. Normal myocardial fibres are seen here in longitudinal section *(Haematoxylin and*

and eosin X 400). *eosin X 100).*



(Plate 3B) Normal heart muscle fibres seen in transverse and oblique session *(Haematoxylin and eosin X 100.)*



(Plate 18) Normal heart. Normal myocardial fibres are seen here in longitudinal section *(Haematoxylin and eosin X 100).*

NOTE: Plates 1A,1B, 2A,2B,3A, and 3B represents controls forliver, kidney, and hearttissues while other plates are for test tissues(i.e. liver,kidney, andheart.) respectively.

The kidney and heart did not manifest serious abnormal histopathological changes as shown in plates 2A and 3A (control) and plates 2B and 3B (test) above.The observedcellular degeneration, hepatocellular necrosis, tubular epithelial cell necrosis and diffuse tubular lumina observed in the liver tissue of Albino rats fed with *D. edulis* pulp oil, cannot be verified from previous studies as it has not been previously documented. Also, the present result of biochemical alteration was not insured by histopatological examination in the liver of the fed Albino rats which reveal degenerative and necrotic changes which were not severe enough to alter the liver function as manifested in the activity of the liver enzymes which show normal liver function.

CONCLUSION

At acute toxicity study, no animal death was observed even at a dose of 10,000mg/kg of the pulp oil administered. Similarly, at sub-acute toxicity study no death was equally observed even at a dose of 5,000mg/kg which indicates that pulp oil is safe orally. *D. edulis* is known to be non- toxic Ajibesin *et al.,* (2002).Obasi and Okolie (1993) supported these findings when they reported lack of toxic principles in the pulp of the plant. These findings are further strengthened by this study which show that petroleum ether extract of pulp oil of *D. edulis* did not cause impair liver function .Heart and kidney tissues were not compromised. The extract did not cause hypernatremia, hyperkalemia; it did not elevate plasma lipid cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides. Haematological parameters are not adversely affected. *D edulis* pulp oil is safe for domestic and industrial uses

REFERENCES

Ajibensin, K.K., Ekpo B.A and Bala N.D., (2002) Antimicrobial activity of the leaves of combretum micranthum and C. Racemosum. *GlobaJ. Med.Sci.* 1:13-17.

Ajibesin, K.K., (2011) *Dacryodes edulis* (G. Don) H.J. Lam: A review on its medicinal, phytochemical and economical properties. *Res. J. Med. Plant*, 5: 32-41

Awvioro , O .G (2002). Histochemistry and Tissue Pathology.*Principles Techniques* 9:154-162.

*Arisi A.C.M, Cornic G, Jouanin L, and Foyer C.H. (*1998*) Over-expression of iron superoxide dismutase in transformed poplar modifies the regulation of photosynthesis at low CO_2 partial*

pressures or following exposure to the prooxidant herbicide methyl viologen. Plant Physiol117:565–574.

Buege J, and Aust, S .D. (1978).MicrosomalLipidPeroxidation.In :Methods in Enzymology.Colowick, S. P,Kaplan, N.O. (eds). Academic Press, New York. pp. 302-311

Chawla R (1999) .Serum total protein and albumin-globulin ratio. In thousand Interpretations) *India .Pract.Clin.Biochem.*pp.106-118.

Cohen, G., Dembiec, D. and Marcus, J. (1970) Measurement of catalase activity in tissues extracts. *Anal. Biochem* . 34 : 30-38.

Doumas B.T., *et al*.(1987) A candidate reference method for determination of total protein in serum. I. Development and validation. *Clin Chem*; 27: 1642-1650

Ennouri, M., H. Fetoui, M. Hammami, E. Bourret, A. Attia and N. Zeghal, (2007). Effects of diet supplementation with cactus pear seeds and oil on serum and liver lipid parameters in rats. *Food Chem*. 101: 248-253.

*Fridovich I. (*1986). *Biological effects of the superoxide radical.Arch Biochem Biophys24:1–11.*

Gaiva,M.H., R.C. Couto, L.M. Oyama, G.E.C. Couto V.L.F.Silveira, E.B. Ribeiro and C.M. Nascimento (2003). Diets Rich in Polyunsaturated Fatty Acids:

Ghasi S, Nwobodo E and Ofili J O (2000) Hypocholesterolemic effects of crude extract of leaf of *Moringa oleifera* Lam in high fat diet fed wistar rats. *Journal of Ethnopharmacology* 69(1):21-2

Hewitt, N. J., Lloyd, S., Hayden, M., Butler, R., Sakai,Y., Springer, R., Facket t, A., Li, A. P. (2002).Correlation between troglitazone cytotoxicity and drug metabolic enzyme activities in cryo-preserved human hepatocytes. *Chem. Biol. Interact* . 142(1–2):73–82.

Kashinath, K.T., 1990. Hypolipidemic effect of disulphide in rats fed high lipids diet and /or ethanol. Ph. D. Thesis, University of Bangalore

Kengue J.C. and Nyangatou J. (1990).Problem of preserving the germination power of the seed Africa pear (*Darcyodes edulis*) *Fruit* 45 (4): 409-412

Ma M, Eaton JW. 1992. Multicellular oxidant defense in unicellular organisms. *Proc Natl Acad Sci USA* 89: 7924–7928.

Misra, H. P.,and Fridovich, I (1989). The role of superoxide anion in the autooxidation of epinephrine and simple assay for superoxide dismutase. *J. Biol. Chem*. 247, 3170-3175

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci* 7**:** 405– 410

Nwaichi E. O. and, Igbinobaro O. (2012) "Effects of Some Selected Spices on Some Biochemical Profile of Wister Albino Rats", *American Journal of Environmental Engineering*, Vol. 2 No. 1, pp. 8-11.

Obasi N.B. and Okolie N.P.(1993). Nutritional constituents of the seeds of Africa pear *Dacryodes edulis.Food Chem*. 46:297-299

Okutucu, B., Dincer, A., Habib, O. and Zihnioglu, F. (2007) Comparison of five methods for determination of total plasma protein concentration, *J. Biochem. Bioph. Meth*., 70, 709–711,

Pereira MD, Eleutherio ECA, Panek AD. 2001. Acquisition of tolerance against

oxidative damage in *Saccharomyces cerevisiae. BMC Microbiology* 1:11–20.

Saka, W.,Akigbe, R.E.,Popoola, O.T, and Oyekunle,O.S. (2012) Changes in Serum Electrolytes, Urea, and Creatinine in *Aloe Vera*-treated Rats *J Young Pharm*4(2); 78- 81

Scandalios J.G. 1993. Oxygen stress and superoxide dismutases. Plant Physiol 101:7–12.

Schalm, O. W., Jain, N.C and Carrol, E.J. (1975). Veterinary Haematology, 3rd Edn., Lea and Febiger, Philadelphia, pp. 197 - 199

Sehrawat A, Sultana S. (2006) Tamarix gallica ameliorates thioacetamide-induced hepatic oxidative stress and hyperproliferative response in Wistar rats. *J Enzyme Inhib Med Chem*. 21(2):215-23.

Sims, G.K. (2006) Using the Berthelot Method for Nitrite and Nitrate Analysis. Soil Sci. Soc. *Am. J.* 70 (3): 1038.

Vasudha K .C, Nirmal Kumar A, Venkatesh T (2006). Studies on the age dependent changes in serum adenosine deaminase activity and its changes in hepatitis. *Indian J Clin Biochem*,; 21: 116 -120.

Yakubu M.T., Akanji MA., and Oladiji AT., (2007). Haematological parameters of male albino rats following chronic administration of aqueous extracts of fedogia agrestis stem. *Pharmacognosy magazine* Vol. 3;34

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NUTRIENT AND NON NUTRIENT CONTENT OF SOME COMMERCIAL VITAMIN C IN NIGERIA

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Abstract

The chemical analysis of some commercially sold vitamin C tablet in Nigeria was investigated. The results showed that sweeteners were in the range of 9.65 – 10.25%, preservative in the samples were found to contain sodium benzoate, sodium metabisulfite, Sorbic acid and sodium propionate with same concentration (25mg/100g). The dye content were found to contain 1.60mg/ml to 4.78mg/ml. Vitamin C content were observed to contain 120.20 -128.59mg/100g of the sample. Sweeteners, preservative s and artificial dye in this study have been implicated to cause some health abnormalities both in infants and adults. The vitamin C content of the various tablets was of excellent recommendable dietary intake RDI value. The additives from this study should be considered by the pharmaceutical/food industries owing to their risk factor in such products. Since vitamin C is essential in our metabolic function which is more or less important in our daily nutrition, natural sources of vitamin C should be inculcated in our dietary lifestyle.

Keyword ; Mineral content, proximate Analysis, Artocarpus aitilis, Phytochemicals

INTRODUCTION

Vitamin C (ascorbic acid) is an anti-oxidant that is essential for human nutrition. It belongs to the water soluble class of vitamins. Humans are one of the few species who lack the enzymes to convert glucose to vitamin C (Groff *et al.* 1995). The importance of vitamin C was first discovered in 1747 during the 16th Century, numerous sea Voyagers died due to the disease known as scurvy. Vitamin C deficiency can lead to scurvy which is characterized by abnormalities in the bones and teeth. James Lind found out that men suffering from scurvy were cured when given oranges and lemons and he published his findings in the treatise of the scurvy in 1753. These findings were not widely accepted by the rest of the world and scurvy continued to lead to wide spread death throughout the 19th century (Jacob *et al.* 1999). The Vitamin is an important aid in the absorption and conversion of iron to it's storage form. It has been proposed by some researchers to have pharmacological benefits in preventing cancer, infections and common cold. The role of vitamin C in preventing cancer is controversial, but has been studied for cancer of oral cavity, uterus, oesophagus, bladder and pancreas.

In Nigeria, the Vitamin C that is commercially sold has been implicated to low vitamin C content. Also most of the manufacturers have comprised the standard as against National Agency for good and Drugs which includes the presence of banned dye, presence of detrimental sweeteners and the use of deadly preservatives during production. As a result of ill standard practices, most of the prescribed vitamin C to patients has resulted to even more severe illness. These reasons have necessitated this research work. Therefore, the aim of this research is to evaluate the content of vitamin C, determination of preservatives, dye and sweeteners used in

some commercially sold Vitamin C tablet in Edo State, Nigeria.

SOURCES OF VITAMIN C

A wide variety of food exists that contains vitamin C. It is widely known by the general public today that the sources of vitamin C are citrus fruits. Fruits with high vitamin C content are not limited to oranges, but also include lemons, peaches, and strawberries, bananas, and grapes fruits. A wide variety of other food also contain sufficient quantities of vitamin C like: cabbage, broccoli, caulis flower, leaf lettuce, tomatoes, potato and beans also have relatively high (7mg/100g to 163mg/100g) vitamin C content (Jacob *et al*. 1999

ABSORPTION AND BIOAVAILABILITY

Absorption of vitamin C is greater when several individual doses of vitamin C, in quantities less than one gram are taken throughout the day rather than one mega dose (Jacob *et al.* 1999). At the intestine and cells ascorbic acid is oxidized to DHAA, which is more quickly transported across the cell membrane. Vitamin C can be impaired by a number of factors. A single large dose saturates the enzymes kinetics for Vitamin C leading to excess ascorbic acid in the intestinal lumen, which causes numerous gastro intestinal problems. Furthermore, the bioavailability of synthetic and natural form of the Vitamin differ very little despite the claims made by manufacturers (Groff *et al.* 1995; Jacob *et al.* 1990)

TRANSPORT

Active transport is the main mechanism of vitamin C distribution within the body. Simple diffusion may occur in the mouth and stomach but accounts for only a very small percentage of uptake (Groff *et al* 1995). Sodium independent transport system shuttle vitamin C across the basolateral membrane of the intestinal cells. In the plasma absorbed ascorbic acid and DHAA can either be transported freely or be bound to albumin, ascorbate can also move into the body cells and tissues (Groff *et al.* 1995) as previously mentioned DHAA is the primary form of vitamin C that crosses cellular membranes.

STORAGE

Vitamin is stored throughout body tissues and blood. Ascorbic acid content of blood component, fluid, and tissues varies widely on an individual basis. Tissues concentration exceeds those found in the plasma by three to ten times. Energy driven transport pumps are responsible for the higher concentration of vitamin C versus the plasma. Both tissue and plasma levels of vitamin C is correlated to intake up to 90mg/day (Groff *et al.* 1995). Vitamin C content of cardiac tissue is between 28 and 85ml/100g wet weight, while that in skeletal muscle is approximately 17ml/100g wet weight, (Howald *et al*. 1975). Other tissues with intermediate level of vitamin C include the kidney, brain, liver, lungs and thyroid. The water-soluble properties of vitamin C prevent it from being stored in the adipose tissue of the body.

EXCRETION

The kidney plays a major role in vitamin C excretion and retention. DHAA and ascorbic acid can be reabsorbed by the kidney tubules as long as body pool levels are equal to or less than 1500mg. The average half-life of ascorbic acid is believed to be between 16 and 20 days (Jacob *et al.* 1999). Its half-life is inversely related to intake. The water-soluble properties of vitamin C lead to urinary excretion of the vitamin.

CURRENT DIETARY REFERENCE INTAKE (DRI)

The recommended daily allowance (RDA) has been replaced by a dietary reference intake (DRI) for vitamin C in the year 2000. In 1989 the recommended daily allowance was established at 60mg for adults. This level was believed to be sufficient enough to maintain body pool levels at 1500mg and does not differ from that established in 1980. A recommended daily allowance for smokers was established because smokers have a higher turnover rate of vitamin C versus non-smoker (Groff *et al.* 1995). The DRI does not differentiate the need between smokers and non-smokers. DRI for vitamin C have been established at 90mg for men and 75mg for women (national Academic of Science et al, 2000).

TOXICITY

The saturable kinetics of vitamin C makes toxicity more likely when multiple large doses (~lg) are consumed throughout a day versus one single dose. A common symptom of unabsorbed vitamin C left in the gastro intestinal tract is osmotic diarrhea (Groff *et al*. 1995). Vitamin C can be transformed in the body to oxalate, which is a common constituent of kidney stones. Doses up to 10g have shown to be associated with higher prevalence of oxalate excretion, but the level does not fall outside of the normal range. As a precaution, people who are prone to kidney stones may want to avoid large doses (10 times the DRI or greater) of the vitamin (Groff *et al*. 1995). People who lack the control to regulate iron uptake should also avoid large doses of the vitamin. As stated earlier vitamin C enhances iron absorption which can lead to toxicity of iron in some people. Furthermore, excess ascorbate in the urine and feaces can falsify laboratory tests such as glucose in urine and fecal occult blood test.

PHYSIOLOGICAL ROLE

Vitamin C has been studied for many years. It participates in numerous biochemical reactions, suggesting that vitamin C is important for everybody process from bone formation to scar tissue repair (Groff *et al*. 1995). The only established role of the vitamin appears to be incurring and preventing scurvy. Vitamin C is the major water-soluble antioxidant within the body. The vitamin readily donates electron to break the chain reaction of lipid peroxidation. The water-soluble properties of vitamin C allow for the quenching of free radicals before they reach the cellular membrane. Tocopherol and glutathione also rely on ascorbic acid for regeneration back to their active isoforms. The relationship between ascorbic acid and glutathione is unique. Vitamin C reduced glutathione back to the active form. Once reduced, glutathione will regenerate vitamin C from its DHAA or oxidized state. A well-known

function of ascorbic acid is the role it plays in hydroxylation reactions that are essential for the formation of collagen. Vitamin C is important in collagen formation as it allows for a tight cross-linking of the triple helix, thereby resulting in stabilization of the peptide. Evidence also suggests that ascorbic acid may be involved in collagen gene expression. Carnitine synthesis prefers to use vitamin C as the reducing agent. Carnitine facilitates the beta-oxidation of fat, through its role transporting long chain fatty acids from the cytoplasm into the mitochondrial matrix of cardiac and skeletal muscle. Vitamin C is directly involved in the enzyme activity of two copper dependent mono-oxygenases which are important in the formation and conversion of iron to its storage form. The protection of neural and endothelial tissue, along with effects on cellular tone can also be attributed to vitamin C. (Groff *et al.* 1995; Jacob *et al.* 1999). Vitamin C has been proposed by some to have pharmacological benefits in preventing cancer, infections, and the common cold. However, these benefits have yet to be reported in the scientific literature. The role of vitamin C in preventing cancer is controversial, but has been studied for cancers of the oral cavity, uterus, oesophagus, bladder, and pancreas. The research is at best equivocal and more studies are needed to further address the role of Vitamin C in preventing cancer.

