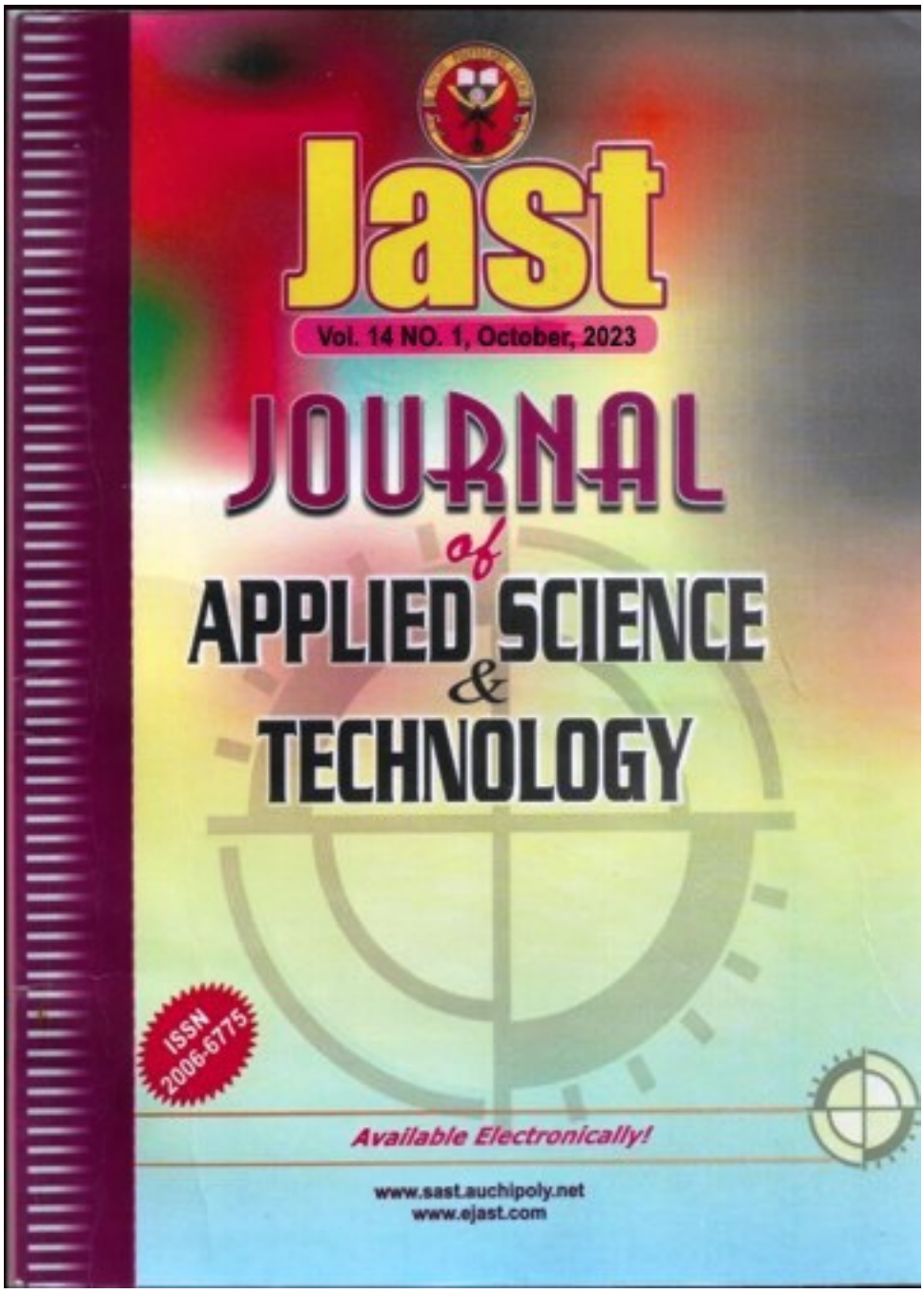
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CONTENTS

CONTENTS

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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,caffeineservesasaningredienti nmanycarbonatedsoftdrinkincludingcolas,pe pper-typebeverages,and citrus beverages.

