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Evaluation of the Total Antioxidant Status of Rats Exposed oo Kerosene, Fuel and Biomass *

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Abstract

This study evaluated and compared the total antioxidant status of rats exposed to kerosene, Fuel and biomass. Twenty-four (24) adult male Wister rats were used for the study. The rats were randomly grouped into four different groups: Group A (control), Group B (exposed to kerosene), Group C (exposed to Fuel) and Group D (exposed to biomass fuel). Blood samples were collected after three weeks and six weeks of exposure by ocular and cardiac puncture respectively. The biochemical analysis was done using Koracevic method. The result of the exposure after three weeks showed an insignificantdecrease (P>0.05) in Group B (1.09 ± 0.06), Group C (1.13 ± 0.15) and Group D (1.14 ± 0.05) when compared with Group A (1.25 ± 0.15). After six weeks of exposure, there was a significant decrease (P<0.05) in Group B (1.00 ± 0.08) and an insignificant decrease in Group C (1.08 ± 0.08) and Group D (1.11 ± 0.06) when compared with Group A (1.33 ± 0.29). The outcome of this study indicated that prolonged or repeated exposure to the above sources of energy especially kerosene could cause reduction in the total antioxidant status. This predisposes the body to oxidative stress, thereby resulting in cell/organ damage.

Keyword: Antioxidants,Biomass,Kerosene, Fuel, oxidative stress, Total Antioxidant Status

INTRODUCTION

Exposure to petroleum products both in and outside petroleum industries have been reported to have some effects on the users, with those who are occupationally exposed being more likely to be affected than their counterparts [1]. Such effects include increased incidence of blood disorders and anaemia, higher cancer risk, renal function impairment and nephrotoxicity [2]. Studies have documented the adverse environmental and health effects of petroleum hydrocarbons over the years. Previous studies reported the cardiotoxic, hepatotoxic, nephrotoxic and haematotoxic effects of hydrocarbons [3]. Mechanism may be dependent on oxidative stress through reduction of antioxidant defense system. Oxidative stress reflects an imbalance between the systemic manifestations of free radicals such as reactive oxygen species (ROS) and the body's antioxidant defense system. This imbalance is due to excessive accumulation of reactive oxygen species or antioxidant depletion or both resulting in cellular damages [4,5,6,7]. Oxidative stress has been associated to the development of a wide range ofdiseases including Alzheimer's disease [8], Parkinson's disease [9], pathologies caused by diabetes [10]and neuro-degeneration in motor neuron diseases [11]. Severe oxidative stress can cause cell injury which results in premature death of cells in living tissues by autolysis [12].

Antioxidants such as glutathione (GSH), uric acid, ascorbate and α-tocopherol are compounds that inhibit oxidation thereby becoming the body's biggest defense against free radicals. Lung effects such as dyspnoea have been associated with high level of exposure to kerosene[13,14]. Exposure to fuel vapor, another product of fractional distillation of crude oil has been reported to increase the risks for acute and chronic health problems [15]. Biomass is an industry term for getting energy by burning wood and other organic matter. Exposure to biomass fuel has been estimated to have caused 0.5% of all deaths and 0.4% of all disability-adjusted life-years in South Africa in 2000[16]. Despite the high use of kerosene, fuel and biomass in Nigeria as sources of energy, knowledge is still sparse on the toxicological effects of their exposure to the human body. Mechanism of action may be through oxidative stress.

METHODOLOGY

Experimental

Twenty-four adult male wistar rats were obtained from the animal breeding unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. After two weeks of acclimatization to the animal house, their average weight was noted. Each animal was randomly assigned to four groups of six animals each: Group A (control, exposed to fresh air), Group B (exposed to kerosene), Group C (exposed to Fuel) and Group D (exposed to biomass fuel). The exposure was done in a whole body wooden inhalation chamber twenty litres in volume. All the groups were kept far from each other and the exposure for groups B and C done five hours daily by placing a plate containing the product of interest in the inhalation chamber. Group D was exposed to smoke produced from heating stove which would burn wood and crop residues in a whole wooden inhalation chamber for one hour. All the animals were allowed free access to feed and water *ad libitum.* Body weight of the animals and mortality data were routinely monitored. The study lasted for six weeks and blood samples collected at three and six weeks for the total antioxidant analysis.

Sample collection

Blood samples were collected after three weeks by ocular puncture and at the end of six weeks by cardiac puncture. The sera were transferred to serum separator tubes, allowed to clot and thereafter centrifuged for 10minutes at 2500rpm.

Total Antioxidant Status (TAS) Determination

The Total Antioxidant Status of the samples were evaluated using Koracevic method [17]. This measure the capacity of the biological fluids to inhibit the production of Thiobarbituric Acid Reactive Substance (TBARS) from sodium benzoate under the influence of the free oxygen radicals derived from Fenton's reaction.

RESULT

The results of the biochemical analysis of the Total Antioxidant Status of the rats exposed to kerosene, fuel and biomass fuel after three and six weeks of exposure are shown in the Tables 1 and 2. The results are presented in mean ± standard deviation.

Table 1: Total Antioxidant Status after three weeks exposure

Groups	TDS (mean ± SD)	p-value
A	1.25 ± 0.15	-
B	1.09 ± 0.06	0.05
C	1.13 ± 0.15	0.26
D	1.14 ± 0.05	0.22

The result of the exposure after three weeks showed an insignificant decrease (P>0.05) in Group B (1.09 ± 0.06), Group C (1.13 ± 0.15) and Group D (1.14 ± 0.05) when compared with Group A (1.25 ± 0.15).

Table 2: Total Antioxidant Status after six weeks exposure

Groups	TDS (mean ± SD)	p-value
A	1.33 ± 0.29	-
B	1.00 ± 0.08	0.04
C	1.08 ± 0.08	0.10
D	1.11 ± 0.06	0.18

After six weeks of exposure, there was a significant decrease ($P<0.05$) in Group B (1.00 ± 0.08) and an insignificant decrease in Group C (1.08 ± 0.08) and Group D (1.11 ± 0.06) when compared with Group A (1.33 ± 0.29).