ANTIOXIDANT

Ascorbic acid is well known for its antioxidant activity, acting as a reducing agent to reverse oxidation in liquids. When there are more free radicals (reactive oxygen species ROS) in the human body than antioxidants, the condition is called oxidative stress (Mayne, 2003) and has an impact on cardiovascular disease, hypertension, chronic inflammatory diseases, diabetes (Tak, 2000; Bruch, 2002; Schorah, 1996; Jacques, 1995) as well as on critically ill patients and individuals with severe burns (Mayne, 2003). Individuals experiencing oxidative stress have ascorbate blood levels lower than 45 μmol/L, compared to healthy individuals who range between 61.4-80 μmol/L (Fortherby *et al.* 2000). It is not yet certain whether vitamin C and antioxidants in general prevent oxidative stress-related disease and promote health. Clinical studies regarding the effects of vitamin C supplementation on lipoproteins and cholesterol have found that vitamin C supplementation does not improve disease markers in the blood. (fortherby *et al*. 2000; Simona, *et al*. 2007) vitamin C may contribute to decreased risk of cardiovascular disease and strokes through a small reduction in systolic blood pressure (Mayer-Daris *et al*. 1997) and was also found to both increase ascorbic acid levels and reduce levels of resisting serum, (Bjelakovi *et al*. 2007) another likely determinant of oxidative stress and cardiovascular risk. However, so far there is no consensus that vitamin C intake has an impact on cardiovascular risks in general, and an array of studies found negative results (Satoh and Sakagami, 1997) meta-analysis of a large number of studies on antioxidants including vitamin C and mortality (Muhulhofer *et al*. 2004).

DAILY REQUIREMENTS

The North American dietary reference intake recommends 90 milligrams per day and not more than 2 grams (2,000 milligrams) per day (Milton, 2003). Other related species sharing the same

inability to produce Vitamin C and requiring exogenous vitamin C consume 20 to 80 times this reference intake (Knowledge of Health, 2004). There is continuing debate with the scientific community over the best dose schedule (the amount and frequency of intake) of vitamin C for maintaining optimal health in humans. A balance diet without supplementation usually contains enough vitamin C to prevent scurvy in an average health adult. A 1999 review suggested a dose of 90-100mg vitamin C daily is required to optimally protect against these diseases, in contrast to the lower 45mg daily required to prevent scurvy.

High doses (thousands of milligrams) may result in diarrhea in healthy adults, as a result of the osmotic water-retaining effect of the unabsorbed protein in the gastro intestinal tract (similar to cathartic osmotic laxatives). Proponents of orthomolecular machine claim the onset of diarrhea to be an indication of where the body's true vitamin C requirement lies, though this has not been chemically verified.

CHANCE OF OVERDOSE

Vitamin C is water soluble, with dietary excesses not absorbed and excesses in the blood rapidly excreted in the urine. It exhibits remarkably low toxicity. The LD50 (the does that will kill 50% of a population) in rats is generally accepted to be 11.9g per kilogram of body weight when given by forced gavage (orally). The mechanism of death from such doses (1.2% of body weight, or 0.84 kg for a 70kg human) is unknown, but may be more mechanical than chemical. The LD50 in humans remains unknown, given lack of any accidental or international poisoning death data. However, as with all substances tested in this way, the rat LD50 is taken as a guide to its toxicity in humans.

MATERIALS AND METHODS

SAMPLE PREPARATION

Eight samples were purchased from different patent chemist in Auchi township of Etsako West Local Government Area of Edo state, Nigeria. The samples are from A(Emzor), B(Cika), C(Esehi), D(Day by Day), E(Emzor White), F(Nutric C), G(Mekophar) and H(Juhel) vitamin C tablets.

DETERMINATION OF VITAMIN C (ASCORBIC ACID)

PRINCIPLE:

Ascorbic acid reduces oxidation:- Reduction indicator dye, 2,6-diichloroindophenol to colourless solution. At the end point, excess unreduced dye is rose pink in acid solution. The determination of vitamin C was done according to AOAC (2005). Vitamin C (ascorbic acid) is extracted and titration performed in the presence of Metaphosphoric acetic acid solution (HPO_3-CH_3COOH) which helps to maintain proper acidity for reaction and to avoid autoxidation of ascorbic acid at high P^H.

EXTRACTING SOLUTION

Meta-phosphoric acid-acetic acid solution: 15g of meta-phosphoric acid was dissolved in 40ml acetic acid and 200ml distilled water, this was then made up to 500ml with distilled water. The solution was then filtered rapidly through a filter paper whatman No. 1 into a glass-stoppered bottle and stored in the refrigerator until required for usage.

ASCORBIC ACID STANDARD SOLUTION

100mg L-ascorbic acid was accurately weighed, dissolved and volume made up to 100ml immediately with meta-phosphoric acid acetic solution ($HPO^{3-}CH_3COOH$).

INDOPHENOLS STANDARD SOLUTION

Procedure

10ml of the sample were immediately extracted with 200ml meta-phosphoric acetic acid solution by blending with warring blender for 3 minutes.

The resulting slurry was then filtered and 50ml of the juice extract was titrated with the dye solution, at the end point the colour changes to rose pink that persist for more than 5 seconds.

Calculation

DETERMINATION OF DYE

The determination of dye was according to the method of Braz (2005).

BROMATED-BROMIDE MIXTURE

A $5mmol^{-1}$ $KBrO_3$ $50mmol^{-1}$ KBr solution was prepared by dissolving accurately weighed 418mg of $KBrsO_3$ (sarabhai M chemicals, Baroda, India) and 3g of KBr (qualigens India Ltd. India) in water and diluted to the mark in a 500ml calibrated flask and this solution was used in titrimetric work. For use in spectrophotometric study, a $1000\mu gml^{-1}$ $KBrO_3$ solution containing a 50mg 2,6-dichlorophenol Na salt was dissolved in 50ml distilled water to which has been added 42mg $NaHCO_3$ and then made up to 200ml distilled water. The mixture was then filtered through filter paper into amber glass-stoppered bottle, stored in the refrigerator and standardizes every day before usage.

STANDARDIZATION OF DYE

To 2ml of standard ascorbic acid solution was added 5ml of metaphosporic –acetic acid. Titration was then performed rapidly with indophenols dye until light but distinct rosepink persists for more than 5 seconds (the titration should require about 15ml indophenols solution and titration check within 0.1m

$$\text{Mg ascorbic acid/100g} = (x - B) \times \frac{F}{E} \times \frac{Y}{F} \times 100$$

Where x= Average volume ml for test solution titration

B= Average unit ml for test blank titration

F= mg ascorbic acid equivalent to 1.0ml indophenol standard solution

E= Weight of sample

V= Volume of initial test solution

Y= Volume of test solution titrated.

large excess of KBr in water and diluting to the mark in 100ml calibrated flask this was diluted stephiose to get $10\mu gml^{-1}$ and $30\mu gml^{-1}$ bromate solutions for use in method B and C respectively.

METHYL ORANGE (50 μgml^{-1})

First, a 500 μgml^{-1} solution was prepared by dissolving 59mg of dye (s.d. fine chem., India, dye content 85%) in water and diluting to the mark in a 100ml calibrated flask and filtered. This was diluted 10-fold to obtain a working concentration of 50 μgml^{-1} .

INDIGO CARMINE (200 μgml^{-1})

A 1000 μgml^{-1} solution was first prepared by dissolving 111mg of dye (s.d. fine chem., India, dye content 90%) in water and diluting to the mark in a 100ml calibrated flask and filtered. The stock solution was diluted appropriately to get 200 μgml^{-1} dye solution with water.

SODIUM TRIOSULPHATE

About 8g of chemical (sharabhai M chemicals, Baroda, India) was dissolved in 1L of water and the solution was standardized iodometrically using a pure sample of potassium dichromate.

HYDROCHLORIC ACID (5mol^{-1})

112 ml volume of concentrated acid. (s.d. Finechem, Munbia, India, SP gr 1.18) was diluted to 250ml with water and mixed well.

POTASSIUM IODIDE (10%)

It's prepared by dissolving 25g of chemical (Qualigens Fine chemicals, India) in 250ml of water.

STARCH INDICATOR (1%)

1g of starch paste made in water was slowly poured into 100ml boiling water, boiled for I minute and cooled.

ASTEMIZOLE STANDARD SOLUTION

A 2gml^{-1} standard drug solution was prepared by dissolving 500mg of pharmaceutical grade astermizole (received from UCB India Ltd., Mumbai, India) in 25ml of glacial acetic acid and diluting to the mark with water in a 250ml calibrated flask and was used in titrimetry. This solution was then diluted with water to get 10 μ gml^{-1} and 25 μgml^{-1} solutions for used in method B and method C respectively.

PROCEDURE

VISUAL TITRIMETRY (METHOD A)

A 100ml aliquot of pure drug solution containing 4-16mg of AST was accurately transferred into a 100ml Erlenmeyer flask. 10ml of bromated-bromide solution (5mmol^{-1} w.r.t $KBrO_3$) was transferred to the flask by means of a pipette. The solution was acidified by adding 7ml of 2 mol^{-1} hydrochloric acid. The flask was stoppered, the content mixed well and kept aside for 15 minutes with occasional swirling. The stopper was then washed with 5ml of water and 5ml of 10% potassium iodide solution was added to the flask. The liberated iodine was titrated with 0.03mooll-1 sodium thiosulphate to a starch endpoint. A blank titration was run under identical conditions.

The amount of drug in the measured aliquot was calculated from:

$$Mg = \frac{(B-S)M\omega R}{X}$$

Where B= volume of thiosulphate consumed in the blank titration in ml

S= volume of thiosulphate of bromated solution in mol^{-1}

$M\omega$= Relative molecular mass of drugs

R= Concentration of bromate reaction with each mole of drug.

SPECTROPHOTOMETRY WITH METHYL ORANGE (METHOD B)

Different aliquot, (0.5 - 4.0ml) of 10 μgml^{-1} AST solution were accurately measured into a series of 10 μl calibrated flasks and the total volume was adjusted to 5ml with water. To each flask was added 1ml each of bromated-bromide solution (10 μgml^{-1} w.r.t $KBrO_3$) and 5mol^{-1} hydrochloric acid. The flasks were stoppered, contents mixed well and let stand for 15minutes with occasional shaking then 1ml of 50 μgml^{-1} methyl orange solution was added to each flask and diluted to the mark with water. The absorbance of each solution was measured at 520nm against a reagent blank after 10minute.

SPECTROPHOTOMETRY WITH INDIGO CARMINE (METHOD C)

Varying aliquots of standard AST solution (0.5-5.0ml, 25 μgml^{-1}) were transferred into a series of 10ml calibrated flasks by means of a micro burette, and the total volume was brought to 5ml followed by 1ml of $5mol^{-1}$ hydrochloric acid. The flasks were stoppered, contents mixed and allowed to stand for 15 minutes with occasional shaking then, 1ml of 200 μgml^{-1} indigo carmine solution was added to each flask and diluted to the mark with water. The absorbance was measured at 610nm against a reagent blank after 10 minutes.

In methods B and C, a calibration graph was prepared by plotting absorbance against concentration of drugs and concentration of the unknown was read from the calibration graph or computed from the regression equation derived from the beer's law data. An amount of powdered tablets and syrup equivalent to 100mg of AST was extracted with chloroform (4x10ml), the combined extracts were evaporated on a steam bath and the residue was treated as for preparation of standard drug solution. A convenient aliquot was then subject to analysis by either method.

DETERMINATION OF SWEETENER

The determination of sweetener was according Nahrung (1985).

HPLC system (waters) consisting of a 600E controller pump, a 717 plus auto sampler, 2996 ASCORBIC ACID detector and an inline-degasser. Millinium 32 software for data processing and silica (waters, spherisorb 5 μm silica, 4.6 x 250mm) analytical column was used for separation. The mobile phase consisted of (hexane: ethyl acetate) (60:40, V/V, PH adjusted to 4.5 using 1% acetic acid), with flow rate of 1ml/min. The analysis was carried out at ambient temperature.

by adding water. Accurately measured 1.5ml of bromated-bromide solution (30 μgml^{-1} w.r.t $KBrO_3$) was added to each flask

STANDARD SOLUTION

Stock solution of aspartame (0.2mg/ml) was prepared by dissolving in water. For drawing calibration curve five different concentrations in linearity range were prepared by diluting stock solution.

SAMPLE PREPARATION

Accurate weight of powdered tablets equivalent to 10mg of drug was transferred quantitatively to 100ml volumetric flask with the aid of 50ml of water. The content of the flask was sonicated for 10 minutes in ultrasonic bath, the flasks were filled up to the mark with water and filtered before derivatization. In a 10ml, screw cap test tube 1ml of each standard solution was added. This was followed by addition of 1ml of 0.2 mol/L sodium bicarbonate ($NaHCO_2$) solution and 1ml of 1% DNFB (a 1ml portion of DNFB was dissolved in 100ml of 1,4-dioxane) these test tubes were then placed on a water bath (60^oC) for 40 minutes. All derivatization reactions were stopped by addition of 0.5ml of 1mol/LHCL. The resulting dinitrophenylanrine (DNP) derivative was extracted with 5ml ethyl acetate and made up to 10ml with mobile phase. All the samples were treated in the same manner as described above for standard. To avoid interference from components soluble in ethyl acetate, all the sample were pre-extracted with 5ml of ethyl acetate before derivatization. Ten (10) μl of each standard sample were injected into the HPLC system.

DETERMINATION OF PRESERVATIVE

PLATE-DIFFUSION ASSAY (THIOMERSAL)

Flat-bottomed petin plates (100mm by 15mm, manufactured specifically for antibiotic assays) were employed. For acceptable results, it is essential that the plates should be placed on a completely level bench or table. To each plate, 21ml of a base agar (BBL antibiotic medium No. 2) 2 were added and allowed to harden for 2 hours; then 4ml of seed agar (BBL antibiotic medium No.1) 2 inoculated with the test organism were layered evenly over the base agar and left for 5-10 minutes. The test organism was maintained on nutrient agar slants (BBL antibiotic medium No.1) by transfer at 14-day intervals. To prepare the test culture, 150ml of the medium were poured into a sterile, cotton-plugged roux bottle and allowed to harden and then dry for 24hours. The medium was then inoculated by spreading evenly over the surface of the growth of a 24-hour slant, which had been washed off with 2.0ml of BBL antibiotic medium No. 3.2. The Roux bottle was incubated at 35^{o}C for 24 hours and the growth was washed off with 80ml of the BBL medium No. 3. Such a suspension may be used for 3-4weeks if it is maintained under refrigeration. To prepare the seed –layer innoculim, 1.0ml of the stock suspension was added to 150ml of seed agar which had been melted and cooled at 52^{o}C. a standard thiomarsal solution was made from reagen-grade crystals and dilutions of 1:2500, 1:5000, 1:10,000 and 1:20000 were prepared, only three consecutive dilution were used for an assay. At the same time, equivalent dilutions of the drug under test were prepared, based on the statement on the label regarding the amount of preservative present. Four plates were used for each dilution; 2 cylinders on each plate were filled to the brim with a given dilution of the standard and the other 2 were filled in a similar manner with the equivalent dilution of the test solution. The plates were covered with porcelain covers glazed only on by one, the outer, side and then incubated at 37^{o}C for 18-24 hours. Subsequently, the zones of inhibition were measured and recorded, the average size of the zone for the different dilutions being determined. Activity regression lines were prepared on semi-logarithm paper and the amount of phenol present in the unknown was determined by direct comparison with the standard.

RESULT

The result of the various experiment carried out are shown below in table 1.

Table 1

Concentration of sweetener (%), preservative (mg/100g), dye (mg/ml) and Vitamin C content (mg/100g) in some commercially sold vitamin C tablets.