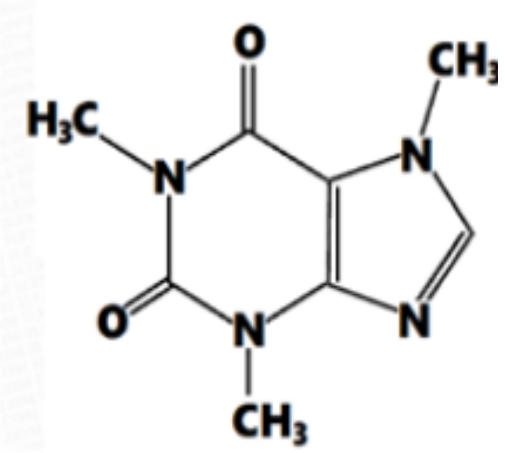

Figure 1 Structure of caffeine

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

MaterialsandMethods

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

ExtractionofCaffeinefromTea

The extraction of the caffeine was determined according to the method of Akinbile *et al.* (2017). The coffee and teasamples (2 g) were eachweighed into conical flask,distilledwater (20 ^{2} wasadded to each of thesampleandthecontentwereheatedandallowed toboilfor 10 min.Sodium carbonate (2g) was added to each sample to precipitate tannins. The samples werefilteredand the filtratesconcentratedto5 mlbyheating, theconcentrate was placed in a separating funnel, chloroform (5 ml) was added to extract thecaffeine 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 Absorbancewasmeasuredat a wavelength of274 nm

Caffeinestockpreparation 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

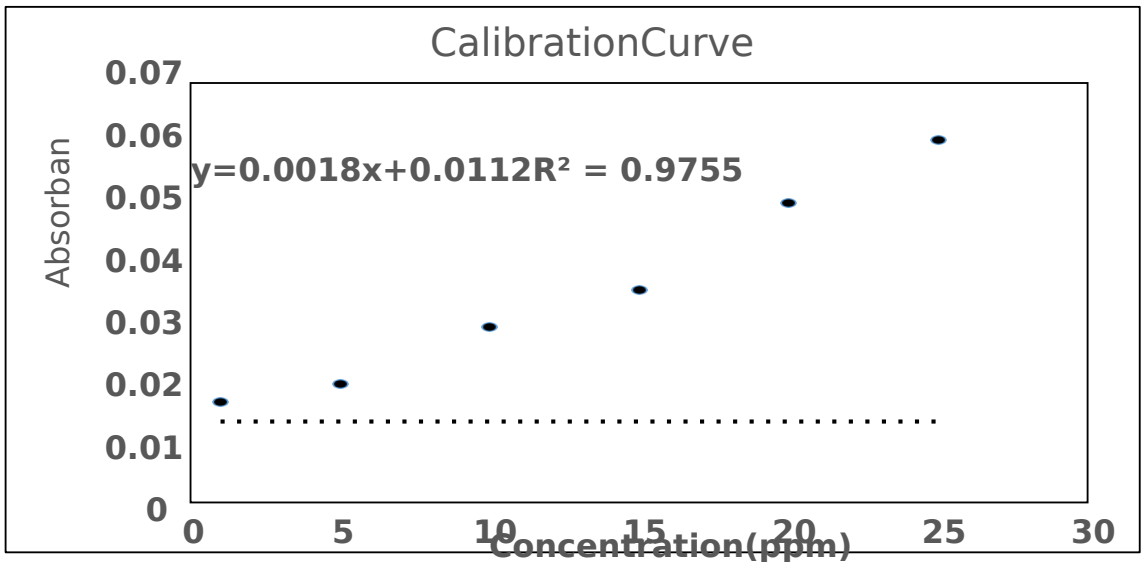

Figure1: Standardcalibrationcurveofcaffeine

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

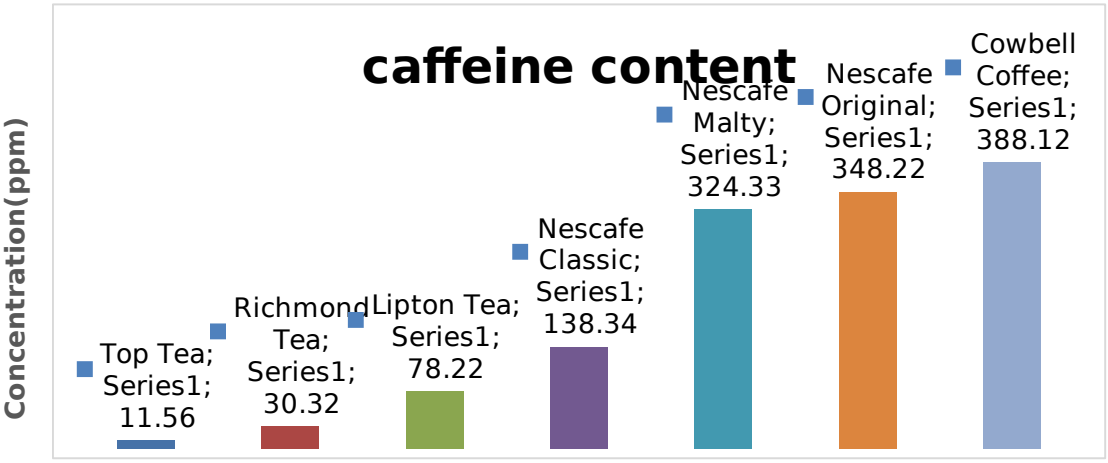

Figure2: Chartshowingcaffeinecontentintheteaandbeveragesamples

Discussion

The standard linear calibration curve obtained from the standard solutionsof caffeine is presented in Table 1 and Figure 1. It showed a good linear relation between theabsorbance and concentrations of standard solutions. Caffeine content levels inteasamplesarepresentedandillustratedinTable2andFigure2.