DISCUSSION

Total Antioxidant Status considers the cumulative effects of all antioxidants present in blood and body fluids [18]. In the present study it was observed that variations exist for TAS level of the study subjects and control. The significant decrease in the TAS of the Group B after six weeks exposure indicates that there was a significant elevation of lipid peroxidation in the wistar rat exposed to kerosene when compared with the control. This observation accords the previous documentations [15]. Exposure to biomass fuel has been estimated to have caused 0.5% of all deaths and 0.4% of all disability-adjusted life-years in South Africa in 2000 [16]. The enhanced lipid peroxidation obtained in petrol attendants when compared with control subjects correlates with decrease in antioxidant defense system in the blood [19].These health effects may be attributed to oxidative stress which may have resulted from the build-up of Reactive Oxygen Species(ROS). The involvement of these ROS in inflammatory responses has also been reported [20].

CONCLUSION

The result of this work suggest that repeated or prolonged exposure to the above sources of energy, especially kerosene may cause reduction in the antioxidant potential of the body, thereby leading to increase in oxidative stress and its associated diseases.

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Decades of Lead, Cadmium and Mercury Contamination in Nigeria Environment, A Review from 1996-2016.

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Abstract

Low-level lead, cadmium and mercury exposure contributes much more toward the causation of chronic disease and impaired functioning than previously thought. Environmental contamination and exposure to them has risen dramatically in the past 50 years as a result of increase in the use of metals in industrial processes and products. A search of the terms mercury, lead, and cadmium exposure, source and poisoning in Nigeria, using Google Scholar search and Africa Journal Online digital library was conducted. The use of industrial processes and products, refuse dumping, absence of poison information centers, and poor record keeping; characterized environmental health in Nigeria. In today's industrial society, there is no escaping exposure to toxic chemicals and metals. The level of mercury, lead, and cadmium in blood depends on the bioaccessiblity rate. Their concentration in blood is a significant factor to public health. Studies conducted regarding their concentrations in the blood and their relationships with environmental exposure will provide useful information to the general public.

Keywords: mercury, lead, cadmium, Nigeria, environment, contamination.

INTRODUCTION

Nigeria has a population of 152,217,341, birth rate of 36.0/1000, infant mortality rate of 92.9/1000, and life expectancy of 50.2 years based on the 2006 census Most of these deaths are preventable because they are associated with the quality of air, water, sanitation, and hygiene that may have been resulted from pollutants in the environment which accumulate unnoticed to toxic levels. In an editorial in the African Journal of Environmental Science and Technology, the top ten environmental challenges of Africa were itemized as follows: water disinfection, air disinfection, solid waste management, lead poisoning, smoke pollution, dust pollution, pesticide pollution, drought and deforestation, petrochemical pollution, and physical injury [QEHIDS WHO 2014, WHO 2014].

However, the Koko Waste Dump episode of 1988 signaled the beginning of consciousness on environmental protection in Nigeria as it led to the establishment of the Federal Environmental Protection Agency in 1988. This Agency has mid-wives many environmental sub-agencies of government at the Federal level, and indeed most states of the Federation now have State Environmental Protection Agencies [Asubiojo 2016].

Sources of Lead in Nigeria

Lead is found in the Earth's crust and has been reported to emit from anthropogenic activities, such as combustion of fossil fuels, mining, paint, batteries production, flooding, drought, eye cosmetics and herbal medicaments [Wright et al 2005, Obi et al 2014], Imported glazed ceramics (e.g., drinking mugs, soup bowls, and cooking pots) [Ketiku and Adeyinka 1999], toys and furniture painted before 1976, children's paint sets and art supplies, pewter pitchers and dinnerware, lead bullets, fishing sinkers, curtain weights Plumbing, pipes, and faucets, hobbies involving soldering, stained glass, jewelry making, pottery glazing, and miniature lead figures [Velaz and O'Connel 2014]. Infant formula, pediatric syrup, canned and non-canned beverage are chief exposure routes for lead poisoning in children [Ikem et al 2002].

Galadima *et al*. (2011) conducted a study on the levels of heavy metals in effluent water from student halls of Usman Danfodiyo University, Sokoto, Nigeria. The results showed the presence of lead at concentrations that are more than 20 times the recommended international limits. The pollution was attributed to continuous use of contaminated water by the students and the disposal of carrier wastes by the sellers of different items in the residential premises of the students. Ladigbolu and Balogun (2011) reported lead concentrations of 405.0 µg/g in soil profile sediments from Ibadan, Oyo State, in which the pollution was attributed to population growth, urbanization, agricultural activities and uncontrolled direct dumping of wastes and sewages into aquatic environment.

In general, some heavy metals are systemic toxins with specific neurotoxic, nephrotoxic, fetotoxic, and teratogenic effects. Moderate exposure adversely affects neuropsychological developments in children leading to decrease in Intelligence Quotient [Huang et al 2014]. High levels of exposure affect haemoglobin synthesis, cause kidney problems and chronic damage to the system.Also abdominal pain and cramping, aggressive behavior, anemia, constipation, slowed body growth, difficulty getting pregnant, difficulty sleeping, headaches, hearing loss, irritability, and loss of previous developmental skills (in young children).

Sources of Cadmium in Nigeria

Cadmium is from a Latin word "cadmia" and Greek word "Kadmeia" which are ancient names for calamine (zinc carbonate). It was discovered in Germany in 1817 by Fredrick Strohmeyer [Hermann 1818]. It is produced mainly as a byproduct from mining, smelting, pigment, cigarettes smokes, automobiles and refining sulphide ores of zinc, and, to a lesser degree, lead and copper. Small amounts of cadmium (about 10%) are produced from secondary sources, mainly from dust generated by recycling iron and steel scrap. It is found in insecticides, fungicides, sludge, and commercial fertilizers which are routinely used in agriculture. Dental alloys, electroplating, motor oil, tobacco smoking and exhaust.

Cadmium found in soil and water can be taken up by certain crops and aquatic organisms and accumulate in the food-chain [IPCS 1992]. Food constitutes the main environmental source of cadmium for non-smokers. Topsoil enrich in sludge contributes to cadmium accumulation in the blood, milk, hair, liver and kidney of sheep, goat, cow, buffalo [Brebner et al 1993, Patra et al 2007, Swarup et al 2005, Balagangatharathilagar et al 2006]. In aquatic ecosystems, cadmium bio-accumulates in muscle of oysters, shrimps, lobsters and fish.