S/N	Sweetener type	%	Preservative type	Mg/100g	Vit. C mg/100g	Dye mg/ml
A	Aspartame	10.25	Sodium benzoate	25.0	125.25	1.60
					125.23	4.58
B	Sucralose	9.98	Sodium propionate	25.0	120.55	4.63
					120.57	4.62

C	Saccharin	9.65	Sodium metabisulphite	25.0	128.59	4.55
					128.57	4.52
D	Asesulfame k	9.75	Sorbic acid	25.0	125.55	4.48
					125.56	4.45
E	Aspartame	10.15	Sodium benzoate	25.0	123.33	4.38
					123.34	4.33
F	Aspartame	10.22	Sodium metabisulphite	25.0	120.47	4.28
					120.46	4.26
G	Sucralose	9.88	Sodium benzoate	25.0	122.2	4.77
					122.24	4.78
H	Aspartame	10.05	Sodium propionate	25.0	120.65	4/60
					120.64	4.58

Values are means of triplicate determination (+SEM)

The above result for sweetener shows that sample A has the highest percentage of sweetener while sample C has the least percentage of sweeteners. In preservative all the samples have equal value. While in vitamin C content sample C has the highest mg/100g value while sample F has the least vitamin C content. In Dye sample G has the highest value of dye while sample A has the least value.

DISCUSSION

PRESERVATIVE

The preservative used in the vitamin C tablets from the study carried out are sodium benzoate, (sample A, E and G), Sodium propionate (sample B and H), sorbic acid (sample D) and sodium metabisulfite (sample C and F). They were observed to have the same concentration (25mg/100g). The present of benzoate in food is very safe for most people, though they cause hives, asthma, and or other allergic reaction in sensitive individuals (FDA, 2008). Sodium Benzoate used in Beverages that also contains ascorbic acids, can react together to form small amount of benzene when present in an acidic solution, a chemical that causes Leukemia and other cancer (FDA, 2008). Some of the food preservatives used to stabilize and protect food can actually harm human when eating in excess, according to the consumer advocacy group, the center for science in the public interest (CSPI). Sodium bicarbonate contribute sodium to food, which can raise a person blood pressure when eating too often. People on low sodium diet for hypertension may experience a worsening of their condition if they consume food with sodium bicarbonate (USDA, 2011). The two food preservative that can cause allergies are sodium bisulfite and sodium metabisulfite in sample C and G. According to (WHO, 2008), the consumption of preservatives may have harmful effect on the pancreas. Elimination of the intake of hazardous preservatives and chemical food additives help to reduce the risk of developing pancreatitis (an inflammation of the pancreas) (FDA, 2008). It also helps to reduce symptoms associated with pancreatitis such as nausea, vomiting, fever, pain, and abnormal tenderness. Among all, sorbic acid observed in sample D is generally regarded as safe (GRAS) (FDA, 2008).

SWEETNER

The sweetner identified in the study are aspartame (sample A, E, F and H), saccharin (sample C), Sucralose (sample B, G) and asesulfume K (sample D). From the result it was

shown that Aspartame have the highest percentage of sweetner. Excess consumption of aspartame might cause cancer or neurological problems such as dizziness or hallucination (Food and Drugs Agency, 2008). Large dosage of Asesulfume K can affect the thyroid gland of human and animals and small amount of it is not harmful to the body (World Health Organisation, 2007). Asesulfume K and Saccharin is about 200 and 350 times sweeter than sugar respectively (United State Department of Agriculture, 2011). From this study, Saccharin was shown to have the least percentage of sweetner. This sweetner is usually found in diet, soft drinks and excess of it can cause cancer of the urinary bladder, uterus, ovaries, blood vessels, skin and other organs and it increase the potency of other cancer causing chemicals (FDA, 2011). Sucralose is an artificial sweetner found in baked food, Ice cream, frozen desert and other food product. Sucralose is safer than Saccharin, asesulfume K and cyclamate (FDA, 20111). The centre for science in the public interest (CSPI) have indicated in a research conducted that sucralose causes premature shrinkage of the thymus gland (part of the immune system). Although other studies carried out have implicated sucralose to be safe (FDA, 2011).

DYE

The presence of Dye (artificial dye) was determined during this study. The highest concentration of Dye was found in sample G while the least concentration was obtained in sample A. the presence of artificial dye in drugs and food products have been implicated to the cause of Cancer (FDA, 2008). The Dye in all the samples were obtained to have almost the same range of concentration (mg/ml). The presence of Dye in food causes liver Cancer, possible carcinogen (USDA, 1956). High level of Dye damage the adrenal cortex of dog and internal organs (FDA, 2008).

VITAMIN C

According to WHO (2007), NAFDAC (2009) and FDA (2008) vitamin C is a good natural antioxidant in the body. Fruit and vegetable are the best sources of vitamin C (USDA, *et al.* 2011). Citrus fruits, tomatoes and tomatoes juice and potatoes and are major contributors of vitamin C to the American diet (Institute of medicine, 2000). The average daily recommended allowance sufficient to meet the nutrient requirement involves (97 -98%) healthy individuals. From this study the vitamin C content investigated are sufficient (120.64 – 125.56 mg/100g) to meet Recommended Dietary Intake (RDI). Excess accumulation of vitamin C causes mist common complaints such as diarrhea, nausea, abdominal cramp and other gastrointestinal disturbance due to osmotic effect of unabsorbed vitamin C in GIT (Jacob *et al.* 2002; Institute of Medicine, 2000). Due to its function as antioxidant and its role in immune function, vitamin C has promoted as a means to help prevent and/or treat numerous health conditions. Vitamin C has low toxicity and is not believed to cause serious adverse effects at high intake (Institute of Medicine, 2000). Therefore the vitamin C contents of the various tablets investigated are found to be rich in vitamin C to meet normal metabolic functions.

CONCLUSION

The relevance of vitamin C cannot be over emphasized. The vitamin C tablet investigated resulted to an excellent recommendable dietary intake RDI value for all samples. The level of preservative, sweetener and dye in the tablet may

pose a risk factor for infant and human health. Further research should be considered on the types of dye used in the vitamin C tablets so as to evaluate the hazardous effect of the artificial dyes, and possibly quantify them.

REFERENCES

Bjelakovic, G.; Nikolova, D.; Gluud, L. L.; Simonetti, R. G.; Gluud, C. (2007). "Mortality in Randomized Trials of Antioxidant Supplements for Primary and Secondary Prevention: Systematic Review and Meta-analysis". JAMA: the Journal of the American Medical Association 297 (8): 842–857. doi:10.1001/jama.297.8.842. PMID 17327526.

Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C (2008). Bjelakovic, Goran. ed. Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. Cochrane Database Syst Rev (2): CD007176. doi:10.1002/14651858.CD007176. PMID 18425980.

Bo, Simona; Ciccone, Giovannino; Durazzo, Marilena; Gambino, Roberto; Massarenti, Paola; Baldi, Ileana; Lezo, Antonela; Tiozzo, Elisa et al. (2007). "Efficacy of Antioxidant Treatment in Reducing Resistin Serum Levels: A Randomized Study". PLoS Clinical Trials 2 (5): e17. doi:10.1371/journal.pctr.0020017. PMC 1865087. PMID 17479165.

Brzozowska A, Kaluza J, Knoops KT, de Groot LC (April 2008). "Supplement use and mortality: the SENECA study". Eur J Nutr 47 (3): 131–7. doi:10.1007/s00394-008-0706-y. PMID 18414768.

C, Anitra; Frei, Balz (1999). Does vitamin C act as a pro-oxidant under physiological conditions?. FASEB Journal 13 (9): 1007–1024. PMID 10336883.

Choi, MD, DrPH, Hyon K.; Xiang Gao, MD, PhD; Gary Curhan, MD, ScD (March 9, 2009). "Vitamin C Intake and the Risk of Gout in Men – A Prospective Study". Archives of Internal Medicine. 169 (5): 502–507. doi:10.1001/archinternmed.2008.606. PMC 2767211. PMID 19273781.

Clemetson (1980). "Histamine and ascorbic acid in human blood". J Nutr 4 (4): 662–8. PMID 7365537.

Cook JD, Reddy MB (January 2001). "Effect of ascorbic acid intake on nonheme-iron absorption from a complete diet". The American Journal of Clinical Nutrition 73 (1): 93–8. PMID 11124756.

Fotherby, Martin; Williams, Julie; Forster, Louise; Craner, Paul; Ferns, Gordon (2000). "Effect of vitamin C on ambulatory blood pressure and plasma lipids in older persons". Journal of Hypertension 18 (4): 541–4. doi:10.1097/00004872-200018040-00009. PMID 10779091.

Fotherby, Martin; Williams, Julie; Forster, Louise; Craner, Paul; Ferns, Gordon (2000). "Effect of vitamin C on ambulatory blood pressure and plasma lipids in older persons". Journal of

Hypertension 18 (4): 411–5. doi:10.1097/00004872-200018040-00009. PMID 10779091.

Goodyear-Bruch, C; Pierce, JD (2002). "Oxidative stress in critically ill patients". American journal of critical care : an official publication, American Association of Critical-Care Nurses 11 (6): 543–51; quiz 552–3. PMID 12425405.

Gomez-Cabrera MC, Domenech E, Romagnoli M, et al. (January 2008). "Oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance". The American Journal of Clinical Nutrition 87 (1): 142–9. PMID 18175748.

Heiner, Kathryn A; Hart, Ann Marie; Martin, Linda Gore; Rubio-Wallace, Sherrie (2009). "Examining the evidence for the use of vitamin C in the prophylaxis and treatment of the common cold". Journal of the American Academy of Nurse Practitioners 21 (5): 295–300. doi:10.1111/j.1745-7599.2009.00409.x. PMID 19432914.

Higdon, Jane, Ph.D. (2006-01-31). "Vitamin C". Oregon State University, Micronutrient Information Center. Retrieved 2007-03-07

Huang, Han-Yao; Caballero, Benjamin; Chang, Stephanie; Alberg, Anthony J.; Semba, Richard D.; Schneyer, Christine; Wilson, Renee F.; Cheng, Ting-Yuan; Prokopowicz, Gregory; Barnes, George J. II; Vassy, Jason; Bass, Eric B. (May 2006). "Multivitamin/mineral supplements and prevention of chronic disease". Evid Rep Technol Assess (Full Rep) (139): 1–117. PMID 17764205.

Institute of Medicine, Food and Nutrition Board, Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. Washington, DC: National Academy Pess, 2000.

Jacques, Paul F.; Sulsky, Sandra I.; Perrone, Gayle E.; Jenner, Jennifer; Schaefer, Ernst J. (1995). "Effect of vitamin C supplementation on lipoprotein cholesterol, apolipoprotein, and triglyceride concentrations☆". Annals of Epidemiology 5 (1): 52–9. doi:10.1016/1047-2797(94)00041-Q. PMID 7728285.

Jacob RA, Sotoudeh G. Vitamin C function and Status in Chronic Disease. Nitr Clin Care 2002; 5:66 -74.

Johnston, Carol S.; Martin, L. J.; Cai, X. (1992). "Antihistamine effect of supplemental ascorbic acid and neutrophil chemotaxis". Am Coll Nutr 11 (2): 172–176. PMID 1578094.

Johnston CS, Solomon RE, Corte C (1996). "Vitamin C depletion is associated with alterations in blood histamine and plasma free carnitine in adults". J Am Coll Nutr 15 (6): 586–591. PMID 8951736.

Linus Pauling Vindicated; Researchers Claim RDA For vitamin C is Flawed (Press release). Knowledge of Health. July 6, 2004. Retrieved October 28, 2010.

Mashour S, Turner JF, Merrell R (August 2000). "Acute renal failure, oxalosis, and vitamin C supplementation: a case report and review of the literature". Chest 118 (2): 561–3. doi:10.1378/chest.118.2.561. PMID 10936161.

Mayne, ST (2003). "Antioxidant nutrients and chronic disease: use of biomarkers of exposure and oxidative stress status in epidemiologic

research". The Journal of nutrition 133 Suppl 3: 933S–940S. PMID 12612179

Mayer-Davis, EJ; Monaco, JH; Marshall, JA; Rushing, J; Juhaeri (1997). "Vitamin C intake and cardiovascular disease risk factors in persons with non-insulin-dependent diabetes mellitus. From the Insulin Resistance Atherosclerosis Study and the San Luis Valley Diabetes Study". Preventive medicine 26 (3): 277–83. doi:10.1006/pmed.1997.0145. PMID 9144749.

Milton, K (2003). "Micronutrient intakes of wild primates: are humans different?". Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology 136 (1): 47–59. doi:10.1016/S1095-6433(03)00084-9. PMID 14527629.

Mühlhöfer, A; Mrosek, S; Schlegel, B; Trommer, W; Rozario, F; Böhles, H; Schremmer, D; Zoller, W G et al. (2004). "High-dose intravenous vitamin C is not associated with an increase of pro-oxidative biomarkers". European Journal of Clinical Nutrition 58 (8): 1151–8. doi:10.1038/sj.ejcn.1601943. PMID 15054428.

Olmedo JM, Yiannias JA, Windgassen EB, Gornet MK (August 2006). "Scurvy: a disease almost forgotten". Int. J. Dermatol. 45 (8): 909–13. doi:10.1111/j.1365-4632.2006.02844.x. PMID 16911372.

Podmore, Ian D.; Griffiths, Helen R.; Herbert, Karl E.; Mistry, Nalini; Mistry, Pratibha; Lunec, Joseph (1998). "Vitamin C exhibits pro-oxidant properties". Nature 392 (6676): 559. Bibcode 1998Natur.392..559P. doi:10.1038/33308. PMID 9560150.

Preedy VR; Watson RR; Sherma Z (2010). Dietary Components and Immune Function (Nutrition and Health). Totowa, NJ: Humana Press. pp. 36; 52. ISBN 1-60761-060-4.

Rivers, Jerry M (1987). "Safety of High-level Vitamin C Ingestion". Annals of the New York Academy of Sciences 498: 445–54. Bibcode 1987NYASA.498..445R. doi:10.1111/j.1749-6632.1987.tb23780.x. PMID 3304071.

Safety (MSDS) data for ascorbic acid". Oxford University. 2005-10-09. Retrieved 2007-02-21.

Sato, K; Sakagami, H (1997). "Effect of metal ions on radical intensity and cytotoxic activity of ascorbate". Anticancer research 17 (2A): 1125–9. PMID 9137459.

Setherah, CJ; Downing, C; Piripitsi, A; Gallivan, L; Al-Hazaa, AH; Sanderson, MJ; Bodenham, A (1996). "Total vitamin C, ascorbic acid, and dehydroascorbic acid concentrations in plasma of critically ill patients". The American journal of clinical nutrition 63 (5): 760–5. PMID 8615361.

Shenkin A (2006). "The key role of micronutrients". Clin Nutr 25 (1): 1–13. doi:10.1016/j.clnu.2005.11.006. PMID 16376462.

Smirnoff, N (1996). "BOTANICAL BRIEFING: The Function and Metabolism of Ascorbic Acid in Plants". Annals of Botany 78 (6): 661–9. doi:10.1006/anbo.1996.0175.

Stanner SA, Hughes J, Kelly CN, Buttriss J (2004). "A review of the epidemiological evidence for the [illegible]dant hypothesis"'. Public Health Nutr 7 (1): 407–22. doi:10.1079/PHN2003543. PMID 15153272.

Tak, PP; Zvaifler, NJ; Green, DR; Firestein, GS (2000). "Rheumatoid arthritis and p53: how oxidative stress might alter the course of inflammatory diseases". Immunology today 21 (2): 78–82. doi:10.1016/S0167-5699(99) 01552-2. PMID 10652465.

Toxicological evaluation of some food additives including Anticaking agents, antimicrobials,

antioxidants, emulsifiers and thickening agents". World Health Organization. 4 July 1973. Retrieved 2007-04-13.

US Recommended Dietary Allowance (RDA)" (PDF). Archived from the original on 2008-05-29. Retrieved 2007-02-19.

U.S. Department of Agriculture, Agricultural Research Service. 2011. USAD National Nutrition Database for Standard Reference, Release 24. Nutrient Data Labouratory Home Page, http://www.ars.usda.gov/ba/bjnrc/ndl.

Vitamin C. Food Standards Agency (UK). Retrieved 2007-02-19.

Vitamin C (Ascorbic acid). Medline Plus. National Institute of Health. 2006-08-01. Retrieved 2007-08-03.

Woodside J, McCall D, McGartland C, Young I (2005). "Micronutrients: dietary intake v. supplement use". Proc Nutr Soc 64 (4): 543–53. doi:10.1079/PNS2005464. PMID 16313697.