The concentration of caffeine in 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 thefactorsthatcanhaveaneffectonthe amount of caffeine content include strength of the brew, growingconditions, processing techniques and other variables such as soil chemistry,altitude and position of leaf on the tea bush,type of plant and cultivationpractices.Caffeine contentionalsovarieswidelydependingonthetypeof beanandthemethodofpreparationused (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.

ConclusionandRecommend

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 recomme 5 limits. Caffeine content should be indic 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.

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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 medicativeproperties, 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 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 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 ke
PDA was k

days. After 24hour it was removed from the incubator and endophytes growth was observed. After 3 days the PDA was

Preparation of Nutrient Agar

02

14g of nutrient Agar powder was weigh
using a weighing balance and dispensed in
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

orked with cotton wool wrapped in luminium 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

11

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 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 Sabou broth. Catalase test was positive 12 Endophytic isolates identified was *Bac 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.

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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 to poor production of vegetation (Cl *et al.,* 2014). Nigeria is generally f rapid deterioration of environmental conditions due to the conventional system of collection and dumping of solid wastes. Therefore, waste management has become a major conc 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 contain concentrations of NaCl, ther increasing tendency among farme 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: can-talupensis, inodorus, conomon, dudaim, flexuosus and momordica (Liu, 2004).

Journal of Applied Science and Tec
2023, Pp 15-21

MA MATERIALS
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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 15th 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	MSW Concentration		seedling emergence (%) in days									
			3		6		9		12		**1515**	
100%	100%	0.000	0.00	40.00	13.30	46.60	46.60	53.30	53.30	53.30	53.30	
	50%	0.000.	0.00	13.30	13.30	20.00	20.00	26.60	26.60	33.30	33.30	
Contr	control	0.000	0.00	0.000.	0.00	6.600	6.60	20.00	20.00	26.60	2 6.60	

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

MSW	MSW Concentration		Height (cm) in days									
			3		**7**		**14**		**21**		**28**	
100%	100%	2.332	2.33	4.834	4.83	5.665	5.66	5.665.	5.66	6.336	6.33	
	50%	0.830.	0.83	3.163.	3.16	4.834	4.83	4.834	4.83	5.865	5.86	
Contr	control	0.66	0.66	2.83	2.83	3.163	3.16	3.163	3.16	5.835	5.83	

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

MSW	MSW Concentration		Leaf area (cm^2) in days							
			7		**14**		**21**		**28**	
100%	100%		8.00	22.87	22.87	76.00	76.00	110.0	110.00	
	50%		4.73	21.37	21.37	46.50	46.50	84.50	84.50	
Contr	control	2.302	2.30	16.23	16.23	46.12	46.12	62.18	62.18	

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

MSW	MSW Concentration		Leaf number in days							
			17		**14**		**21**		**28**	
100%	100%		1.30	5.005	5.00	6.306	6.30	7.607	7.60	
	50%		0.60	3.003.	3.00	4.604	4.60	7.307	7.30	
Contr	control	0.600.	0.60	2.602.	2.60	3.603	3.60	6.006	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.

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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)

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

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

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.

Table2 : 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

Samples	Colours	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 4.0×10^3 while dried tomato had the least with 1.8×10^3 and paste tomatoes came after it having a bacteria load of 2.6×10^3. 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.

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

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

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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 (as1^0, 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^{0}C 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 37ºC 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 37ºC. The obtained microorganism is the broth were standardized using normal saline to obtain a population density of 10^8cfu/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 121ºC for 15mins.The media were allowed to cool for 45ºC 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 37ºC for 30mins respectively.

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 at121ºC 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 37ºC 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 121ºC for 15mins and poured into sterile Petri-dishes and left to cool and solidify.The contents of the mean inhibitory concentration (MIC) test tubes (that is the test tubes that showed no growth)were then sub-cultured into the media and incubated at 37ºC 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.

PHYTOCHEMICAL SCREENING OF CASTOR OIL SEED EXTRACT.