The levels of cadmium exposure through food, water, and air that are typical for most people are not of major health concern. For example, the intake of cadmium from the diet is usually about 0.0004 mg/kg/day, roughly ten times lower than the typical amount needed to cause kidney damage by this route Oral LD50 values for animals, range from 225 to 890 mg/kg for elemental cadmium, 63 to 88 mg/kg for cadmium chloride, 72 mg/kg for cadmium oxide, and 590 to 1125 mg/kg for cadmium stearate [ASTSDR 2008].

Presence of cadmium at higher concentration than the maximum allowable limits in water, vegetation and food have been reported [Agrawal and Raj 1978, Khandekar et al 1980, Allen 1995, Kumar et al 2008, Asagba 2010]. Arinola et al. reported a plasma level of 0.06 µg/dL in male adults in Ibadan, and a mean concentration of 9 µg/dL for adult men in Nkpor and Nnewi (Anambra State, southeastern Nigeria) [Arinola et al 2014,Orisakwe et al 2014]. The mean blood cadmium level of pregnant women/nursing mothers in southeastern Nigeria was 0.99 µg/dL, while that of non-pregnant women was .80µg/dL, with ranges between 0.1–2.8 and 0.1–2.9 µg/dL, respectively [Nkolika and Benedict 2009].

The toxic effects of oral cadmium exposure have been well studied in animals, and a significant body of data from exposed humans has also been accumulated. In humans, most severe cases of oral cadmium toxicity have been associated with ingestion of foods or fluids contaminated by storage in

cadmium-plated containers. Oral exposure to cadmium may result in adverse effects on a number of tissues, including kidney, liver, bone, testes, the immune system, and the cardiovascular system [Jarup 1998].Cadmium has been reported to exert deleterious effects in terms of thyroid function,nephrotoxic, cytotoxic, genotoxic, immunotoxic and carcinogenic [].

It can be detected when about 10 milligrams of cadmium contents has been absorbed either through the skin, inhalation or ingestion. The following signs can help to detect cadmium level in the body levated levels of creatine in the blood and urine may confirm cadmium poisoning generally. When a person is exposed to cadmium over a long period of time and in smaller ATSDR 1999, Lippmann 2000, Risso-de-faverney et al 2001], prostate cancer Martin *et al.* [Martin et al 2002] doses, he or she starts noticing shortness of breath, tooth-staining and weight loss resulting in damaged liver and kidney, sweet or metallic taste in the mouth, increased amount of saliva, vomiting, choking, anemia, abdominal pains, spasm of ingested cadmium, chest pain, wheezing, inflammation of the lungs, weakness in muscles and leg pain, diarrhea, stomach pains, severe vomiting, bone fracture, reproductive failure and infertility, damage to the central nervous system, damage to immune system, psychological disorders, possibly DNA damage or cancer development and eventually lead to death. Cadmium is one of six substances banned by the European Union's Restriction on Hazardous Substances (RoHS) directive [Abimbola et al 2012].

Sources of Mercury in Nigeria

Mercury is one of the most toxic elements and a threat to human being because it accumulates and magnifies to unsafe levels in food chains [Madison 2007], it cannot be removed and is rapidly transformed by microorganisms into organic compounds (methyl mercury) that tend to bioaccumulate and biomagnify in animals [Roncheti et al 2006]. All mercury species is toxic, with organic mercury compounds generally being more toxic than inorganic species [Leopolda et al 2010].

Mercury is a major non-essential trace metal not needed in food. Mercury and its compound are non-biodegradable and so have adverse impact on the environment as they bio-accumulate in the ecosystem. So, they persist in the environment for an extended period killing important microorganisms in the environment It can travel globally through air, soil and water bodies and accumulates in organisms through food chain.

Air- Metallic, or elemental mercury, is liquid at room temperature and like any other liquid it evaporates into the air, where it can be inhaled. The remaining emissions are in the form of gaseous inorganic ionic mercury (such as mercuric chloride) or bound to emitted particles. Thermometers and sphygmomanometers contain mercury and so do many medical batteries, fluorescent lamps and electrical switches. These forms have a shorter atmospheric lifetime and will deposit to land or water bodies within roughly 100 to 1,000 kilometres of their source. Skin lightening cream, combustion of fossil fuels, where mercury is emitted, coal combustion, is currently the main source of mercury pollution.Coal combustion is worldwide, contributing between 750 and 1500 tons per year. The electrical and electronic manufacturing industry has been one of the largest users of mercury in the world for at least two decades. According to Electronic Industries Alliance [EIA 2005], mercury can be found in at least 26 categories of electrical and electronic devices, including electrical lighting, switching devices, control instruments, thermostats, laptop/notebook computers, mobile phones, and semiconductors [EIA 2005].

According to the minister, studies mercury used in dental sector harms the environment through wastewater from dentists' offices, improper disposal of mercury amalgam, releases of mercury vapor, cremation of people with mercury in their teeth and fecal excretion from patients with amalgam fillings. Speaking to icirnigeria.org, Oladele Osibanjo, executive director of Basel Convention and Leslie Adogame of Sustainable Research and Action for Environmental Development observed that poor awareness, lack of education about Mercury, and weak regulation all encourage importation of mercury products thus driving pollution. As such its presence in food suggests contamination [Leopolda et al 2010]. Mercury concentration permissible limit is 0.001 mg/L as specified by World

Health Organizationand Standard Organization of Nigeria.

Mercury has no known function in human biochemistry but causes damage to the brain and the central nervous system. It is a heavy metal that exists in liquid form, at room temperature. It is of major public health concern because it is inimical to human health. Exposure to high levels of metallic, inorganic, or organic mercury can permanently damage the brain, kidneys, cardiovascular systemand developing fetus (neuro-developmental deficits). Effects onbrain functioning may result in irritability, shyness, tremors, changes in vision or hearing, and memory problems [ASTDR 1999]. It affect fertility, blood pressure regulation and can cause tremors, and numbness of the fingers and toesLow concentration of mercury can induce the major constituent of intracellular protein inclusions in dopaminergic neurons and can lead to some generative disease such as Alzaimer's and Parkinson's diseases [Zahi et al 2005]. Reduced sensation and strength in the arms and legs, muscle cramps and decreased nerve conduction have been observed.