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Nutrient and anti-nutrient Compositions of new variety of the African Bread fruit (*Artocarpus atilis*) seed

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Abstract

Non-conventional plant foods are possible good sources of nutrient. The current study was conducted to evaluate the proximate, mineral and anti-nutrient contents of new variety of the African Bread fruit in Edo North, Nigeria. The new variety of the breadfruit (*Artocarpus altilis*) was identified by the International institute for tropical Agriculture, Ibadan, Nigeria. Samples of the new variety of the African breadfruit (*Artocarpus altilis*) were collected from Igarra, Akoko-Edo Local Government area of Edo state, Nigeria. Carbohydrate, crude protein, ash, crude lipid contents were determined using standard methods. Determination of phytate, oxalate, tannin and saponin were also conducted. Among the minerals investigated were phosphorus, calcium, magnesium, potassium and sodium. The various analyses were expressed as mean ± standard deviation. Tannin content had the highest (6.36mg/100g) concentration while Phytate recorded the lowest (1.65mg/100g). However saponin and oxalate had 3.04mg/100g and 3.05mg/100g respectively. Carbohydrate and crude protein recorded high value of 75.92% and 14.98% respectively. Crude fibre contained 1.42%, crude lipid 4.60% and ash 3.02%. The mineral content amounted to phosphorus (0.312mg/100g), calcium (0.164mg/100g), magnesium (0.0485mg/100g), potassium (0.482mg/100g) while sodium (0.0476mg/100g) had the lowest value in the composition. The study showed that the new variety of the African breadfruit (*Artocarpus altilis*) is a foodstuff with appreciable levels of protein, carbohydrate and minerals as well as safe levels of antinutritional factors

Keywords: African bread fruit (*Artocarpus altilis)*, proximate composition, mineral composition, anti-nutritional factors.

INTRODUCTION

The Breadfruit *(Artocarpus altilis)*, a native to Polynesia, is a common forest tree that can be cultivated. The plant belongs to the Moraceae, a family of about 50 genera and over 1000 species (Eusoso and Bamiro, 1995), and was introduced in Venezuela as a food for slaves. In Nigeria, *Treculia africana* species of the Breadfruit is common in the eastern part (Ifeanachho and Uzoukwu, 2008). *Artocarpus altilis* is a species of breadfruit in Edo north of Nigeria whose parts have various nutritional and medicinal uses. Reports have it that the roots, leaf, bark of some varieties of bread fruit are used as component in the treatment of some disease (Aghoha, 1971). The seeds are usually roasted and eaten as such or dulled before consumption. This is usually a delicacy among the people of Igarra especially when eaten with palm kernel nut or coconut. Some varieties in Nigeria (*Treculia africana*) have been studied and are appreciated for their nutritional properties (Ifeanachho and Uzoukwu, 2008; Olugbenga *et al*. 2008). When cooked, the taste is described as potato-like, or similar to fresh-baked bread (Morton and Julia, 1987). Many people in other parts of the world have heard of *Artocarpus altilis*, few have eaten it among. Most varieties of the seeds are purgative if eaten raw. Adequate information on the chemical composition of a material is a pre-requisite for its effective utilization in animal nutrition. Determination of nutrient composition of foods had in no small measure helped in identifying the quality and usefulness of such foods in nutritional

management and clinical care (Olugbenga *et al*. 2008). Given its remarkable nutrition potential, there is need for detailed nutrient evaluation. In Nigeria, no data is available for *Artocarpus altilis* unlike *Treculia africana* that is consumed by the eastern Nigerians. The aim of this study therefore seeks to evaluate the nutritional and anti-nutritional composition of the seed of *Artocarpus altilis* grown in Igarra, Nigeria.

MATERIALS AND METHODS

Source of material

Fresh Bread fruit (*Artocarpus altilis*, identified by the International institute for tropical Agriculture, Ibadan, Nigeria, IITAA) were collected purchased from Igarra in Akoko-Edo Local Government Area in Edo State, Nigeria

Sample preparation

The seeds were separated from the pulp by handpicking. The seeds were washed clean with distilled water and oven dried at 55^0C. The clean seeds were then kept in a covered plastic container at ambient temp until required.

Chemical analysis

Moisture, crude protein, crude fat, crude fibre, and ash contents were determined in triplicate by the methods of AOAC (2005). Carbohydrate was determined by the difference. Nitrogen was determined by wet digestion analysis of the micro Kjelhdal method, and nitrogen multiplied by 6.25 to estimate crude protein content (pearson, 1976).crude fat was estimated by exhaustive extraction of sample (5 g) with petroleum ether (40-60^0C) using Tecator soxhlet apparatus. The anti-nutrient, Oxalate content was determined by Day and Underwood (1986). Saponin was determined according to Obadoni and Ochuko (2001). Phytate content was acoording to the methods of Nkama and Gbenyi (2001), while tannin content was determined by Van-Burden and Robinson (1981). The minerals, calcium and magnesium were determined using atomic absorption spectrophotometer (AAS). Potassium and Sodium were analyzed using flame photometry method while phosphorus was determined calorimetrically with spectrophotometer using phospho-vanadomolybdate method (AOAC, 2005)

Statistical analysis

Means and standard deviations were calculated for all samples using the procedure of Obi (1986).

RESULTS AND DISCUSSION

The results of proximate, antinutrients, mineral compositions are presented in Tables 1-2.

Table 1 shows the nutrient composition of *Artocarpus altilis* seedling. The crude protein 14.98%, crude fat 4.60%, crude fibre 1.42%, ash content of 3.02% and carbohydrate content of 75.92% with an energy value of 405.00kcal/100g. The meal of *Artocarpus altilis* seeds can be described as a high carbohydrate diet compared to the values investigated for *Treculia africana* seed (13.66%) as reported by Olugbenga *et al*. 2008 and pulp(12.69%) (Ifeanacho and Uzoukwu, 2008). This study also reveals high protein (14.98%) which exceeds the minimum protein requirement. This value is higher than previously reported data for African Breadfruits seed (Ejiofor, 1988; Olugbenga *et al*. 2008) and pulp (Ifeanacho and Uzoukwu, 2008) and comparable with values reported (Aletor and Omodara, 1994). The fibre and fats content were found to have lower values than previously reported data for *Treculia africana* seed (2.82% and 2.50% respectively) (Olugbenga *et a*l. 2008). It had been suggested that the fibre naturally occurring in foods might reduce the rate of small intestinal digestion by impeding the penetration of food by digestive enzymes. This phenomenon is thought to manifest in low GI of foods (Osilesi et al. 1991). The mineral contents (Table 1) were found to contain Phosphorus 0.312mg100g, calcium 0.164mg/100g, Potassium 0.482mg/100g, magnesium 0.485 mg/100g and Sodium 0.0476 mg/100g. This values are comparable with data for phosphorus and lower than that of calcium and sodium(ifeanakwo, 2008). Phosphorus is stored in plant seeds as phytate during seed development (Lott *et al*., 1995; Mubarak, 2005).African breadfruit constitutes very impotant sources of minerals which may be utilized in the diet of the low income groups in Nigerian. The relatively low levels of potassium (0.482%) for Artocarpus altilis seed suggest that it could be suitable for patients with cardiovascular and renal disorders whose dietary management in many cases hinges on sodium and potassium restriction. The potassium value is significantly lower than the values for African breadfruit (Ifeanacho and Uzoukwu, 2008).

Table 2 shows the result of antinutritional factors present in a seeds. The values were found to contain Oxalate (3.05mg/g), phytate (1.65mg/g), Saponin (3.04mg/g) and Tannin (6.36mg/g). Tannin had the highest concentration while Phytate had the lowest. The phytate content in this study is the comparable with the range of previously reported literature data (0.660 − 3.302 mg/g) for cereal and legume seeds (Marshall *et al*. 2011). Phytate accounts for approximately 50-80% of the phosphorus in seed-feed stuffs (Ravindran *et al*., 2000; Fredrikson *et al*., 2001). The seed contains significant values in antinutritional factors and toxicant; and this suggest the reasons why the seeds are subjected processes such as boiling and roasting before consumption which has been the usual practice with the people who comsumed them. The digestility of foods may be reduced by the presence of antinutritional factors which are present in plant seeds (Kumar *et al*., 2010). This antinutritive factors lead to the formation of indigestible complexes with some nutritionally important minerals (Konietzny and Greiner, 2003; Khattak *et al*., 2007) thereby impairing the bioavailability, absorption and utilization of minerals. Result of proximate and antinutrient supply clues in research which is paramount to human an animal nutrition.

Table 1: Proximate and Mineral composition of the African bread fruit (*Artocarpus altilis)* seed

Parameter	Composition
Crude protein (%)	14.98± 0.01
Crude fibre (%)	1.42±0.01
Lipids (%)	4.60±0.06
Ash (%)	3.02±0.01
Carbohydrate (%)	75.92±0.01
Energy (kcal/100g)	405.00±0.67
P (mg/100g)	0.312 ± 0.01
K (mg/100g)	0.482± 0.01
Ca (mg/100g)	0.164± 0.01
Mg (mg/100g)	0.0485± 0.01
Na (mg/100g)	0.0476± 0.01

Values are Means ± Standard deviations of triplicate determinations

Table 2: Some anti-nutritional factors of the African bread fruit (*Artocarpus altilis)* seed

Parameter	Composition(mg/100g)
Phytate	1.65±0.007
Tannin	6.36± 0.010
Saponin	3.04±0.007
Oxalate	3.05±0.013

Values are Means ± Standard deviations of triplicate determinations

CONCLUSION

The seed of the African bread fruit (*Artocarpus altilis*) contain substantial amount of nutrient, enhancing human nutrition. The high non-protein nitrogen which is shown to be present in the analyzed seeds could contribute to the building of non essential amino acids, therefore a large

proportion of the individual protein requirement could be met by the bread fruit subject to adequately high rate of intake. *Artocarpus altilis* seeds like the common bread fruit (*Treculia africana)* variety are known to contain vitamins and phytochemical, it is therefore recommended that investigation into levels of the phytochemical and vitamins be carried out so as to further ascertain its health potential and safety. Investigation should also include processing such as germination or sprouting of the seeds for possible nutrient improvement

REFERENCES

Aletor,V..A. and Omodara, O. .A. (1994) Studies on some leguminous browse plant with particular reference to their proximate , mineral and some endogenous antinutritional constituents. *Animal Feed Science Technology*. 46:343-348

Aghoha (1971). Medicinal plants of Nigeria. Oxford University Press, Ibadan Nigeria

AOAC (2000) Association of Analytical chemistry, Washington DC, USA

AOAC (2005) Association of Analytical chemistry, Washington DC, USA.

.Balick, M and Cox, P. (1996). Plants, People and Culture. *The Science of Ethnobotany*, New York.

Chowdhury FA, Raman Md. A, and Mian A. J.(1997) Distribution of free sugars and fatty Acids in Jackfruits. Food Chem. 60 25-28.

Duke J., and Alan A. (1986) Handbook of Proximate Analysis Tables of higher Plants. CRC Press Inc. Boca de Raton,.P.21.

Eusoso K. and Bamiro F. (1995) Studies On The Baking Properties of Non-Wheat Flours I. Breadfruit (Artocarpous altilis), Int. J Food Sci. and Nutr. 46: 267-273.

Gebre-Mariam T. and Ababa A. Some Physicochemical Properties of Dioscorea Starch from Ethiopa. Starch/Starke, 1998: 60:241-246.

Hagerman A. E. and Buttler L G. (1978). Precipitation Method For The Quantitative Determination Of Tannin *J. Agric. Food Chem*. 26: 809-812.

Hill F. W, Anderson D. L, Renner R, and Carew Jr L B. (1960). Studies 159 The Metabolizable Energy of Gr.... and Grain Products for Chicken *Poult.Sci*. 39: 573- 579.

Ifeanacho, M.O. and Uzoukwu, C.O. (2008) Nutritional composition of African Breadfruit (Trecula Africana) pulp. Nigerian Journal of Nutritional Sciences. 29(2): 190-194.

Konietzny, U. and Greiner, R. (2003) Phytic acid: Nutritional impact. In B. Caballero, L. Trugo, and P. Finglas (Eds.). *Encyclopaedia of food science and nutrition*. London, UK: Elsevier. 4555-4563.

Khattak, A. B., Zeb, A., Bibi, N., Khalil, A. S. and Khattak, M. S. (2007) Influence of germination techniques on phytic acid and polyphenols content of chickpea (*Cicer arietinum* L.) sprouts. *Food Chemistry* 104: 1074-1079.

Kumar. V, Sinha. A.K, Makkar. P.S. and Becker, K. (2010). Dietary roles of phytate and phytase in human nutrition: A review. *Food Chemistry* 120: 945-959.

March J. G, Villacampa A. I and Grases F. (1995).Enzymaticspectrophotometric Determination of Phytic Acid With Phytase from Aspergillus ficuum. Analytica Chimica Acta 300 (1-3): 269-272.

Marshall A Azeke; Rafaat M Elsanhoty; Samuel J Egielewa and Mary U Eigbogbo (2011). The effect of germination on the phytase activity, phytate and total phosphorus contents of some Nigerian-grown grain legumes. *J Sci Food Agric*. 91: 75–79.

Obadoni and Ochuko (2001). Saponin Methodology, reported by Onimawo and Egbekun (1998). O'Brian, Patrick (1998) Joseph Banks, A Life: Explorer, Plant Hunter, Scientis. Collins Harvill, London.

Obi I. U. (1986) Statistical methods of detecting differences between treatment means. Snapp Press (Nig) Ltd. Enugu

Osilesi, O., Jenkins, D.J.A., Jaiyesimi, A.E., Adebawo, O.O., Osiyemi, F.O. and Kuti, J.A. (1991) Glycemic index of cowpea varieties in rural Nigerian diebetics. Nig. Med. Pract. 22(5-6): 91-95.

Olugbenga, O.A., Paul, O.O. and Odutola, O. (2008) Nutrient compostion and Glycemic index characterization of Trecula Africana (African Bread-fruit). Nigerian Journal of Nutritional Sciences. 29(1): 249-255.

Pearson D. (1976) Chemical Analysis of Food, 7th Edition Churchill Livingstone, London P.3-11.

Rahman M. A, Nahar N. Jabbar Mian A. and Mosihuzzaman M, Variation of Carbohydrate Composition of Two Forms of Breadfruit.

Rincon A. M, Padilla F. C, Aranijo C, Tillet, S. and Myrosma (1991) Cannifolia, J Sci. Food and Agric.; 79:532-36.

Rincon AM, Perez E, Gonzalez Z, and Rodriguez P. (1999) Microstructural Changes of Canavalia Ensiformis Starch Modified by Thermal Methods. *Food Sci. Tech. Int*. 5:31-40.

Tumaalii F and Wooton R. (1998) Properties of Starch Isolated from Western Samoan Breadfruit Using a Traditional Method, *Starch/Starke.* 40:7-10.

Van-Burden and Robinson (1981). Determination of Tannin Content of Breadfruit flour. JSci Fd. 2342-2350.

Yan Z, Xing G, Zhi-Xian L (2003). Quantitative Determination of Oxalic Acid Using Victoria Blue B based on a Catalytic Kinetic Spectrophotometric Method. Microchimica Acta 144(1-3): 199-205.

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COMPARATIVE ANALYSIS OF SUPERVISED LEARNING ALGORITHMS FOR THE PREVENTION OF CYBER FRAUD

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ABSTRACT

The study trained and tested five supervised learning algorithm namely, K Nearest Neighbour Classifier, Decision Tree Classifier, Random forest classifier, Gradient boosting classifier and Extra Tree classifier was implemented using the sci-kit library in python to evaluate the performance of selected machine learning model as a tool for preventing cyber fraud.. Dataset used for the study was gathered from github, a data repository platform. Exploratory data analyses were conducted using panda packages in python. Base on the experimental results of the supervised learning algorithms for the prevention of cyber fraud. Extra Tree Classifier shows an accuracy of 99.51% which was the highest accuracy. Random forest achieved the second highest accuracy both with an accuracy of 99.48%. Decision Tree also achieved a high accuracy of 99.23%, followed by K Nearest Neighbor with accuracy of 99.08% and lastly, Gradient boosting achieving the lowest accuracy of 98.72%. Hence, we conclude that the highest accuracy that we can achieve is 99.51% and there is no further need in using more models to predict the accuracy. In future work, it would be interesting to compare neural network performance with Extra Tree Classifier. Distributed computing tools such as OpenMP, MPI, Hadoop and Spark might also be helpful in reducing the cost of computation time.