Test for Alkaloids

Wagner's reagent or Dragendoff's reagent

Can be used. The formation of reddish brown precipitate and orange precipitate respectively indicate the presence of alkaloids. (Trease and Evans, 1989**)**

Test for Reducing Sugars and Glycosides

Fehling's solutio 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 presence of steroids. (Trease and Evans,1989) **Test for Saponins**

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)

Te 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		0	100	
Escherichia coli	20±0.5		0	200	
Enterobacter aerugenes	12±0.4		0	400	
Klebsiella pneumonia	21±0.6		0	100	
Pseudomonas aeruginosa	20±0.0		0	100	
Staphylococcus aureus	29±0.1		0	100	
Proteus mirabilis	17±0.4		0	300	

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 (Komarov *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

HEMICAL

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.

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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

*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 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 160^{o}C 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. (Munro, 2000; AOAC, 2023).

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

SAPONIN DETERMINATION

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

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

FIBRE DETERMINATION

Defatted sample was weighed into a 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

ue the tion 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:

Strain, 1999). The assay was performed in triplicates.

ID VALUE DETERMINATION

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.

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

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 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°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.

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

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 ABSORBANCE AT 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

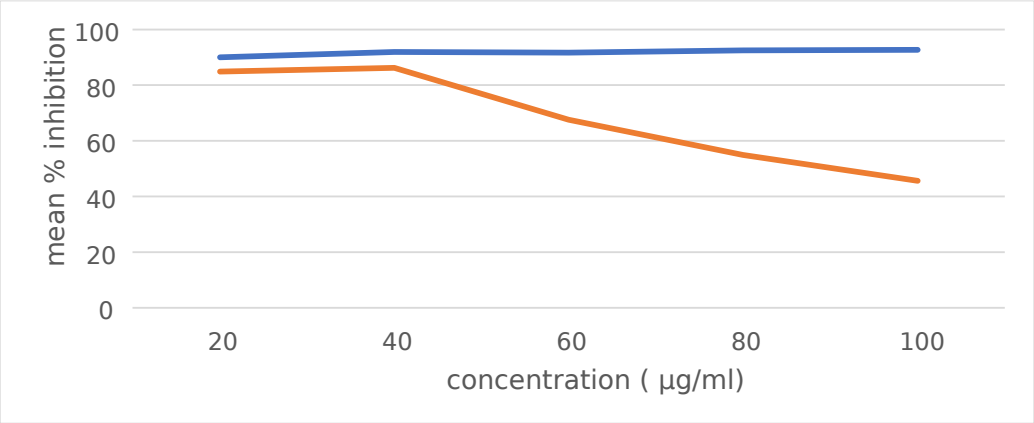

Fig1: DPPH Radical Scavenging Activity of both standard (Vitamin C) and castor oil seed extract

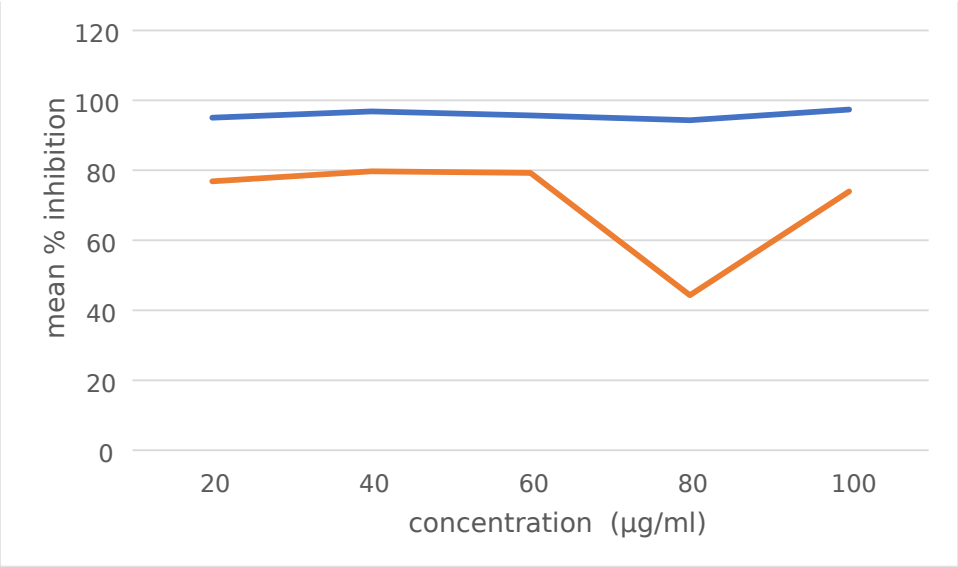

Fig2: Lipid Peroxidation Activity of both standard (Vitamin E) and castor oil seed extrac