The minamata diseases presented mostly with neurological symptoms that include prickling, tingling sensation, paresthesia; impair peripheral vision, hearing, small and fast; slurred speech, unsteadiness of gait and limb, muscle weakness, memory loss, irritability, inability to sleep and depression. Renal damage occurs because mercury bio-accumulates in the kidney and often manifest as frank proteinuria, hematuria and oliguria; these could results to acute renal failure, with degeneration or necrosis of the convoluted tubules [Odinioha 2012].

Mercury determination can be achieved by pre-concentration, separation and detection of total dissolved mercury. The most common technique used to monitor total mercury in natural waters is cold vapor atomic fluorescence spectrometry [Salameh et al 2013].

Role of Nigerian Government

Nigerian government through the Federal Ministry of Environment should engage in variety of activities to phase out these contaminants: promoting use of clean energy in industrial activities, enforcing proper use and disposal of products that contain them, discouraging the use of mercury in gold mining, developing laws to protect the health of Nigerian citizens and finding lasting alternatives to their use

Both environmentalists who are currently attending the week-long (Nov 3rd-7th) Mercury Negotiations in Bangkok, Thailand, advocate for a mercury-free Nigeria and call upon African countries to impress upon the exporting nations and funding organizations to halt the toxic trade of dental mercury into Africa [Abiose 2014].

In order to phase out mercury, Nigeria became a signatory to the Minamata convention on mercury; a global environment treaty, on 10th October, 2013.

The Role of Individual in Lead, Cadmium and Mercury Contamination Control

A good strategy for reducing emitted these contaminants are by using flue gas cleaning during combustion. Therefore, all new generation sources using fossil fuels and waste incinerators should be equipped with efficient flue gas cleaning systems at the time of construction. Do not buy product that contain them. Also discard every found product under your care that contain any of them

Conclusion

There is need to assess elevated blood lead, mercury and cadmium levels across populations in Nigeria by undertaking sound scientific studies using appropriate sample sizes and methodologies. From the information provided, heavy metals under study are much more released due to the use of industrial processes and products, refuse dumping, absence of poison information centers, and poor record keeping. Nigeria Government and their citizen must put hands together to see that these contaminants are reduced to minimal by playing their respective roles as stated, to improve health of the masses

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Antimicrobial Activity And Phytochemical Analysis Of Methanolic Extract Of *Psidium Guajava* (Guava Leaf)

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ABSTRACT

Methanolic extract of leaves of Psidium guajava (Guava leaves) was screened for its phytochemicals and antimicrobial activities on some clinical bacterial isolates, such as Pseudomonas Spp, Staphylococcus aureus, Proteus mirabilis, Escherichia coli, and Vibrio cholera. Agar diffusion technique was used to assay the growth inhibition against the five bacterial isolates. The antibacterial effect of the methanolic extract of Psidium guajava was compared to that of some selected commercially available antibiotics. Results obtained showed the extract inhibits the growth of the test isolates with diameter of zones of inhibition of 5.2mm to 19.0 mm for Vibrio cholera, Escherichia coli, Proteus mirabilis, S. aureus and Pseudomonas spp respectively. The Broth microdilution assay gave minimal inhibitory concentration values ranging from 12.5ugml to 16.0 ug/ml. The result of the phytochemical revealed the presence of saponins, alkaloids, carbohydrates, etc.

Keywords: Guava, Phytochemical, Antimicrobial, methanol

INTRODUCTION

Plant leaves have been used as herbal medicine for their healing properties since ancient times [2]. The medicinal activities of various plant materials and extracts have been recognized since the beginning of the 6th century[4]. Some bioactive compounds within these plants are responsible for their medicinal value[1]. The most prominent of these bioactive compounds are alkaloids, saponins, tannin, flavonoid, phenolic compounds and carbohydrates[5]. Their concentrations may vary in different plants which result in unique medicinal properties for a specific plant.[6]

Leaves and bark of guava plant are well recognized for the treatment of gastrointestinal disorders, diarrhea, colds, tooth aches and inflammations[2].

Plants extracts have been used in folk medicinal practices for the treatment of different types of ailments since antiquity[7]. In spite of the great advances achieved in modern medicine, plant still make an important contribution to health care.This is due to the recognition of the value of traditional medicinal systems [8]. Constant consumption of guava leaves is considered to provide protection against lung, esophagus, pancreas, liver, breast, colon and skin cancers induced by chemical carcinogens[5]. Guava leaves are capable of preventing hepatitis and controlling diabetes. And it is also known to be highly effective in healing of burns and bruises.[10]

Medicinal plants are of great importance to the health of individuals and communities.[1] During the last few decades, the global interest has increased rapidly due to their antibacterial and antioxidant activities, low toxicity and the potential to be a cheaper alternative to costly synthetic drugs.[11] The determination of antibacterial activities of different medicinal plants is of special interest these days due to the current global issue of increasing antibiotic resistance of microorganisms.[2] It is assumed that the drug resistance in pathogenic microorganisms is developing due to indiscriminate use of commercial antimicrobial drugs.[3] Antimicrobial resistance threatens the prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi.[6] Therefore, it is highly imperative to determine compounds which can be used to develop novel medicines with higher antimicrobial properties.[11]

Psidium guajava is indigenous to India but grown widely in West Africa including Nigeria [12]. Recent studies on *Psidium guajava* proved it to be a useful medication for people living with upper respiration

tract infection and pneumonia.[7] This study was conducted to determine the antimicrobial properties in Guava (*Psidium guajava*) leaves available in Nigeria. The primary objective of the study was to add to the progressive research works related to the antimicrobial activity of plants.

MATERIALS AND METHODS

Sample Collection

Guava (*Psidium guajava*) leaves were collected from farmlands at Nodu Village, Okpuno, Awka South L.G.A. Anambra State, Nigeria.

SOURCE OF TEST BACTERIAL ISOLATE

The clinical isolates of *Pseudomonas Spp*, *Staphylococcus aureus, Proteus mirabilis, Escherichia coli and Vibrio choleriae* were obtained from a Diagnostic and Bacteriology Laboratory, 133 Zik Avenue, Awka, Anambra State, Nigeria.