Keywords: *Cyber-Fraud, Supervised Learning Models, Python*

INTRODUCTION

The cyber-attacks are increasingly getting sophisticated with zero-day exploits and malware that evade Fraud measures. (Tao et al., 2021) observed that cyber fraud systems are increasingly getting used to improve operational efficiencies and reduce costs in critical areas, such as finance, transportation, defense, and healthcare. Cybercrimes are monotonically increasing and there is a growing concern on the Fraud, confidentiality and computer assurance of the stored data. (Eswaran et al., 2021) affirms that Intrusion Detection and Prevention Systems (IDPS) include all protective actions or identification of possible incidents, analyzing log information of such incidents, how to block them in the beginning itself and generate reports for the concern of Fraud personnel. A significant challenge is the design of anomaly detectors that effectively detect failures and cyber-attacks in Cyber Physical System (CPS). (Ghafouri, A., 2018) observed that in the design and evaluation of anomaly detectors, realistic attack models that represent the harmful effects of cyber-attacks on CPS are needed. Resilience in CPS is defined as protecting the operational goals (e.g., stability) as well as other non-operational goals (e.g., privacy) in the presence of both expected events (e.g., failures) and unexpected events (e.g., cyber-attacks). According to Ghafouri, (2018) resilience in CPS must attain three goals: Integrity which represents the trustworthiness of data or resources, Availability which is the ability to access and use information on demand as specified, and confidentiality, which is the ability to keep information secret or private from unauthorized users. The use of wireless technology has increased the vulnerability and accessibility of networks. Standard

Fraud measures have proved to be grossly inadequate under the circumstances. The major attention on cyber fraud has now shifted to the use of different machine learning model for use in network anomaly detection such as neural networks, genetic algorithms, fuzzy logic, support-vector machines, finite automata, and other diverse data-mining-based approaches. This study sought to analyze the performance of ensemble modelling for the prevention of cyber fraud.

According to Fehling *et al.,* (2014), the cloud symbol is usually used to symbolize the internet. Cloud computing is now frequently used to describe the delivery of software, middleware platforms, infrastructure, whole business processes and storage services over the internet. These services are delivered when they are needed in the quantity needed at a certain time. Put differently, cloud computing is very much similar to the rent-a-car model. The cost effectiveness and efficiency of the cloud platforms is tempting most organizations to migrate to the cloud and enjoy a wide range of benefits (Chhabra, N., Bala, M., & Sharma, V. 2022) which according to (Kabanda, 2020) include, free capital expenditure, accessibility from anywhere at any time, no maintenance headaches, and improved control over documents as files will be centrally managed.

Cyber Fraud is also a key challenge in this industry as cybercriminals use cloud services as warehouses to store their malicious software and as targets that will be used as launch pads for Denial of Service (DOS) attacks (MacAfee, 2018).

Cybercrime has matured with a big market with several stakeholders and is unlikely to stop as it is very rewarding. Online criminal marketplaces have gone to the extent of selling ransomware services and products. End users of technology continue to fail to adhere to basic Fraud norms and this sustains the cybercrime market (MacAfee, 2018). Cybercrime features on the top 10 global risks together with terrorist attacks, natural disasters and extreme weather patterns (Kabanda, 2018). According to MacAfee (2018) and World Economic Forum (2017) as cited by (Bozkus & Caliyurt, 2018). Cybercrime costs the world $US 575 billion annually which constitute 0.5% of the world's Gross Domestic Product. The damage caused by cybercrime is also expected to reach US$6 trillion by 2021 (Kabanda, 2018). Cybercrime is expected to grow taking advantage of poor Fraud of the Internet of Things (IoT) devices (MacAfee, 2018). Cybercriminals are also riding on Artificial Intelligence (AI) to make and replicate malicious software as well as identifying weak targets. Ransomware erupted in 2015 and is likely to continue to be very popular going forward whilst improving in sophistication. It is anticipated that businesses are going to be facing ransomware attacks every 14 seconds by 2019 and the attacks on healthcare systems is expected to quadruple by 2020 (Concierge Fraud report, 2018). Information Fraud culture consist of perceptions, attitudes, assumptions, values and knowledge that guide the interaction of people with organizational information assets with the mandate of securing information (Al Hogail, 2015).

METHODOLOGY

A Python anaconda navigator which has multiple data science packages embedded in it, and updated library like Numpy, Pandas, Matplotlib, Seaborn, Sci-kit learn, StandardScaler, Keras, etc was adopted for this study. the method involve training and testing five (5) machine learning model namely: K Nearest Neighbour Classifier, Decision Tree Classifier, Random forest classifier, Gradient boosting classifier and Extra Tree classifier was implemented using the sci-kit library in python.

Dataset Acquisition

The dataset used for this research is the famous NSL-KDD dataset downloaded from github. The NSL-KDD dataset is a

successor of DARPA's KDD'99 dataset which was obtained from the previous 1998 DARPA intrusion detection evaluation program. Although it has been noted that this newer version of the KDD data set has some flaws as pointed out in (Lin, Ke, & Tsai, 2015), it is also a good candidate as a benchmark dataset in the Intrusion Detection field (REIS, KAYA, & SAHINGOZ, 2019). The goal is to the performance of selected machine learning model for the performance of cyber Fraud. The NSL-KDD data set has a total number of 39 different attack types. All the attacks can be grouped into four main categories as DoS, Probe, R2L, and U2R. While the NSL-KDD data set contains a total of 39 different attack types, the number of unique attack types contained in the train set and test set differentiate from each other. The attack types contained in the train set which are called as "known attacks" have a total number of twenty-two attack types. On the other hand, the test attacks have a total number of thirty-seven attacks. The additional attacks in the test set which are not present in the train set are called "novel attacks." Having unknown attack types in the test set shows if the system can adapt to different scenarios when the testing is done. The train dataset includes 125973 records and 43 attributes while test data includes 22544 and 43 attributes making the total of the whole data. There are no missing values in the dataset. There is also no redundancy in the dataset.

Data Pre-Processing

Exploratory data analyses were conducted using panda packages in python. The frequency distributions of attack_type, protocol type were visualized using matplotlib and seaborn python package. The correlation scores between features were measured using Pearson correlation and is displayed using seaborn heatmap.

Frequency Encoder

It is a way to utilize frequencies of categories as labels. It helps the model to understand and assign the weight in direct and inverse proportion, depending on the nature of the data.

Label Encoder

Label encoders are used to convert categorical data, or text data into numbers, which our predictive models can better understand. In this study, the sci-kit learn "label_encoder()" is used to convert the "attack type" columns to numeric data type.

One Hot Encoding

One hot encoding is the process of creating additional features based on the number of unique values in the categorical features, in other words it is the process of creating dummy variables. One hot encoding allows the representation of categorical data to be more expressive. In this study, the pandas "get_dummy()" is used to implement one hot encoding.

Feature Scaling

Feature scaling is a method used to standardize the range of independent variables or features of data. In data processing, it is also known as data normalization. Sci-kit learn "MinMaxScalar()" was used to scale the dataset used in this project.

Algorithm

Machine learning approach relies mostly on algorithms, a set of rules that when followed leads to a desired output. The algorithm of this study is written below.

Step 1: Start.
Step 2: Import libraries to be used and dataset.
Step 3: Data Cleaning.
Step 4: Formation of new/clean dataset.
Step 5: Visualization of data.
Step 6: Train and test data split (70% to 30%).
Step 7: Algorithm using feature subset.
Step 8: Training and testing the model.
Step 9: Model Evaluation.
Calculate model accuracy

Step 10: Report.
Step 11: Stop.

Flowchart

163 wchart shows different shapes and diagrams that are connected by arrow. Each shape represents a step in the process, and the arrows shows the order in which they occur in the study. The flowchart for this study is shown in **Fig 1** below.

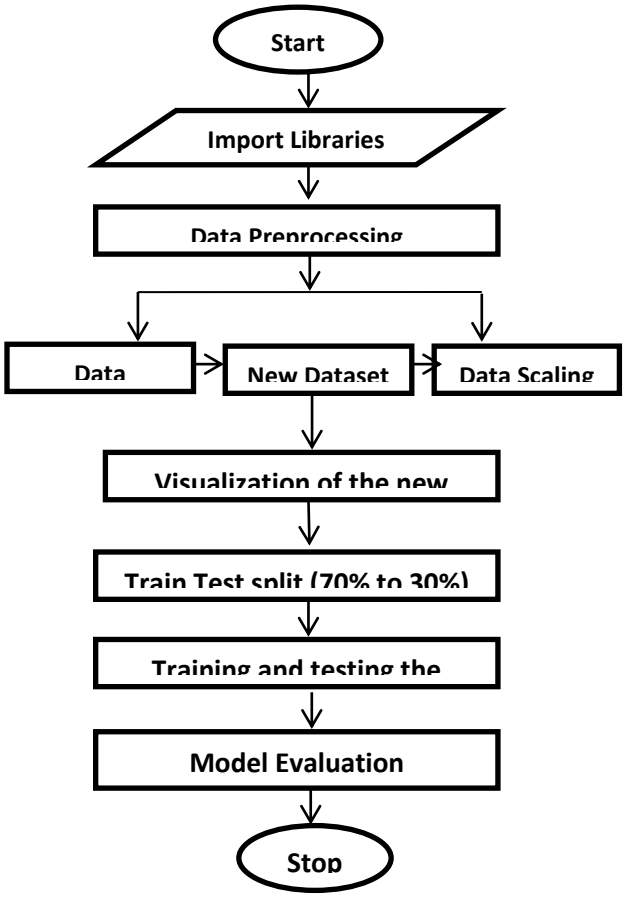

Figure 1: Depicting the Model Flowchart

Exploratory Data Analysis (EDA)

The EDA was implemented majorly to have a detailed understanding of the dataset, it enhances data cleaning and visualization. The data was divided into the train and test set, Thereafter the data was normalized using the sci-kit learn **MinMaxscalar()**. The process of dividing the dataset was done using the **train_test_split()** method in the sci-kit learn package. The trained dataset comprises 125973 records and 43 attributes while test data includes 22544 and 43 attributes making the total of the whole data. There is no missing value in the dataset. The dataset indicates the attribute information present in the instance and the attribute class values present. Graph was adopted to show the major information of the dataset.

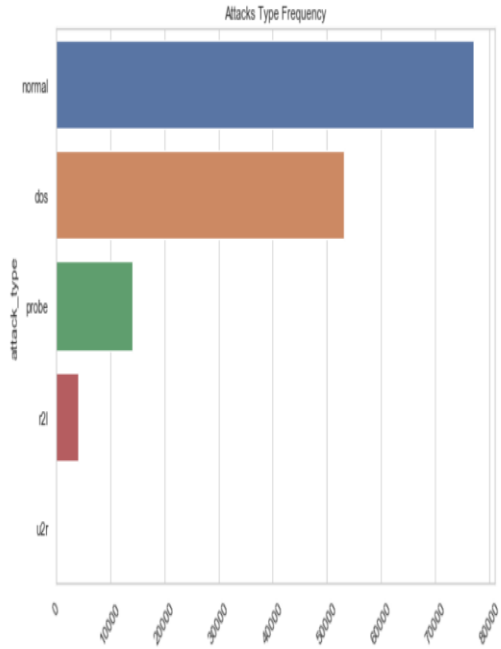

Figure 2: Showing frequency of attack types

RESULTS AND DISCUSSIONS

The study was implemented using Intel Core i5, 2.7 GHz processor, 8GB RAM, 1 terabyte hard disk drive (HDD), and Windows 8.1, a 32-bit operating system, the models were developed on **python Anaconda Navigator**. Five different classifiers were used for evaluating the performances of selected machine learning models for the performance of cyber fraud. These classifiers were trained using the training data.

Result Analysis and Evaluation

Table 1.1: Showing the Result Analysis
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Models	Accuracy Score
KNN	99.08
DTC	99.23
RFC	99.48
GBC	98.72

From table 1, the highest accuracy is Extra Tree Classifier with an accuracy of 99.51%. Random forest achieved the second highest accuracy both with an accuracy of 99.48%. Decision Tree also achieved a high accuracy of 99.23%, followed by K Nearest Neighbor with accuracy of 99.08% and lastly, Gradient boosting achieving the lowest accuracy of 98.72%. Hence we conclude that the highest accuracy that we can achieve is 99.51% and there is no further need in using more models to predict the accuracy.

CONCLUSION

Machine learning algorithms can be effectively used to extract relevant information from the huge amounts of data generated from the Information and Communication Technology industry. This study shows that proper selection of the combination of machine learning models and accurate implementation of those techniques on the data set yields a fast and effective implementation of a system for evaluating the performance of selected machine learning models on cyber fraud. Overall, comparing the five machine learning model building K Nearest Neighbour (KNN), Decision Tree Classifier (DTC), Random Forest Classifier (RFC), Gradient Boosting Classifier (GBC), and Extra Tree Classifier (ETC) model for evaluating the performance of selected machine learning model for the performance of cyber Fraud achieved differences in accuracy; with Extra Tree Classifier having the highest Accuracy. In future work, it might be interesting to compare neural network performance with Extra Tree Classifier. Distributed computing tools such as Open MP, MPI, Hadoop, and Spark might also help reduce the cost of computation time. Overall, there is a belief that if the quality of studies continues to improve, the use of machine learning classifiers will likely become much more commonplace in many organization settings.

References

Al Hogail, M. (2015). How is the ministry fostering public-private partnerships (PPPs) with local private developers.

Bozkus Kahyaoglu, S., & Caliyurt, K. (2018). Cyber security assurance process from the internal audit pers pective. *Managerial Auditing Journal*, *33*(4), 360-376.

Chhabra, N., Bala, M., & Sharma, V. (2022). Implementation and Validation of the Optimized Deduplication Strategy in Federated Cloud Environment. *Computers, Materials & Continua*, *71*(1).

Eswaran, S., Srinivasan, A., & Honnavalli, P. (2021). A threshold-based, real-time analysis in early detection of endpoint anomalies using SIEM expertise. *Network Security*, *2021*(4), 7-16.

Fehling, C., Leymann, F., Retter, R., Schupeck, W., & Arbitter, P. (2014). *Cloud computing patterns: fundamentals to design, build, and manage cloud applications*. Springer Science & Business Media.

Ghafouri, A. (2018). *Resilient Anomaly Detection in Cyber-Physical Systems*. Vanderbilt University.

Kabanda, G. (2021): Performance of machine learning and big data analytics paradigms in cybersecurity and cloud computing platforms. Global Journal of Computer Science and Technology: G Interdisciplinary, 21(2), 1-25.

Kabanda, G (2020). Performance of Machine Learning and other Artificial Intelligence Paradigms In Cybersecurity. *Oriental journal of computer science and technology*, *13*(1), 1-21.

Kabanda, G. (2018). A Cybersecurity culture framework and its impact on Zimbabwean organizations. *Asian Journal of Management*, *Engineering & Computer Science*, *3*(4), 17-34.

MacAfee, (2018). https://www.mcafee.com/consumer/ensg/store/m0/catalog.html

Tao, F., Akhtar, M. S., & Jiayuan, Z. (2021). The future of artificial intelligence in cybersecurity: A comprehensive survey. *EAI Endorsed Transactions on Creative Technologies*, *8*(28), e3-e3.

SAHİNGOZ, O. K. (2019). A clustering approach for intrusion detection with big data processing on parallel computing platform. *Balkan Journal of Electrical and Computer Engineering*, *7*(3), 286-293.

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HIGH-LEVEL FEATURES FOR A ROBUST SPEAKER RECOGNITION SYSTEM

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Abstract

Speaker recognition is the process of automatically identifying an unfamiliar speaker by removing the information unique to that speaker from the speech wave. In automatic speaker recognition, higher level features based on linguistic or distant information have drawn a lot of interest. In order to provide a technological perspective in this crucial domain of speaker recognition, this study proposes an idea of choosing a robust parameter for text-independent speaker verification and identification in order to achieve an accurate result and limits faced. Cepstral features, for example, have been found to have relatively low error rates (particularly in quiet settings), but they are more prone to error in noisy environments. This study presents a design approach and a number of features for an effective speech identification system. This study offered many features and a design approach for a reliable speech identification system. This model's implementation was done in Matlab, where both low-level and high-level features were combined. It has been discovered that high-level characteristics or hyper-parameters increase the accuracy of Automatic Speech Recognition (ASR) by providing the system with more data.