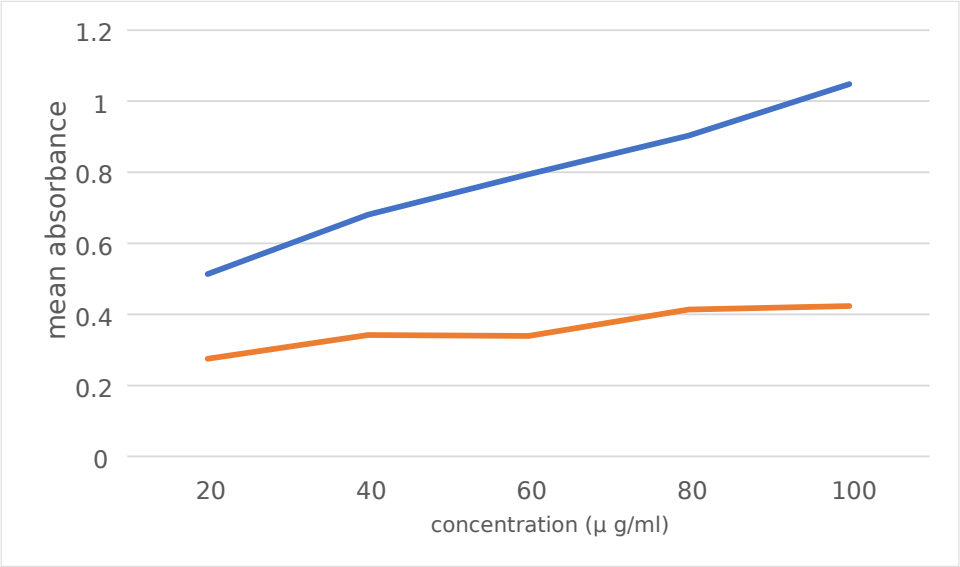

Fig3: Ferric Reducing Power Activity (FRAP) of standard (Vitamin C) and castor oil seed extract.

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 quality of plant fc through reducing certain 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 sapon[illegible]ıg 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

(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 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)

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 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.

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CONSTRUCTION AND PERFORMANCE EVALUATION OF AREMOTE 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.

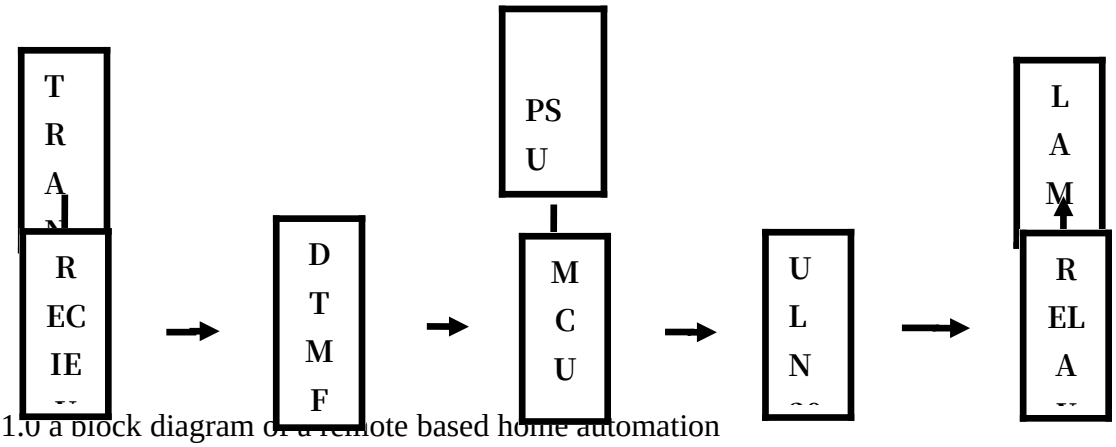


Figure 1.0 a block diagram of a remote based home automation

- **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.

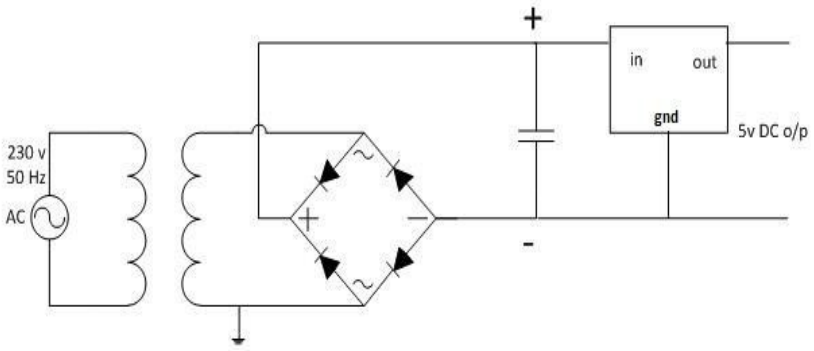


Figure 2.0. a Bridge Rectifier Circuit.

- **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 DT takes a number code from the num 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.