Pure cultures of each of the bacterial isolates were obtained by culturing the isolates on their selective media. The biochemical and physiological tests were performed to re-identity and confirm the purity of the isolates.

PREPARATION OF PLANT EXTRACT

The fresh leaves of Guava (*Psidium guajava*) were harvested and washed carefully in running tap water and then rinsed in distilled water.The leaves were air dried at room temperature (25^{o}C) before grinding into fine powder using mortar and pestle. The provided powder obtained was store in airtight glass containers protected from sunlight until required for analysis.

CRUDE-EXTRACT PREPARATION

For the preparation of crude extract 500g of grinded materials of *Psidiumguajava* leaves weresoaked in methanol in round bottom flask for about 24 hours. The extracts was filtered into a round bottom flask. The process of filtration was recurring for 3 days using supplementary concentration of methanol (300ml, 200ml and 100ml) earlier in the filtration process. The extracts were dried using evaporationunder water bath.

FRACTIONATION OF CRUDE EXTRACT

10g of crude plant extract was added into a round bottom flask. Different concentration of distilled water (200ml, 150ml and 100ml) was poured to the extract contained in flask for three times. Then through separating funnel, filtration was done. For concentration of water extract, process of rotary evaporation was performed under reduced pressure. **PHYTOCHEMICAL SCREENING**

Methanol extract were assayed for phytochemical constituents such as alkaloids, saponins, flavonoids, phenol, glycosides, terpenoids, steroids, reducing sugar, tannin, emodin, fatty acid, anthocyanin, coumarin, starch and protein using standard procedures.

TEST FOR ANTIBACTERIAL PROPERTY OF *PSIDIUM GUAJAVA*

Susceptibility tests were carried out using the modified agar diffusion method of Garrod, et al (1981) and Irobi (1992). Commercial antibiotics were used as positive reference standard to determine the sensitivity of the isolates.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

The minimum inhibitory concentration was determined using the method of Rusell and Fur (1977). The methanolic extract at concentration of 45mg/ml, 85mg/ml, 145mg/ml and 155mg/ml were added to molten sterile nutrient agar (oxoid) aseptically and thoroughly mixed together in a sterile petri dish. This was then allowed to set, the surface of the agar was allowed to dry properly before streaking with the appropriate bacterial isolate. The plates were then incubated at 37^{o}C for 72 hrs. The lowest concentration preventing all visible growth was taken as the minimal inhibiting concentration.

RESULTS

The methanolic extract of *Psidium guajava*showed antimicrobial activity against all the test organisms with the highest activity on *Escherichia Coli* and the least with *Pseudomonas spp*(Table 1). Partial purification of the crude extract by TLC showed two components with Rf values: 0.85cm and 0.88cm (Table 3) respectively.

The phytochemical screening of the crude extract revealed the presence of alkaloids, saponins, carbohydrates, etc. (Table 2). The presence of these phytochemicals/bioactive compounds is a confirmation of the importance of *Psidiumguajava*, serving a good purpose on the treatment of diarrhea and dysentery.

TABLE1: Susceptibility of Test Organism to the Crude Extract and the Standard Reference Antibiotics

Test Organisms/ Crude Extract/ and Antibiotics	Diameter Zone of Inhibition (mm)				
	Pseudomonas spp	***Staphylococcusaureus***	***Proteusmirabilis***	***Escherichiacoli***	***Vibriocholerae***
Methanolic Crude extract	5.2	8.50	11.0	12.0	6.0
Ampicillin	6.3	17.0	8.80	9.50	18.0
Oxacillin	17.0	10.0	-	10.0	-
Nitrofurentin	16.0	-	-	11.0	15.0
Tetracycline	15.0	14.0	-	-	-
Vancomycin	17.0	-	-	12	-
Piperacillin	-	-	19.0	-	-

Key:- = no zone of inhibition

TABLE 2: Phytochemical Composition of *Psidium guajava*Extract

Phytochemical compounds	Status
Flavonoids	+
Phenolic Compounds	-
Carbohydrates	+
Tannin	+
Saponins	+
Alkaloids	+
Reducing Group	-

TABLE 3: Purified Spots of the Two Components the Rf Factor

Spot	R_f – Factor
C_1	0.85
C_2	0.88

DISCUSSION

Plant extracts have been used in folk and even modern medical practices for the treatment of different ailments, most of which are due to the bioactive components of plants. Naturally occurring substance of plant origin have been reported to inhibit the growth of microorganisms. Bacterial infections seems especially controllable due to the availability of effective antimicrobial drugs. The inevitable consequence of the application of antibacterial drugs is a development of resistance to antibiotics.[5]

In traditional medicine, a plant is simply eaten raw, cooked or infused in water or even prepared as food while in orthodox medicine, a plant may be subjected to several chemical processes before its active ingredients are extracted.[4] The results presented in this paper shows the crude extracts of *Psidiumguajava*possesses antimicrobial activity against the common gram-negative and gram-positive organisms, thus confirming the use of the plant in the treatment of diarrhea and dysentery.

The antimicrobial & phytochemical screening and quantitative estimation of crude extracts of the chemical constituents of *Psidiumguajava*studied showed that the extract was rich, in alkaloids, saponins and carbohydrates. *Psidiumguajava*is a potential source of useful drugs.

CONCLUSION

All the extracts of *Psidiumguajava*are rich in various phyto-constituents. The methanol extract can act as standard drug against bacterial strains as it showed more inhabitation zone than the standard drug piperacillin. The test gave validity to the traditional use of *Psidiumguajava*as a natural antimicrobial.

It is therefore, recommended that further studies should be carried out on the efficacy of the crude plant extract to enhance the primary health care delivery systems in the developing countries.