Keywords: Speaker recognition, Speaker verification, High level features, Cepstral features, Automatic speaker recognition

INTRODUCTION

Speaker recognition is the process of recognizing automatically who is speaking on the basis of individual information included in speech waves. The broad field of speaker classification makes use of a wide range of properties of spoken language ranging from lower-level features reflecting voice parameters to higher level features that capture phonetic, prosodic and lexical information. This technique uses the speaker's voice to verify their identity and provides control access to services such as voice dialing, database access services, information services, voice mail, security control for confidential information areas, remote access to computers and several other fields where security is the main area of concern (Singh, & Pandey, 2003). In forensic phonetics, it is a common practice for human experts to use not only voice characteristics but also speaker characteristics based on pronunciation, prosody, and lexical information to access the match between a suspect's speech and speech in a recording of interest (Elizabeth, 2007). Speaker verification is the process of rejecting or accepting the identity claim of a speaker. In most of the applications, voice is used as the key to confirm the identities of a speaker are classified as speaker verification (Sreedharan & Eswaran, 2019). In this paper, the main contribution is to provide a brief overview of work on higher- level/ hyper parametric features and design a model that would compare voice samples as regards to their hyper parameters derived from various sample database. Early text-dependent speaker recognition systems utilized information from short-time spectrum to provide unique features for speaker recognition. These features consisted of energy measurements from the outputs of a bank of a filter. LPC was one of the feature extraction method based on the source-filter model of speech production. Alku, & Saeidi, (2017) used linear prediction model for parametric representation of speech derived features. The predictor coefficients and other speech parameters derived from them, such as the impulse response function, the auto-correlation function, the area function, and the cepstrum function were used as input to an automatic speaker recognition system, and

found the cepstrum to provide the best results for speaker recognition. Mathur et al., (2012), made use of all-pole LP (linear prediction) to model a signal by a linear combination of its past values and a scaled present input. Gupta, K., & Gupta, D. (2016) compared different features useful for speaker recognition, such as Mel frequency cepstral coefficients (MFCCs), linear frequency cepstral coefficients (LFCCs), LPCC (linear predictive cepstral coefficients) and perceptual linear prediction cepstral coefficients (PLPCCs). Both MFCC and LPCC coefficients were used to extract vocal track information, but used different technique to extract the features. MFCC extraction is similar to the cepstrum calculation except that one special step is inserted, namely the frequency axis which is warped according to the Mel-scale using mel filter bank. The filter bank outputs are then converted to cepstral coefficients by applying the inverse discrete cosine transform (IDCT). In case of LPCCs, first, LPCs are obtained for each frame using Durbins-recursive method, and then these coefficients are converted to cepstral coefficients. The predictor coefficients themselves are rarely used as features and can be transformed into robust and less correlated features such as LPCC, line spectral frequencies (LSFs) and perceptual linear prediction cepstrum coefficients (PLPCC) or the *Eigen-MLLR coefficients*. Experimental evaluation of recognition accuracy of the MFCC, LPCC and PLPCC has been made and result of this report is that all features perform poorly without some form of channel compensation, however, with channel compensation MFCC slightly outperform other types.

These features above are also called short-term (spectral) or low-level features. These features are used in most state-of-art speaker recognition systems as they are easy to compute and yield good performance (Li et al., 2022). However, it has its own disadvantages. The main disadvantage of the cepstrum is that it is quite sensitive to the environment and noise. Adami et al., (2003), presented two new approaches that demonstrated effective ways to model and apply prosodic contours for text independent speaker verification tasks. In the first approach, the relation between dynamics of fundamental frequency (fo) and energy trajectories were used to characterize the speaker's identity. In this, global distribution of energy and fo features such as log fo, log energy and their first order derivatives were created. Accent and intonation information from a known set of frequently and naturally occurring words found in conversational speech were also known. The use of n-grams was used to model the sequence.

From source-filter model, it was shown that speech signal can be decomposed into two parts: the source part and the system part. The system part consists of the smooth envelope of the power spectrum and is represented in the form of cepstrum coefficients, which can be computed by using either the linear prediction analysis or the mel-filter-bank analysis. Ezzaidi, & Rouat, (2004) proposed an approach which jointly exploits the information of the vocal tract and the glottis source. This approach synchronously takes into account the correlation between the two sources of information. The fundamental frequency and the MFCC coefficients were also used to represent the information of the source and the vocal tract, respectively. It was also shown that systems based on voiced segments yield good scores. However, when the dependence of the source and vocal tract is taken into account, the best results were observed for durations T lower than 500ms. It is well known that these features are characteristics of each person, so that they carry information about the speaker. Ferrer, Scheffer, & Shriberg, (2010), introduced the use of continuous prosodic features for speaker recognition and showed how they can be modeled using joint factor analysis. Shriberg, Ferrer and Kajarekar (2005) described a new approach to modeling idiosyncratic prosodic behaviour for automatic speaker recognition. This approach computes various duration, pitch, and energy features for each estimated syllable in speech recognition output, quantizes the features, forms N-grams of the quantized values, and models normalized counts for

each feature N-gram using support vector machines (SVMs) referred to as SNERF-grams (N-grams of Syllable based Nonuniform Extraction Region Features) S.R.

Prasanna, Gupta, & Yegnanarayana, . (2006) used the features from spectral, duration and pitch. It was shown that not only that the performance of verification improved, but also the non- spectral features such as duration and pitch were found to be robust for variations due to channel.

High level features are based on voice timbre and accent/ pronunciation of speaker and also on lexicon - the kind of words the speakers tend to use in their conversations. Works on such "high-level" conversational features was introduced where a speaker's characteristic vocabulary, the so-called ***idiolect***, was used to characterize speakers. The idea in "high-level" modeling was to convert each utterance into a sequence of ***tokens*** where the co-occurrence patterns of tokens characterize speaker differences. Shriberg (2007) demonstrated how higher-level features can contribute to performance in a state-of-the-art system. Various features such as cepstral and cepstral-derived, phonetic (acoustic tokenization), prosodic, lexical features along with their performance was discussed. Higher-level features also have the potential of increased robustness to channel variation, since lexical usage or temporal patterns do not change with the change of acoustic conditions. Mainly pitch and energy dynamics were investigated. However, types of prosodic features such as (explicit) syllable-based prosody sequences, interpause/conversation level statistics, and durational features, could also be considered. In summary, long-term features have been investigated for several years and indications have been provided that they can be useful for speaker recognition.

METHODOLOGY

This section shows the general architectural design that illustrates the methods and techniques used in higher-level feature modeling for the proposed design model. Each speaker recognition system has two phases: Enrollment and verification. During enrollment, the speaker's voice is recorded and typically a number of features are extracted to form a voice print, template, or model. In the verification phase, a speech sample or "utterance" is compared against a previously created voice print. The Architectural framework for the Speaker recognition sysem proposed in this paper as described in Figure 3.1 is basically simple and can give more accuracy to speaker verification and identification system integrating low-level features and high-level features extracted from a speech signal. This model is geared towards:

- Providing a high-level feature which can be integrated into the state-of-the-art ASR.
- Improving the accuracy of ASR through the use of a prosodic feature extraction of speech signals from long-term information. The process flowchart is presented in Figure 3.2.

Architecture of proposed model for speaker recognition:

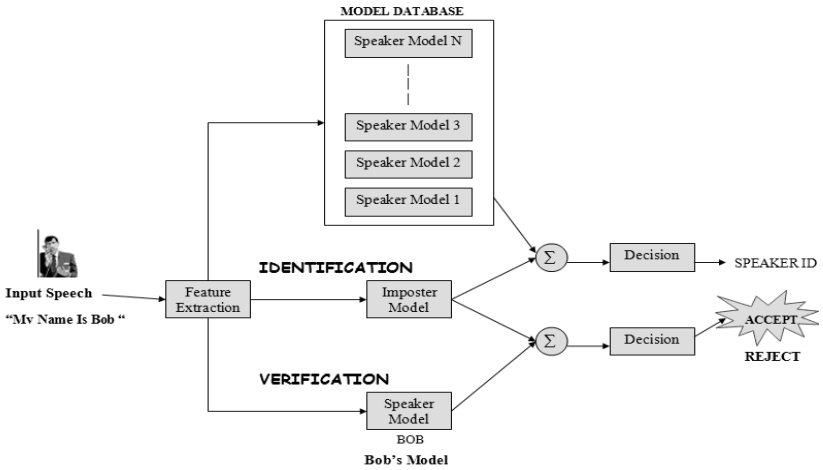

Figure 1.0: speaker recognition architecture

Speech signal: this is the data sample to be analyzed. Speech signals should be in a compatible formant for feature extraction.

Feature extraction: this step is usually done inside the model. Here extraction models or techniques could be used in extracting speech signal features and converting it to i-vectors used by the system for the database modeling or imposter or speaker model. Features are also broken down according to their parameters.

Model database: this is more or less than a codebook containing various speaker codebooks till N-speaker codebook.

Imposter model: this maps an identity of 1:1 mapping. That is for each speaker model there is only one of the set of N-speaker .model that has been enrolled. Hence there is an identity match.

Speaker model: this was proposed by bob and it uses a mapping of 1:N mapping. This means that for every signal enrolled there might be a possible match, else speaker is not verified. Hence it either rejects or accepts using a likelihood ratio hypothesis.

Decision: this shows the result of computed signals and their output, that is whether to accept or reject the hypothesis based on likelihood ratio. **Three main process phase:**

Enrollment: a user is added to the system.

Verification: claim to users identity is compared with a claimed user model.

Identification: a database of user models is searched for the N-most likely sources.

Process flow chart

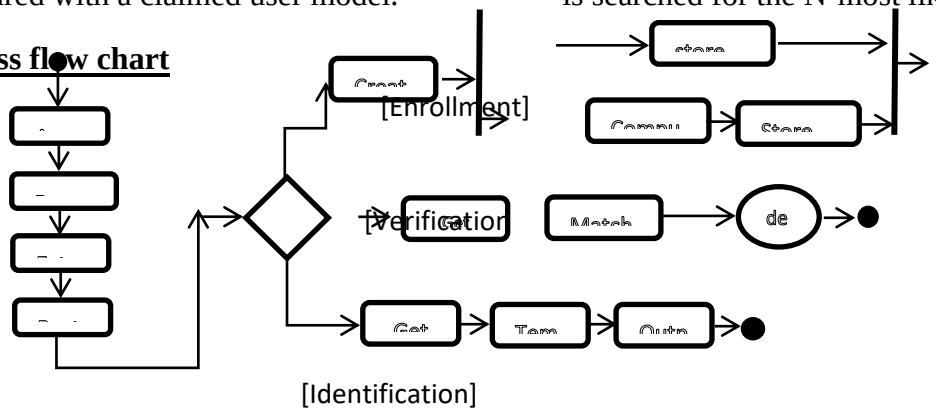

Figure 2.0: Process Flowchart

Process steps:

The following are the process steps to be taken in designing a model for speaker recognition:

Capture or acquisition: this is also called data representation. Speech signals are represented in this phase which is digitized via an input device (microphone, pen tablet,..) and stored in the memory.

Preprocessing: the signal-domain acquired data is prepared for feature extraction. This is used for normalizing the signal-domain data and remove biases or sources of corruption in a systematic way.

Feature extraction: different features are then extracted from the preprocessed data. This process reduces the dimensionality of the input data to create a feature-level representation of input patterns that will be used by classifiers to perform pattern recognition.

Postprocessing: here the features are normalized to remove bias or to adapt them to classifiers.

Template creation: templates are created from training feature sets to obtain a generic representation of a user that will be used for future comparisons. Many algorithms could be used depending on modality/features and model classes.

Background model: this is also known as a world model or anti-model needed by some algorithms to provide normalization for user presentation scores. They mostly represent averages of the user from a population of the system.

Template storage: after the estimation of the parameters, user models are stored in a secure location for later usage.

Template matching: a presentation is compared using a user's template. This results in presentation scores which is usually related to that user. Dependin g on the model and classifier type this processing step will vary.

Threshold computation: prosecutors presentations belonging to a user and impostors presentations are compared or matched to that user model to determine a hard limit(threshold), which would not be considered belonging to the user.

EVALUATION RESULTS

The proposed speaker recognition system is implemented using a MATLAB based program specifically developed for speaker recognition. The program is used initially to create a database of known speakers where the speech signals of these speakers are stored as ".wav" files in the database. Then, the framing, Hamming windowing, MFCC feature extraction, and VQ feature matching methods are used to identify unknown speakers. The following describes the details of the program developed.

Speaker recognition GUI:

The developed program is a Graphical User Interface (MENU type) and is shown in below. Full MATLAB code listing of the program is given in Appendix A. The program consists of a main module (speaker.m) and a number of function files called by the main module.

The program consists of 13 GUI (MENU type) items, and each item is described below briefly:

Load a New Sound File From Disk:

This is the first option of the MENU and it allows the user to add sound files to the database. The files must already exists on a digital medium (e.g. on the hard disk) and must have ".wav" file extensions, duration of the speech file is between 1 and 2 second all the speaker speak the same word "zero". In a typical speaker recognition application a microphone is used to record and save the speech signals

as files on a digital medium. Figure 1 shows the speaker recognition system menu.

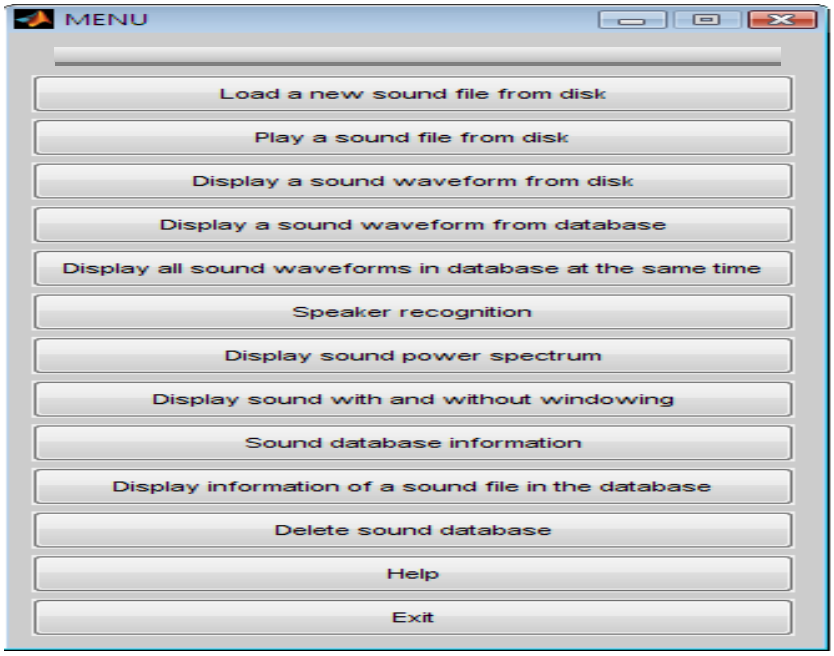

Figure 3.0: Speaker Recognition System Menu

Play a sound from a disk:

This option allows the user to play a selected sound file on the speakers of the PC. After selecting this option, a Dialog Box as in Figure 8 is displayed where the required file can be selected. Figure 2 shows the dialog box for selecting a new sound

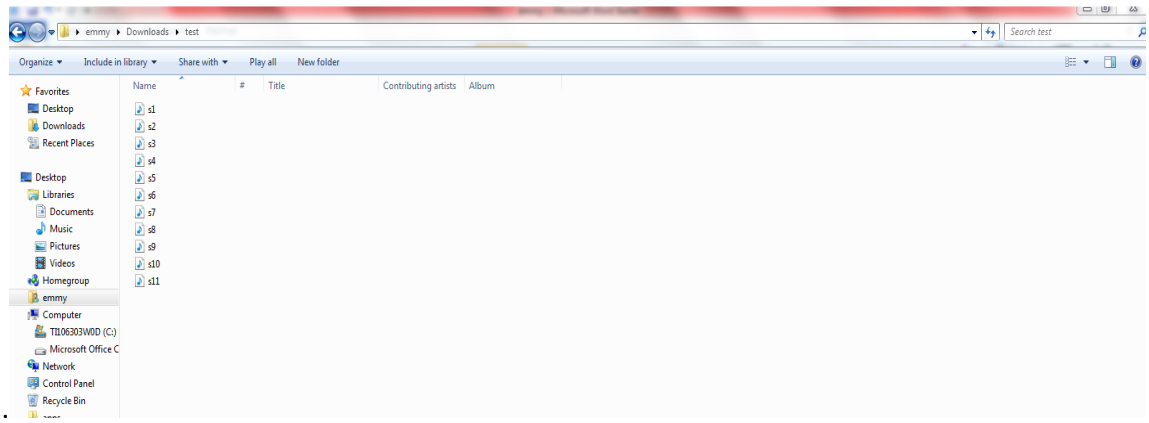

Figure 4.0: Dialog Box for selecting a new sound file

Display a sound waveform from a disk:

This option allows the user to select and display a sound file from a Dialog Box. Figure 3 shows a typical output when sound file called “s2.wav” was selected and displayed

.