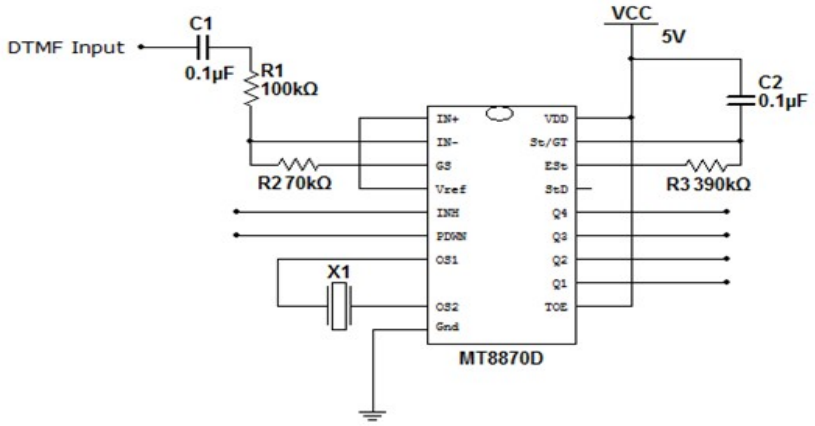


Figure 3.0. DTMF Decoder IC (MT8870**)**

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)

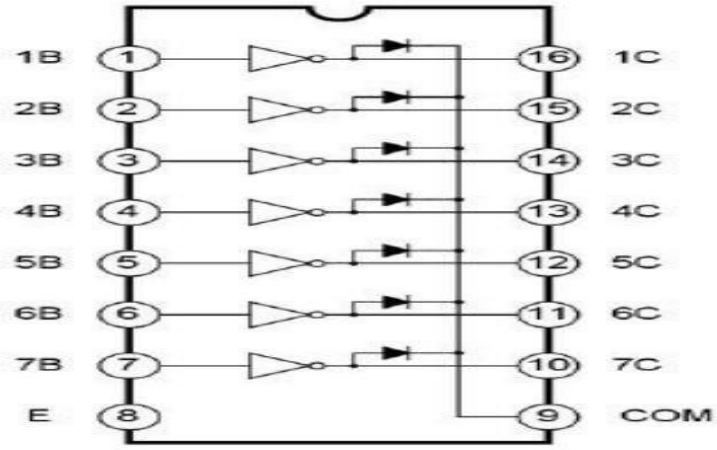


Figure 4.0 ULN2003 IC Logic Diagram

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.

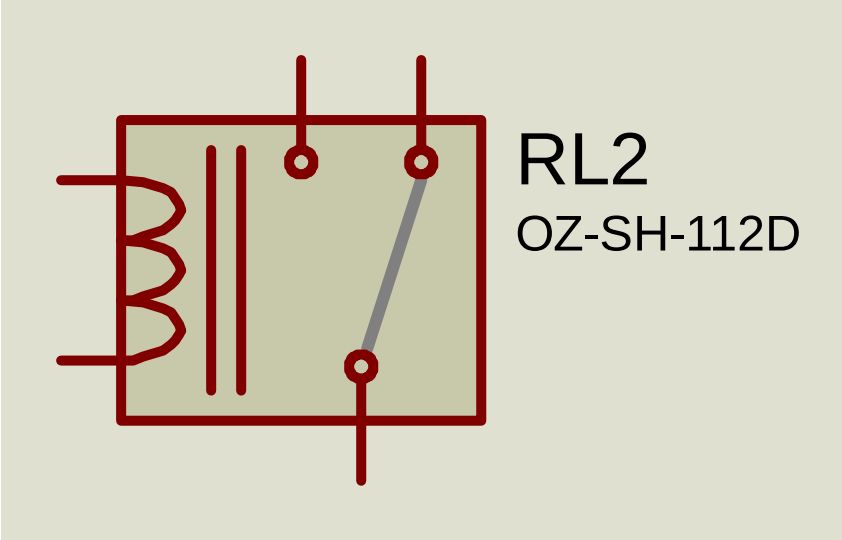


Figure 5.0. A relay

- **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.