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Proximate Assessment And Heavy Metal Load Of Two Major Instant Noodles Sold In Aguata Local Government Area, Anambra State. Nzekwe, ABC & Mgbechi, C.E

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Abstract

Proximate and mineral analyses were conducted using standard methods on two major instant noodle samples namely indomie and tummy-tummy. The proximate result for indomie were: moisture (15.10%); ash (6.87%); fibre (4.50%); fat (8.04%); protein (11.55%); carbohydrate (54.01%) while for tummy-tummy: moisture (19.31%); ash (1.34%); fibre (2.50%); fat (14.14%); protein (13.30%) and carbohydrate (49.41%). The mineral content of indomie samples were: magnesium (17.009ppm), iron (19.152ppm), cadmium (0.050ppm), lead (0.000ppm), copper (0.200ppm) and zinc (2.268ppm) while that of tummy-tummy are: magnesium (8.064ppm), iron (2.846ppm), cadmium (0.022ppm), lead (0.037ppm), copper (0.076ppm) and zinc (5.594ppm). The various concentration of these parameters are within the permissible limit though periodic checks should be carried out.

Introduction

Instant noodles are one of the main staple food consumed in Asian countries and worldwide consumption is on the rise. Historically speaking, noodles originated from Northern China and consequently introduced to other Asian countries by traders, seafarers and migrants. Noodles have now become more adopted for everyday uses and its storage has been facilitated by the introduction of dried noodles[1].

Instant noodles known as staple food in Malaysia lack some nutritional component such as dietary intake fibre in appropriate proportion[2]. Therefore, incorporation of lentil on noodles gives additional properties like increase in fibre content and indirectly contributes to protein and mineral content.

Instant noodles are widely consumed throughout the world and their consumption is second only to bread. India stands fourth in the global instant noodles consumption listing about 5.5 billion servings per year. China tops the list at a staggering 44.5 billion servings consumed annually. It is a fast growing sector of pasta industry. This is because instant noodles are convenient, easy to cook, low cost and have relatively longer shelf-life and they are highly processed.

They are low on nutritive content: high on fat, calories and sodium, and are laced with artificial colours, preservatives, additives and flavourings[3].

The main raw materials for instant noodles are wheat flour, water, common salt (sodium chloride) and alkaline salt (typical sodium and potassium carbonate. Other ingredients such as gum, colourant, antioxidant, enzyme, emulsifiers, starch, monosodium glutamate etc may be incorporated to the formulation in order to enhance the structure, texture and flavor[4].

A major problem with instant noodles is the fact that it is made from white flour. White flour is essentially pure starch. Many essential nutrients are lost when wheat is processed into white flour[5].

Experimental

Sample Collection

Two popular brands of instant noodles – indomie and tummy-tummy (ascertained through a questionnaire prepared to determine the popular brand and distributed amongst the sellers and buyers of instant noodles) were bought from Eke Oko market at Oko, Anambra State.

The noodle samples were dried and stored differently for the proximate analysis and heavy metal determination.

Proximate Analysis

The moisture content, ash content, fat content, crude protein, crude fibre and carbohydrate content were determined using standard methods described by AOAC, 1998.

Heavy Metal Determination

Heavy metal analysis were conducted using VARIAN A240 Atomic Absorption Spectrophometer as described by APHA, 1995.

Results and Discussion

Table 1: Percentage levels of proximate parameters in Indomie and Tummy-Tummy noodles

Parameters	Indomie	Tummy-tummy	NAFDAC (2006)
Moisture %	15.10	19.31	NS
Ash %	6.87	1.34	NS
Fibre %	4.50	2.50	NS
Fat %	8.04	14.4	NS
Protein %	11.55	13.30	NS
Carbohydrate %	54.01	49.41	NS

NS – No Specified

Table 1 presents the results on the proximate analysis of indomie and tummy-tummy noodles.

The relative low amount of moisture content in these noodles (15.10% for indomie; 19.31% for tummy-tummy) are in line with the common knowledge that the higher the moisture content of food sample, the lower its shelf life because of its high susceptibility to bacterial attack. In comparism, micro-organisms can survive and grow well in tummy-tummy than indomie.

The ash content of indomie (6.87%) suggests that is has a higher mineral content than tummy-tummy (1.34%), as the ash content of food sample gives an insight into the inorganic (mineral) content of the sample.

The fibre content of indomie and tummy-tummy are 4.50% and 2.50% respectively. Crude fibre, on its own, do not have definite or unique nutritive benefits but simply help in bowel movement; indomie containing higher fibre content than tummy-tummy can aid more in food digestion than tummy-tummy.

The fat content of the noodles (8.04% indomie and 14.14% tummy-tummy) shows that tummy-tummy stands a chance to serve as source of fat than indomie. The implication of this is that noodles that have more fat has more energy than the one with less fat.

However, this might pose a health risk as frequent consumption of these noodles as witnessed today

would lead to the development of more fatty acid in the adipose tissue and would contribute to the cholesterol level in humans with their associated health implication such as obesity and the risk of heart disease.

The low protein content (11.55% and 13.30%) indicates that they could serve as protein sources. Protein are needed for growth and development especially in children.

The high carbohydrate content of indomie and tummy-tummy (54.01% and 49.41%) are in consonance with expected results as these noodles are usually source of energy giving the raw material for their production is wheat flour[8].

Table 2: Levels of metals in indomie and tummy-tummy instant noodles.

Metals	Indomie	Tummy-Tummy	NAFDAC (2006)
Magnesium (ppm)	17.009	8.064	20.010
Iron (ppm)	19.152	2.846	5.000
Cadmium (ppm)	0.050	0.022	10.000
Lead (ppm)	0.000	0.037	30.000
Copper (ppm)	0.200	0.076	5.000
Zinc (ppm)	2.268	5.594	5.000

The mineral contents play important metabolic and physiological roles in the living system.

The level of magnesium was higher in indomie (17.009ppm) than that of tummy-tummy (8.064ppm), though both were less than the recommended value of NAFDAC 2006. Magnesium helps to maintain normal nerves and muscle function. It also regulates blood glucose level[9].

Iron content in indomie is higher compared to tummy-tummy. It is an important component of haemoglobin, if not enough the body can't make enough healthy oxygen carrying red blood cell.

Heavy metal like cadmium is of no use to human body and it is toxic even at low level. On the other hand, lead like cadmium are poisonous, easily detected even at negligible amount. Both values were lower than the recommended values of 10.000ppm and 30.000ppm respectively for cadmium and lead.