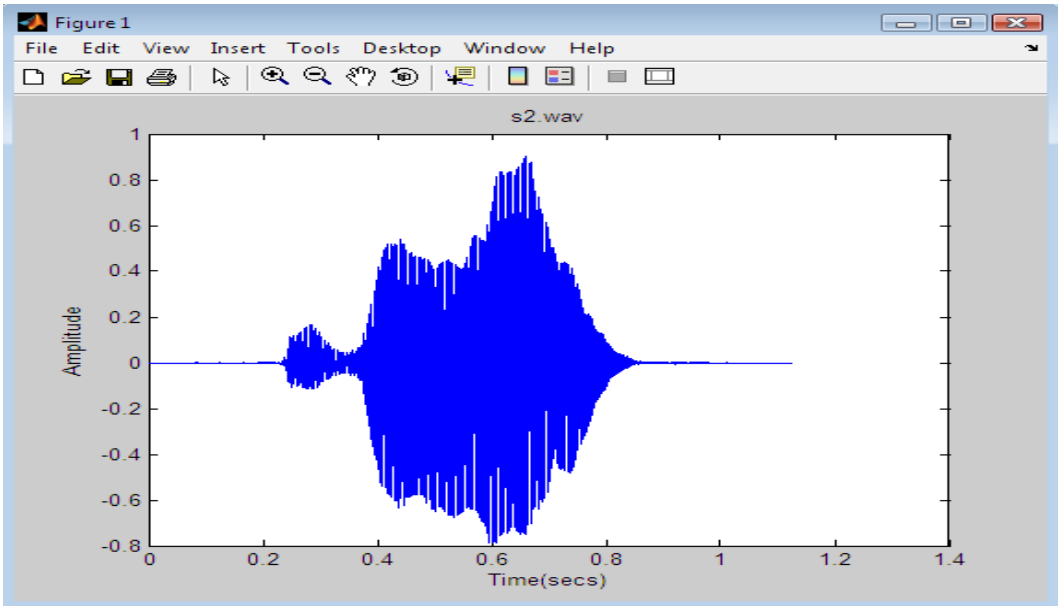

Figure 5.0: The waveform for sound file s2.wa

Display a Sound Waveform From the Database:

This option is similar to option 3, but the sound file to be displayed is selected from the sound database rather than from the disk. As shown in Figure 4, the user is first required to enter the ID number of the file to be displayed.

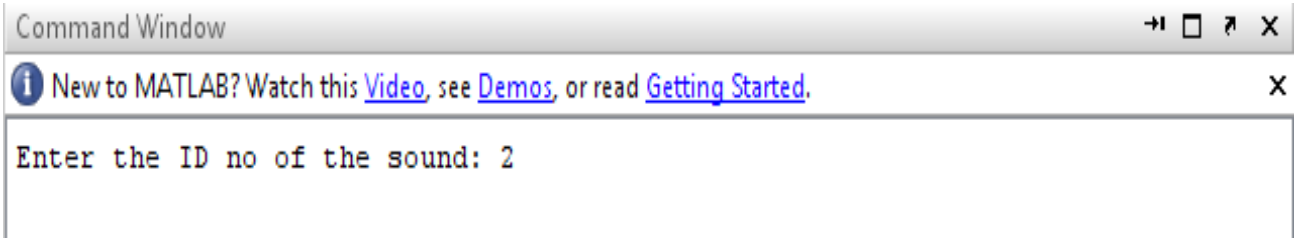

Figure 6.0: Entering ID of the sound file to be displayed

Display All Sound Waveforms in the Database at the Same Time:

This option displays the waveform of all the sound files stored in the database. Figure 5 shows an example output when this option is selected (in this example there were only 3 files in the database named “s1.wav”, “s2.wav” and s10.wav”).

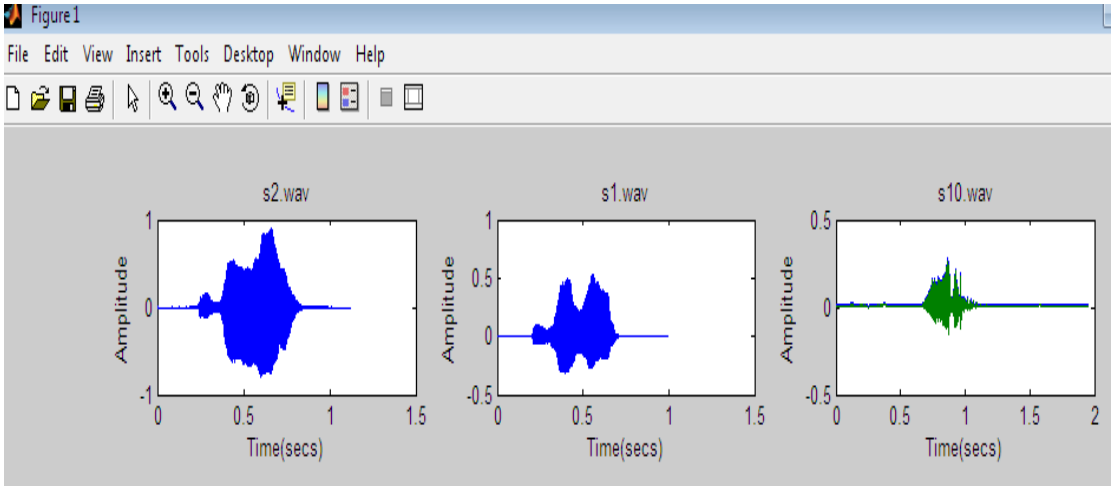

Figure 7.0: Displaying waveforms of all files in the database

Speaker Recognition

This is the main option in this thesis which implements the actual speaker recognition process. When the user click this option a Dialog Box as in Figure 4.6 is displayed, prompting the user to select a sound file to be identified. The MFCC algorithm is implemented in this option to extract the features of the sound file. Then these features are compared using Vector Quantization feature matching algorithm. The identity of the matching speaker is output and displayed by the program.

In Figure 6, sound file with ID of 2 (file "s2.wav") was selected for recognition. As can be seen from the figure, at the end of the program the file has been identified and its ID is displayed on the screen. In this example, there were 3 sound files in the database named "s1.wav2, "s2.wav", and "s3.wav". The program displays informative messages during its execution, such as the creation of matrices containing all the frames, application of the Hamming Window and the FFT, and the determination of the MFCC coefficients.

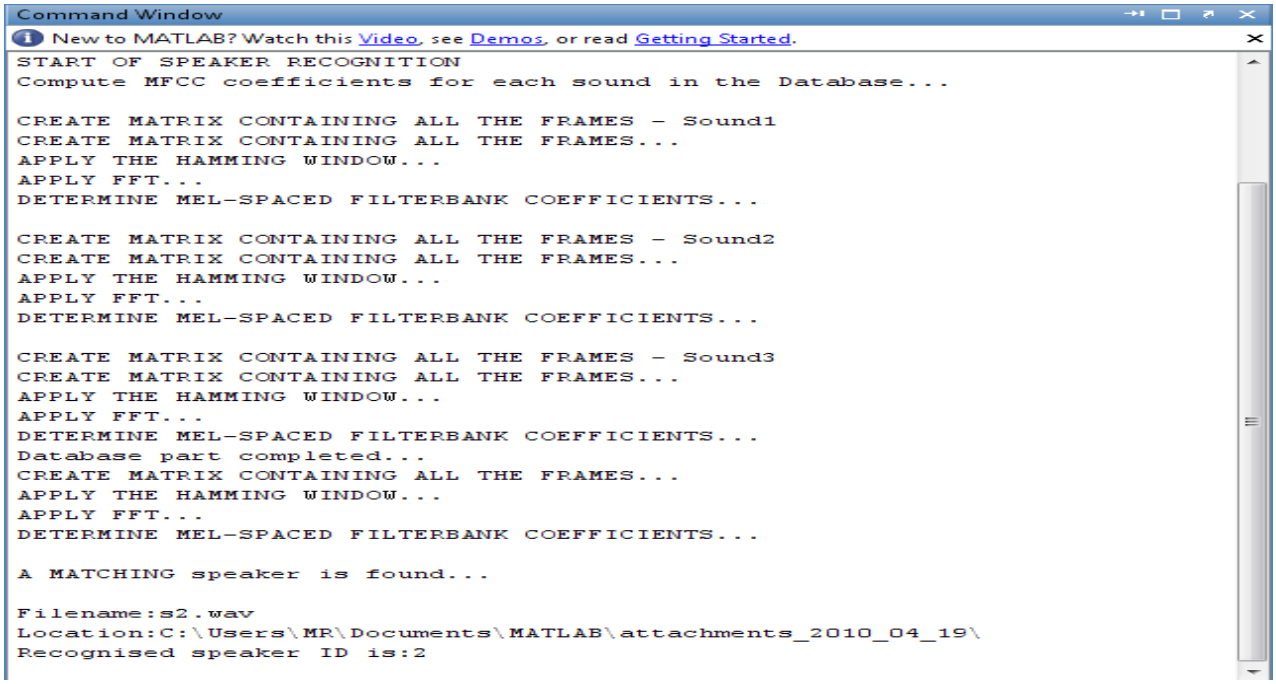

Figure 8.0: The system recognize the selecting file s2.wav

Display Sound Power Spectrum:

This option displays the power spectrum of a sound file selected by the user. Both the linear and the logarithmic power spectrums are displayed. Figure 7 shows a typical output displayed when this option is selected. MATLAB function file "pspectrum.m" is used.

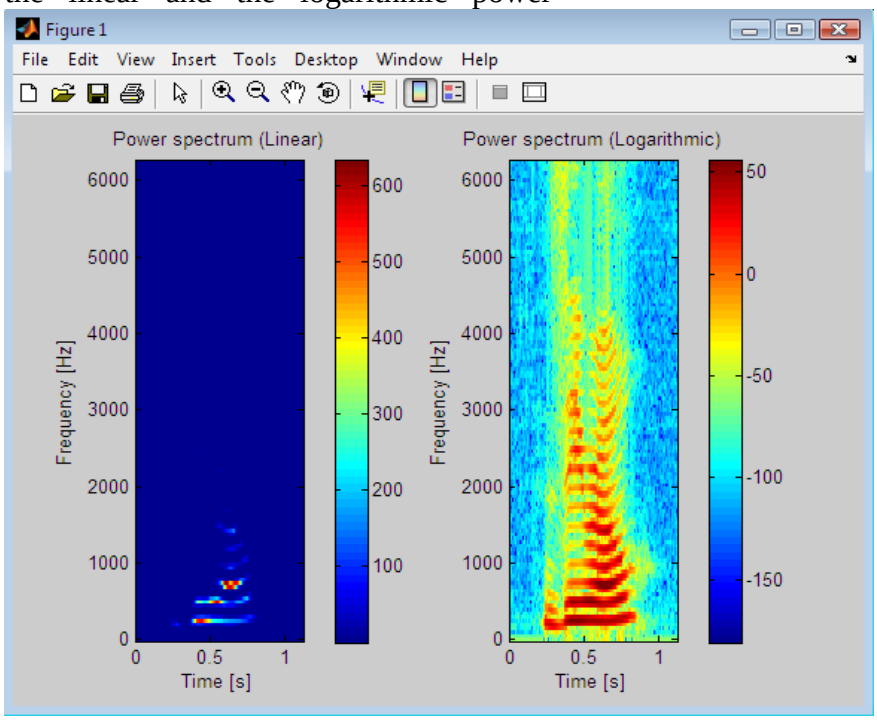

Figure 9.0: Typical Power Spectrum output from the program

DISCUSSION

The developed system was tested using a database with 10 speech signals, stored in the database with names "s1.wav", "s2.wav",……,"s10.wav". using short speech samples taken from the internet, an attempt to recognize a speech sample (s3.wav), among the files in the database was done and clearly yielded a positive result through the program. Though it was time consuming but identification of the speaker was achieved.

Expression of results:

Probabilities of evidence of acceptance or rejection are based under two rival assumptions:

- **Prosecution hypothesis**: the unknown or the test sample is originated from the given source.
- **Defense hypothesis**: the unknown sample originates from some other members of a potential population.

Hence, the ratio between the two hypothesis is known as the **likelihood ratio** and is expressed mathematically as:

Likelihood ratio = Hp/Hd

Where Hp = prosecution hypothesis

Hd = defense hypothesis.

It is important to know that these high numbers do not indicate how likely the voice sample is to have originated from the suspect. It only expresses the relative strength of the evidence.

Modifying the centroids (codebook size)

It was decided to modify the number of centroids used (i.e. the codebook size) in the algorithm and see its effects on the speaker identification rate. The speaker identification rate is defined as the percentage ratio of the number of recognized speakers to the total number of speakers in the database. Table 1 shows the change of the identification rate as the

number of centroids (k) is changed from 1 to 64. It is clear that a higher identification rate is obtained with larger values of k. When k is greater than or equal to 4, the identification rate was 100%. i.e. all the speakers in the database were identified correctly.

Table 1: Identification rate and number of centroids

Number of centroids (k)	Database size (number of speakers)	Identification rate (%)
1	10	80
2	10	80
4	10	100
8	10	100
16	10	100
32	10	100
64	10	100

Table 1: shows the results for code book sizes of k = 1, 2, 4, 8, 16, 32 and 64, and with the noise variance of 0.001. It is clear that the effects of noise is reduced as the number of centroids is increased and the identification rate was 100% when k was greater than or equal to 16. As expected, the identification rate is very high when the noise is small and large numbers of centroids are used. As the noise level increases the identification rate falls sharply, even for number of centroids.

CONCLUSION

The proposed system is a significant research on high level features for speaker recognition and it has proved to be effective in modeling the state of the art recognition system. The proposed system was implemented using MATLAB by integrating both low-level and high-level features. This research has been able to establish that higher level features provide significant complementary information for speaker identification. Higher-level features are increasingly useful as training data increases. Furthermore, because certain higher-level features are inherently more invariant to channel and noise characteristics than are spectral features, they offer the possibility of additional robustness for speaker recognition under degraded acoustic conditions. For the wider area of speaker classification, higher-level features in speech provide far more information about a talker than only his or her identity.

REFERENCES

Adami, A. G., Mihaescu, R., Reynolds, D. A., & Godfrey, J. J. (2003, April). Modeling prosodic dynamics for speaker recognition. In *2003 IEEE International Conference on Acoustics, Speech, and Signal Processing, 2003. Proceedings.(ICASSP'03).* (Vol. 4, pp. IV-788). IEEE.

Alku, P., & Saeidi, R. (2017). The linear predictive modeling of speech from higher-lag autocorrelation coefficients applied to noise-robust speaker recognition. *IEEE/acm transactions on audio, speech, and language processing*, *25*(8), 1606-1617.

Ezzaidi, H., & Rouat, J. (2004, May). Pitch and MFCC dependent GMM models for speaker identification systems. In *Canadian Conference on Electrical and Computer Engineering 2004 (IEEE Cat. No. 04CH37513)* (Vol. 1, pp. 43-46). IEEE.

Ferrer, L., Scheffer, N., & Shriberg, E. (2010, March). A comparison of approaches for modeling prosodic features in speaker recognition. In *2010 IEEE international conference on acoustics, speech and signal processing* (pp. 4414-4417). IEEE.

Gupta, K., & Gupta, D. (2016, January). An analysis on LPC, RASTA and MFCC techniques in Automatic Speech recognition system. In *2016 6th international conference-cloud system and big data engineering (confluence)* (pp. 493-497). IEEE.

Li, L., Liu, R., Kang, J., Fan, Y., Cui, H., Cai, Y., ... & Wang, D. (2022). multi-genre speaker recognition. *Speech Communication*, *137*, 77-91.

Mathur, A., Reddy, S. M., & Hegde, R. M. (2012). Significance of parametric spectral ratio methods in detection and recognition of whispered speech. *EURASIP Journal on Advances in Signal Processing*, *2012*, 1-20.

Prasanna, S. M., Gupta, C. S., & Yegnanarayana, B. (2006). Extraction of speaker-specific excitation information from linear prediction residual of speech. *Speech Communication*, *48*(10), 1243-1261.

Shriberg (2007). "Higher-Level Features in Speaker Recognition," in *Speaker Classification I*, ser. Lecture Notes in Artificial Intelligence, C. Müller, Ed. Heidelberg, Germany: Springer, 2007, vol. 4343.