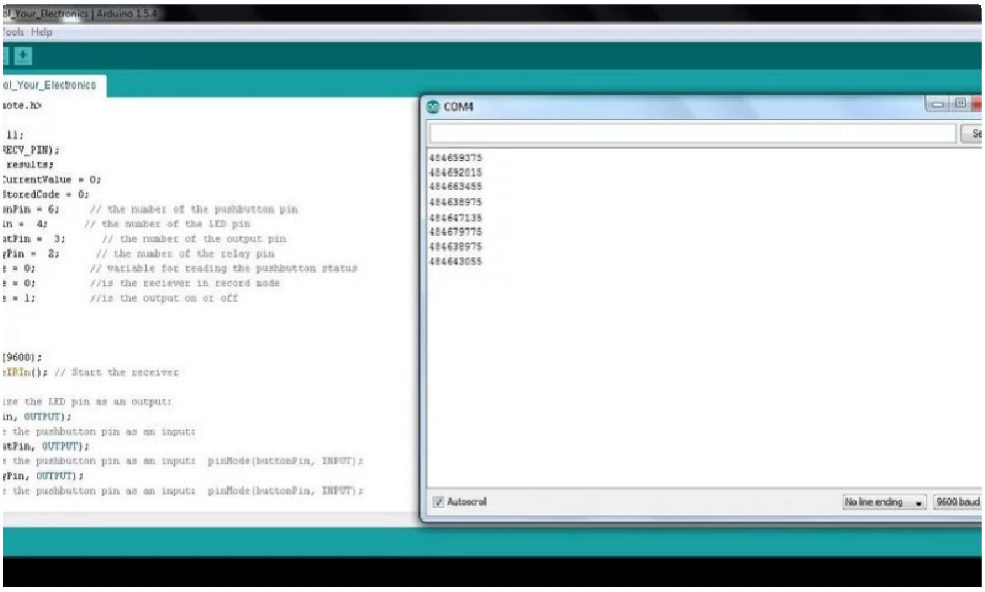


Figure 6.0. ARDUINO IDE showing the HEX number and the codes

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

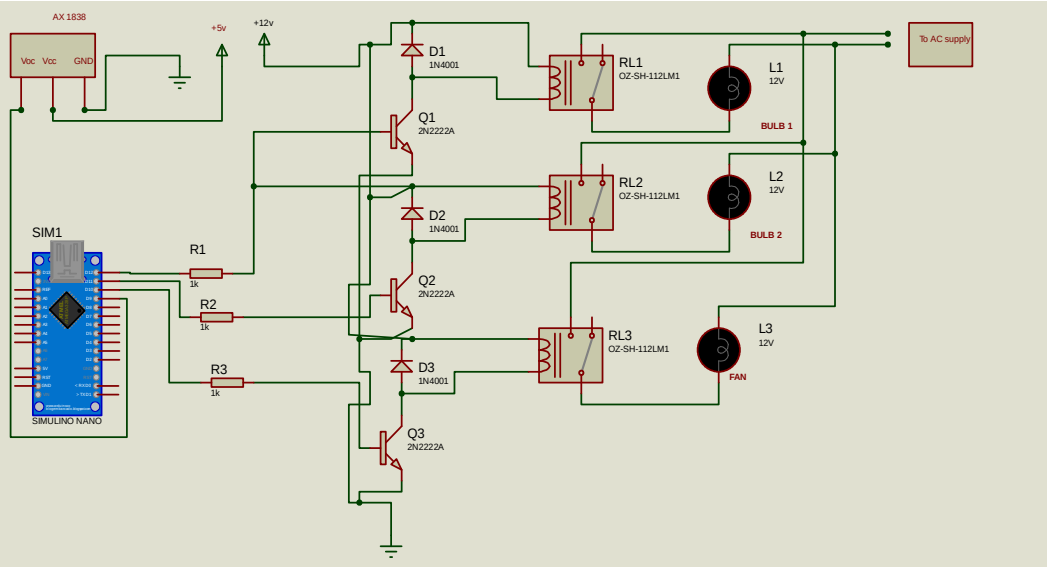
Cutter etcetera.

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.

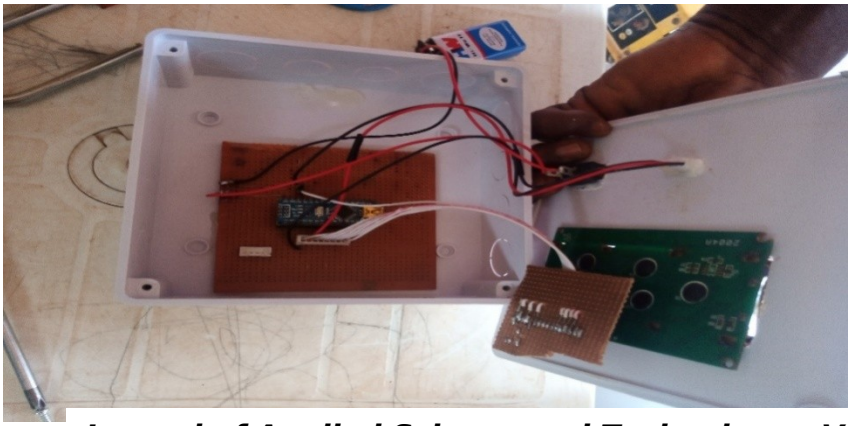


Figure 8.0: The casing of the circuit

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

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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