Copper and zinc strengthens the immune system as antioxidant enzyme co-factor[10]. The overall observation on heavy metal level on indomie and tummy-tummy shows that the two noodles fell below NAFDAC permissible limit for heavy metal in food. Heavy metal are toxic metal having density more than five times than that of water.

Conclusion

From the results obtained, tummy-tummy are richer in fat and protein while indomie has more of carbohydrate and fibre. Also they both contain some important minerals that are good to the human body.

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The Influence Of Gender On The Readability Of Recommended Chemistry Textbooks In Enugu State.

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Abstract

This paper investigated the influence of gender on the readability recommended chemistry textbooks in Enugu State. The recommended chemistry textbooks were used to determine the extent of understanding of some chemistry textbooks by chemistry students. The study was guided by one research question and a corresponding purpose of study. The sample of the study were three chemistry textbooks and three hundred and thirty SS1 and SSII chemistry students, (330), drawn from ten schools out of forty-three (43) secondary schools in Agbani Education zone of Enugu State. The research design was a descriptive survey which was a plan of study that enables researchers to use reliable techniques to collect data from a well defined population. The instrument for data collection was close test of readability of chemistry textbooks (CTRCT) which was developed from the three textbooks. The major findings of the study were that none of the textbooks has enough human diagrams and common examples that were gender free and most of the textbooks though segmented but lacked detailed explanations. It was recommended that authors of chemistry textbooks should endeavour to include enough human pictures in favour of both males and females in experiments to avoid gender bias among science students.

Keywords: Readability, Gender, Chemistry textbooks.

Introduction/Background

The relevance of chemistry in the technological advancement of a nation is enormous, owing to the fact that the knowledge of chemistry is applied in almost all the activities in the homes, schools, markets,

For instance, the knowledge of chemistry has aided man in the establishment of chemical industries, improving agricultural processes and food storage, innovative and improved medical assistance for health benefit among others. Consequent upon this, there is need for encouragement towards the study of chemistry.

In addition, Eke (2010) opined that for Nigeria to meet up with other industrialized nations and to achieve vision 20:20:20 the place of chemistry should not be overlooked, since it is a vital plat form that propagates everything that makes life worthy of living. This has nexus with the goals of science education as encapsulated in the national policy on Education (2004). This policy outlines the goals of science education to include:

- Cultivate inquiry and rational mind.
- Produce scientist for national development in other words, this involves producing individuals in discovering things in the environment and to making meaningful ideals out of them so as to make life comfortable and interesting.
- Service studies in technology and technological development among others.

These goals are necessary for scientific and technological growth of our nation. Inherent in these goals is a clarion call for the nation to harness the requisite textbooks and materials in the teaching and learning of chemistry. Chemistry education being part of science education requires improved knowledge in other to attain these goals.

Considering the performance of students in chemistry in public examinations like west African Examination council (WAEC) one can argue that these goals are not yet fully achieved. The poor performance of students in chemistry in Senior Secondary School Certificate Examination as reported by Chief WAEC Examiner (2008, 2009 and 2010), revealed that the knowledge and skills expected of the students are not yet acquired . these knowledge and skills which students are expected to acquire are found in chemistry textbooks. One can now see the need for chemistry textbooks to be readable.

Inadequate instructional materials appear to be the main cause of poor performance of students, since the quality of educational materials such as textbooks is the most fundamental where information presented is reliable, valid and authoritative. A textbook is seen as a tool for a subject without which a child finds himself backward in the subject.

Similarly, the appropriateness of the vocabulary and presentation of facts to the level of the learner, the learner's clarity of diagrams and pictures, procedures are also very indispensable qualities that characterize good educational textbooks (Ali, 1996).

In addition, Nweze (2003) defined textbook as a pedagogical instruments used to disseminate information about teaching and learning for attainment of educational goals. In the worlds a textbook is a printed material that contains facts use for teaching and learning. A chemistry textbook is a printed material that contains chemical concepts, principles and experimental activities that guide teaching and learning of chemistry. Chemistry textbook being the main source of information should have attributes of good textbook and such attributes are appropriateness of vocabulary, presentation of facts, clarity of objectives and procedures, enough teachers and students activities, among others.

Appropriateness of vocabulary means that the content will use good and simple sentences, clear diagrams and illustration so as to make it readable. In addition Kelly(1997)maintained that the qualify of textbook pertains principally to its readability.

Readability refers to the easiness of the sentence or passage to reading with understanding Hargis(1998) stated that readability, the 'ease of reading words and sentences', is an attribute of clearity. The ease of reading sentence with understanding depends on the following; appropriate vocabulary, simple sentences, proper presentation of facts, netural languages, relevant diagrams among others. Any chemistry textbook that does not contain these qualities is not readable and may create difficulty to the reader/student. This is why Nweze (2003) opined that a good chemistry textbook whose activities are relevant and brought to the understanding of the students can help them achieve most of the stipulated objectives.

Since readability is concerned with the problem of matching between the reader and text, the readers (chemistry students) are made up of males and females and it is likely that readability may be influenced by gender. Ogba and Ndaba (2006) defined gender as all characteristics and expected behavioural roles of men and women which a society has determined and assigned each sex. Readability of chemistry textbook by male and female students may or may not be same since the language is masculine. Masculine language may have differential effect on readers.

Most chemistry textbook contain masculine terminologies and pictures which may create sex bias in the books and differential effect towards gender on readability.

Since readability has been identified as an important criterion under which a textbook can be assessed, lots of text for measuring readability are available and are Flesch-kincard test, Dale-chall test, Fry test, Cloze test, Harrison among others.

Purpose of the study

This study aimed to:

Determine the influence of gender on the readability of recommended chemistry textbooks in Enugu state

Research Question

This question guided the study;

What is the influence of gender on the readability of recommended chemistry textbooks in Enugu state.

Statement of research hypothesis

Ho The readability scores of the chemistry textbooks used in Enugu state do not significantly depends on gender.

Review of Related Literature

Akubue and Enyi (2010) agreed that gender is a psychological construct associated with either male or female that results from social, physical and psychological traits attribute to each and all that has to do with their respective role in the society. In other words, gender refers to males and females that stem from peoples idea due to their respective roles. Simply gender means males and females based on social roles and behaviour associated with them.