Shriberg, E., Ferrer, L., Kajarekar, S., Venkataraman, A., & Stolcke, A. (2005). Modeling prosodic feature sequences for speaker recognition. *Speech communication*, *46*(3-4), 455-472.

Singh, S. K., & Pandey, P. P. (2003, November). Features and techniques for speaker recognition. In *M. Tech. Credit Seminar Report, Electronic Systems Group, EE Dept, IIT Bombay submitted Nov* (Vol. 3).

Sreedharan, S., & Eswaran, C. (2019). A review on speaker verification: Challenges and issues. *Int. J. Sci. Technol. Res.*, *8*(8), 956-960.

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PREDICTION OF NIGERIA'S GROSS DOMESTIC PRODUCT (GDP) USING SUPERVISED LEARNING TECHNIQUES

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ABSTRACT

Analyzing an historical data demands computational approaches for inferring dependencies between past and short-term future values. The problem of developing adequate models and prediction techniques is pertinent to the scientific community, financial industry statisticians, and government parastatals. Therefore, this study sought to predict the Gross Domestic Product (GDP) of the Nigerian economy. A dataset was obtained from the Central Bank of Nigeria (CBN) and the National Bureau of Statistics (NBS) ranging from 1991 to 2021, which contains six features and thirty instances used. Four Supervised Learning Algorithms "K-Nearest Neighbor (KNN), Logistic Regression (LR), Support Vector Machines (SVMs), and AdaBoost Classifier (ABC)" and Facebook prophet were used to train and test the dataset. The Model Accuracy (MA) and Cross Validation (CV) were evaluated. LR has a CV score of 27% and MA of 61%, and the nearest model to LR was KNN with a CV score of 11%, and MA of 50%. This shows that the LR is suitable for the study. However, it was observed that the trend shows a downward movement for the prediction, which was a clear indication that the GDP would continue to decline until the year 2031. Furthermore, the researcher recommends that various financial industries like CBN, NBS, Crypto-currency, and FOREX exchange firms should carry out thorough research and embrace the various benefits they can derive from this study.

Keywords: Gross Domestic Product (GDP), Supervised Learning Techniques, Prediction

INTRODUCTION

In the recent time, all modern predicting applications use analytical method by applying different models such as Artificial Neural Networks (ANN), Support Vector Machines (SVM), Hidden Markov Models (HMM), Autoregressive Integrated Moving Average (ARIMA), etc. (Taylor and Letham, 2018) affirms that the major feature of all these different time-series forecasting models is that they work under the reasoning that history does repeat itself thereby using previous data to predict the future. Thereby, they are commonly known as pattern-based forecasting models. In the last decade, forecasting economic time series have been the focus of many researchers. This area of study continues to show relevance from both theoretical and analytical views. Statisticians, financial analysts, and government parastatal use economic historical data to predict current and future investments. Moreover, policy decision-makers sometimes have to make speed choices and are often found to be wrong due to the lack of knowledge of the current economy in their country (Jervis, 2017). Financial policies could be adjusted together with tax policies to curb the recession on the real economy. (Cicceri et al., 2020) believes that major statistical indicators that are publicly released offer many doubts and uncertainties for their economic forecasts as they are released very late and are frequently subject to a major review. To reduce this gap, the problem of developing adequate predictive approaches is relevant to the scientific field, financial analysts, data scientists, and the government parastatal.

He et al., (2021) used the most advanced pre-processing technology, machine learning model, and training algorithm to create a seasonal-trend decomposition-based dendritic neuron model (STLDNM) to address this issue. A different part of the model is to use loess style-based decay (STL) as a pre-processing technology. In particular, STL can extract seasonal and trend characteristics from real data, in order to use a simple polynomial measurement method to manage the sub-series. Next, the remaining components were predicted by a dendritic neuron model (DNM) used to remove overfitting which was trained by an effective back-splitting algorithm. Sezer et al., (2020) categorized various studies according to their target predictive areas, such as indicators, forex, and asset predictions, but also included them based on their DL model decisions, such as Convolutional Neural Networks (CNNs), Deep Belief Networks (DBNs), and Short-Term Memory (LSTM). We also try to visualize the future of the industry by highlighting potential backlogs and opportunities for the benefit of interested researchers.

He et al., (2021) proposed a dual RNN strategy, namely Robust Dual Recurrent Neural Networks (RDRNN), for predicting a series of noisy time series. They built and trained two RNNs at the same time and used the loss rate to separate the different samples to become noise-free samples and noisy samples. They exchanged samples of small losses (possibly non-noise data) to match the main data sequence of the time series, and re-evaluated samples of large losses (potentially noisy data) to minimize the impact of sound. Strong results in three popular Chinese stock market indicators show that the new learning paradigm goes far beyond the basics.

Zhang et al., (2019) introduced a stock market forecast method based on high order HMM. Unlike the first-order HMM that is commonly used, the short-term and long-term dependence are both considered as high-order HMM. By introducing a feature reduction method that can transform a high-velocity vector of HMM into a single, they are introducing HMM trading strategy for high-order predictable trading and trading CSI 300 and S&P 500 index the next day provided historical data. In our approach, we calculate daily returns in

history to show the relationship between hidden regions and price change trends. The CSI 300 test and the S&P 500 index show that high-order HMM has the preferred ability to identify market price than the first order. The high-quality HMM has higher accuracy lower risk than the first-order model in predicting the price index. Katarya and Mahajan (2017) focus on ANN-based financial market prediction studies, while Hu et al. (2015) focused on EC use of stock forecasting and trading algorithmic trading models. Xing et al. (2018) survey natural language-based monetary studies. Finally, there have been application-specific survey papers focusing on specific financial time-series forecast implementation. Among these studies, stock market predictions were qualitative with great interest. Several stock market research forecasts have been published based on various soft computer systems at different times. Bahrammirzaee et al., (2010) analyzed financial prediction and planning studies and other financial applications using various Artificial Intelligence (AI) techniques such as ANN, Expert Systems, and hybrid models. Cao et al., (2019) proposed two-hybrid predictive models that include two types of empirical decay (EMD) and short-term memory (LSTM). A financial timeline is a type of indirect and indirect signal, which can be divided into several internal mode functions of different time measurements with real EMD and complete rotation durability with a variable volume (CEEMDAN). To confirm the impact of historical data on the predictive outcome, LSTM speculative models are developed across the series of features from EMD and CEEMDAN inclusion. The final result of the prediction was obtained by reconstructing each series of predictions. The predictive performance of the proposed models was confirmed by a linear regression analysis of major stock market indicators. Compared to a single LSTM model, a vector support system (SVM), a multi-layer perceptron (MLP) and other hybrid models, the test results show that their proposed models show better performance in predicting a single step forward financial series.

METHODOLOGY

A Python anaconda navigator which has multiple data science packages embedded in it, and updated library like Numpy, Pandas, Matplotlib, Seaborn, Sci-kit learn, StandardScaler, Keras, etc was adopted for this study.

Dataset

The dataset used for this study was gathered from the Central Bank of Nigeria (CBN) and the National Bureau of Statistics (NBS) in 2020. The dataset contains thirty attributes (1991 to 2020) and six features which are year, Unemployment rate, Gross domestic product (target), Inflation rate, foreign direct investment and official exchange rate.

Steps adopted for the Study

1. Data Preprocessing: This is a technique that is used to transform raw data, the steps to deal with the handling of missing data, noisy data, and dataset cleaning. To use the machine learning classification models, the value of the GPD was rounded up to an integer value.
2. Exploratory Data Analysis (EDA): The benefit of this stage is to know hidden information about the dataset such as mean, standard deviation, quartile, minimum and maximum value. Also, the correlation chart among the features of the dataset was produced using the heat plot library.
3. Evaluation Stage: In the methodological evaluation, the classification report and confusion matrix were used as the accuracy metrics. For the forecast model, Root Mean Squared Error (RMSE), Mean Average Error (MAE), Mean Squared Error (MSE), Mean Percentage Error

(MPE), Median Absolute Percentage Error (MDAPE).

4. Testing Stage: To know if the model performed well, unlabeled data (data without Gross Domestic Product (GDP) was fed into the best classification models and the output was explained using LIME. For the prediction, the future GDP of Nigeria was estimated which captures ten years into the future.

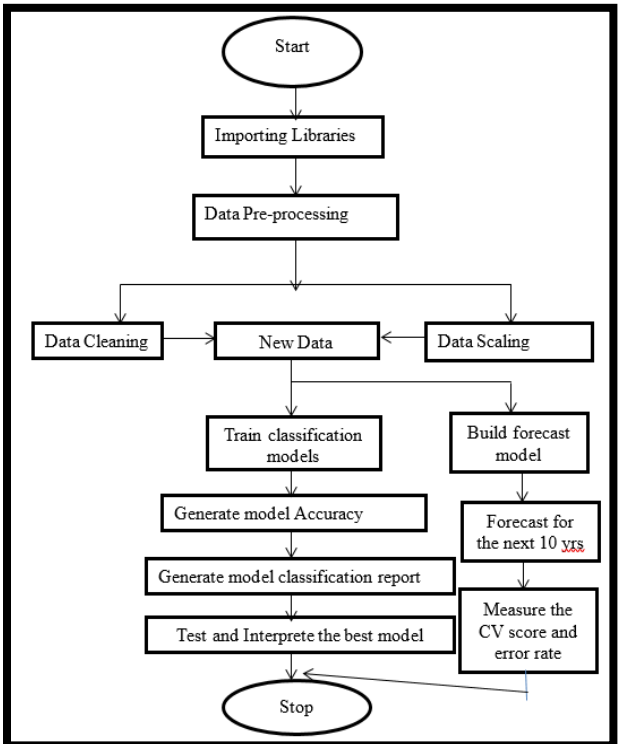

Figure 1: Flowchart of the System (Authors Idea, 2023)

RESULTS

This study was implemented using Intel Core i7, 2.7 GHz processor, 16GB RAM, 500 GB hard disk drive (HDD), and Windows 10, a 32-bit operating system, the model was developed on **python Anaconda Navigator**. Table I shows the Cross Validation (CV) and Model accuracy obtained for the prediction of Nigeria's GDP.

Table 1: Showing the CV score and the Accuracy of the classification models.

Model	CV score (%)	Model Accuracy (%)
ABC	27	39
SVM	28	44
LR	27	61
KNN	11	50

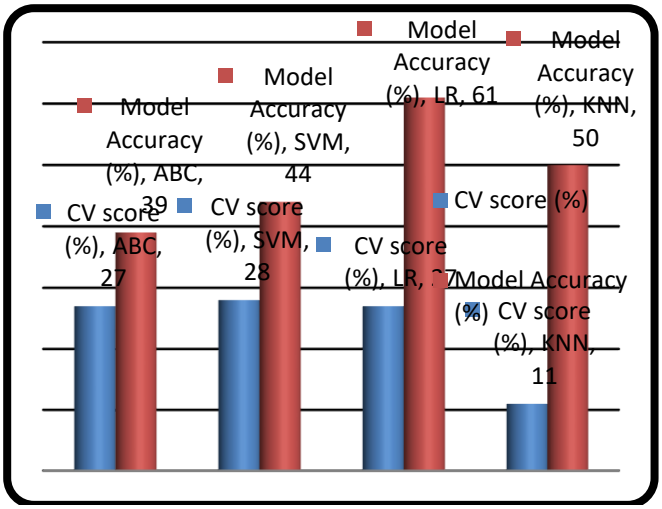

Figure 2: A Clustered Cylinder Depicting the CV and Model Accuracy score.

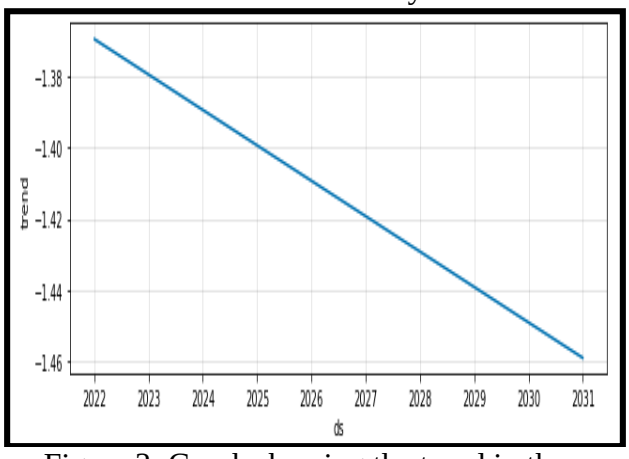

Figure 3: Graph showing the trend in the future GDP

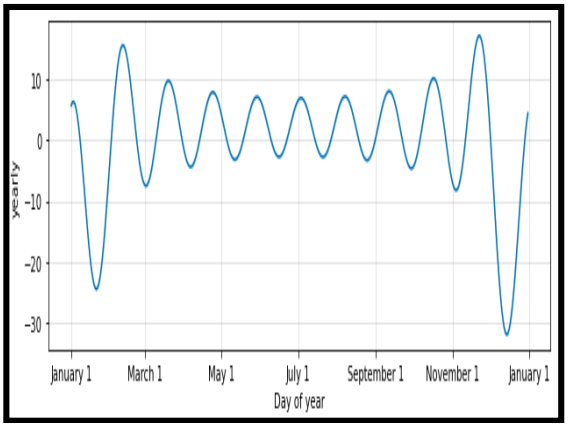

Figure 4: Graph showing monthly trend in the GDP

DISCUSSION

After each model was built, the CV score and the model accuracy for the classification models were measured. It was observed that Logistic Regression has the highest CV score of 27% and model accuracy of 61% while the closest model to it is KNN with a CV score of 11% and model accuracy of 50%. This shows that with this type of data, the Logistic Regression model used (LR) outperformed other models (KNN, SVM, ABC).

CONCLUSION AND FUTURE WORK

This study focuses on the prediction of Nigeria's Gross Domestic Product using supervised learning techniques, after the analysis of the dataset, a downward trend was observed from the year 2022 to 2031, which was a clear indication that the GDP would continue to fall until the year 2031. However, forecasting models such as LSTM, ARIMA, and SMA are yet to be evaluated on the dataset as well as other machine learning techniques such as vectorization and pipelining might prove to be more effective for the study. Also, forecasting the GDP of other countries trading with Nigeria such as the USA and China can be done to know how those countries' GDP affects that of Nigeria to plan for the future.

REFERENCES

Cao, J., Li, Z., & Li, J. (2019). Financial time series forecasting model based on CEEMDAN and LSTM. *Physica A: Statistical Mechanics and its Applications, 519*, 127-139.

Cicceri, G., Inserra, G., & Limosani, M. (2020): A machine learning approach to forecast economic recessions an Italian case study. Mathematics, 8(2), 241.

He, H., Gao, S., Jin, T., Sato, S., & Zhang, X. (2021). A seasonal-trend decomposition-based dendritic neuron model for financial time series prediction. *Applied Soft Computing, 108*, 107488.

He, J., Khushi, M., Tran, N. H., & Liu, T. (2021). Robust Dual Recurrent Neural Networks for Financial Time Series Prediction. In Proceedings of the 2021 *SIAM International Conference on Data Mining (SDM)* (pp. 747-755). Society for Industrial and Applied Mathematics.

Hu, Y., Liu, K., Zhang, X., Su, L., Ngai, E. W. T., & Liu, M. (2015). Application of evolutionary computation for rule discovery in stock algorithmic trading: A literature review. *Applied Soft Computing, 36*, 534-551.

Jervis, R. (2017). Perception and misperception in international politics. Princeton University Press.

Katarya, R., & Mahajan, A. (2017, December). A survey of neural network techniques in market trend analysis. In *2017 International Conference on Intelligent Sustainable Systems (ICISS)* (pp. 873-877). IEEE.

Sezer, O. B., Gudelek, M. U., &Ozbayoglu, A. M. (2020). Financial time series forecasting with deep learning: A systematic literature review: 2005–

2019. *Applied Soft Computing*, *90*, 106181.

Taylor, S. J., & Letham, B. (2018). Forecasting at scale. The American Statistician, 72(1), 37-45.

Tkáč, M., & Verner, R. (2016). Artificial neural networks in business: Two decades of research. *Applied Soft Computing*, *38*, 788-804.

Xing, F. Z., Cambria, E., & Welsch, R. E. (2018). Natural language based financial forecasting: a survey. *Artificial Intelligence Review*, *50*(1), 49-73.

Zhang, M., Jiang, X., Fang, Z., Zeng, Y., & Xu, K. (2019). High-order Hidden Markov Model for trend prediction in financial time series. *Physica A: Statistical Mechanics and its Applications, 517, 1-12.*