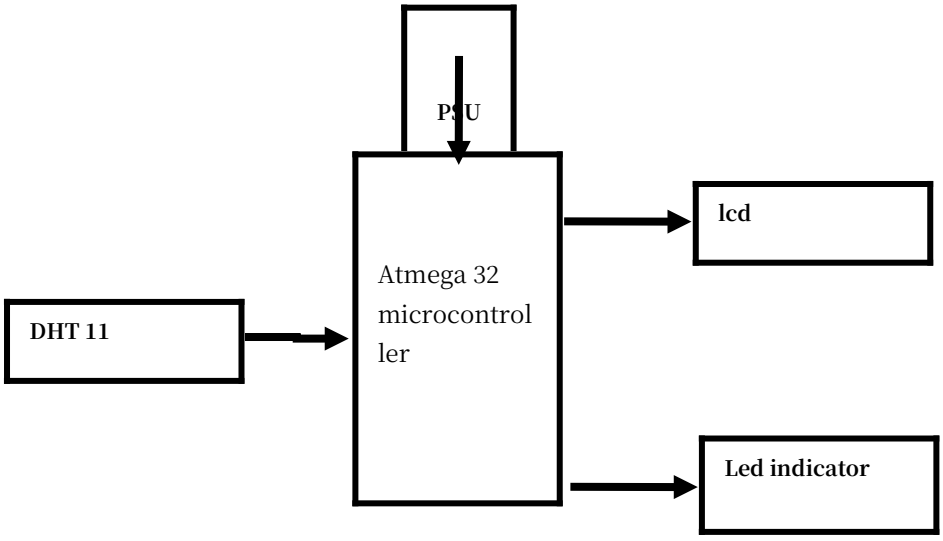

Figure 1.0 Block diagram of Temperature and Humidity Measurement System.

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

the users.

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.

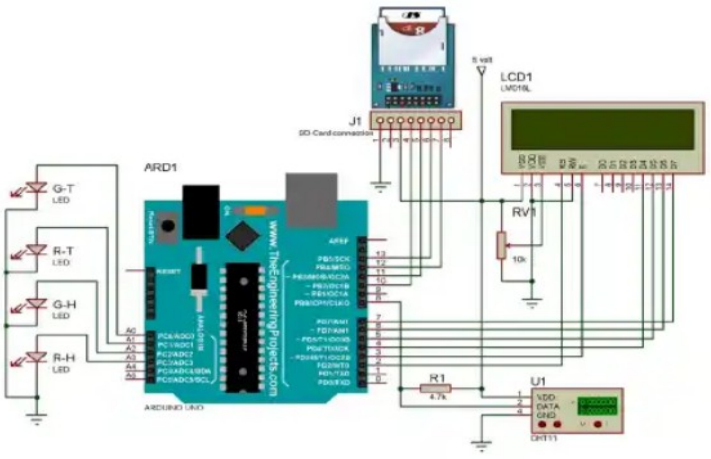

Figure 2.0. Circuit Diagram of Temperature and Humidity Measurement System; source (Sonawane *et al*., 2019)

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

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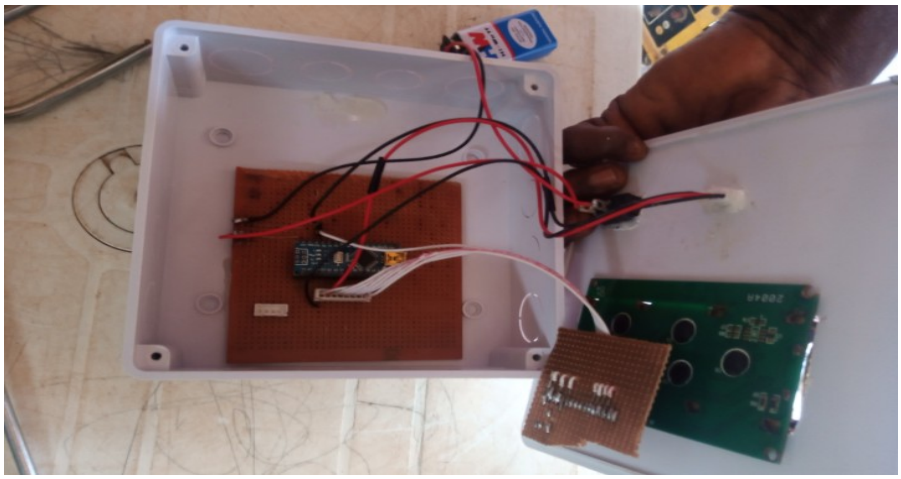

Figure 3.0. Developed Temperature and Humid

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 Pe

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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.

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