The understanding of chemistry textbook by chemistry students (males and females) depends on the style of writing which includes, neutral language, natural and common diagrams, simple sentence structure among others. When a chemistry textbook is sex biased, then the readability by students (males and females) may be affected positively or negatively.

It may be assumed that since males and females are of different psychological construct, they should exhibit different behaviour and perform different activities. On the other hand Inyang and Jegede (1991) observed that gender has no effect on students achievement in science.

Considering the above ideas, it can be argued that gender being a socially constructed role, learned behaviour and expectation associated with males and females may influence readability of chemistry textbooks (Nwagbara, 2010).

Research Method

Research design:- The study employed a survey design and according to Nwagu (20105), survey research design is a plan of study which enables researchers to use reliable techniques to collect data from a well defined population.

Sample and Sampling Techniques

The target population comprised of three commonly used chemistry textbooks out of five and senior secondary chemistry students (SS1- SS11) in Agbani Education zone of Enugu state.

Instrument

The instrument used for data collection was cloze test of Readability of chemistry textbooks (CTRCT). This is because cloze measures the interaction between the reader and the textbook cloze readability tests are constructed by deleting every fifth word from each passage and three passages of approximately 500 words were selected from each text. Every fifth deleted will be substituted with a black space. To ensure fair representation of all the sections of the text, the passages from each textbook were randomly drawn from the beginning , middle and end of the text respectively.

The Result

The result of the study was shown in the table below:

Table 1: readability scores of recommended chemistry textbooks in use in Enugu state secondary by gender.

Chemistry Text book	Mean Readability scores male	female	Interpretation
Ababio chemistry	61.25	67.86	Standard
STAN chemistry	28.52	29.98	very confusing
Bajah chemistry	23.22	28.88	very confusing

The readability scores from table 1 regarding males for the chemistry textbooks, Ababio, STAN and Bajah are 61.25,28.52 and 23.22 and females were 67.86,29.98 and 28.88 respectively. These data above were interpretated to mean standard, very confusing and very confusing respectively for both males and females.

Table 2: the t-test result of the readability scores of recommended secondary school chemistry textbooks in use in Enugu state by gender.

Chemistry Textbooks	variable	n	$\bar{x}$	SD	t-value	degree of freedom	sig	decision
Ababio	male	183	61.25	37.88				
Chemistry	female	147	67.86	37.19	-1.23	328	.220	accepted
STAN	male	183	28.53	17.00	-0.57		.571	accepted
Chemistry	female	147	29.98	18.89		328		
Bajah	male	183	28.22	21.21	-1.90	328	.059	accepted
Chemistry	female	147	28.88	20.39				

It] was indicative from table 2 that t-calculated value (-1.23) has a probability value of .220 for the influence of gender on Ababio textbook since the probability value of .220 is greater than .05 level of significant ($p>.05$), the null hypothesis was accepted. For STAN chemistry textbook the associated value of t(-0.57) is .571. similarly the null hypothesis was accepted since .571 is greater than .05level of significant. The probability associated with the calculated value of t(-1.90) for the influence of gender on Bajah chemistry textbook is .059. since .059 is greater than .05 level of significant the null hypothesis was accepted. These implied that the readability of chemistry textbooks was not dependent on gender.

Conclusion

From the findings of this study, It can be concluded that gender had no effect on the readability of these textbooks. It was then recommended that chemistry authors should ensure that the pictures and diagrams should be gender free, illustrative and natural.

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Phytochemicals and Proximate analysis of *KaempferiaGalanga*

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ABSTRACT

The chemistry of West African timbers are not known unlike that of the developed countries. An attempt is thus made to address this problem by investigating the chemical constituents of a timber (KaempferiaGalanga). The wood sample was obtained from the timber market in Edo State. At the point of the purchase, it was ascertained by the dealers and confirmed by literature.The proximate analysis were carried out with the following results: moisture content (27.0%), carbohydrate (2mg/g), protein (2.8%), fiber (2%),ash content(3.4%) and lipids (7.7%). Physical properties such as pH (6.54), specific gravity (0.23), charring temperature (93-113°C), porosityIndex (1.19 %) and colour (Lemon Chiffon) of the timber powder sample were determined. Phytochemical screening was also carried out to determine the presence of secondary metabolites in the wood powder samples. The chemical constituents and extractive studied gave the following results which includes: flavonoids(3.8%), alkaloids(3.6%), saponins(6.2%),lignin(20.5%),hemicellulose(32.0%),cellulose (43%), tannins (700mg/100g), glycosides (680mg/100g) phenol (1.8mg/g). The proximate analysis results reveal the effect of some factors on the wood while the phytochemical analysis shows the presence of secondary metabolites. . Thus from the chemistry of wood, it was observed that thechemical constituents and proximate analysis reveals the importance of timber to medicine.

INTRODUCTION

Wood is the hard fibrous material that forms the main substance of the trunk or branch of a tree or shrub. It is an organic material, a natural composite of cellulosefibres embedded in a matrix of lignin which resists compression. Wood is produced as secondary xylem in the stems of trees and other woody plants. In a living tree it performs a support function, enabling woody plants to grow large or to stand up for themselves. It also mediates the transfer of water and nutrients to the leaves and other growing tissues.

Wood when reduced to charcoal creates temperatures hot enough to extract metals from stone. Metals in turn revolutionized the implements for agriculture, construction and warfare. Much research has been carried out on the phytochemicalsand chemical constituents of plants, plant leaves and seeds [1].Others include the antibacterial, phytochemical and antioxidant activities of the leaf extracts of *Gliricidiasepium* and *Spathodeacampanulata*, [2,3], proximate composition of amino acid profiles[4], proximate study of the mineral and anti-nutrient composition of *Moringaoleifera*[5] and phytochemical screening of *Lophiralanceolata*seeds. From the literature, a lot of work has been done on the chemical constituents of the seeds, leaves and plants of these tropical timbers but it appears there is no record on the chemical constituents of woods of these tropical timbers.Therefore, the aim of this study is to determine thephytochemical and proximate composition of *KaempferiaGalanga.*

MATERIALS AND METHOD

Materials:

The wood sample was obtained from timber market in EdoState, Nigeria. The wood sample was carefully selected from sawed healthy timber, identified and its local name obtained from the timber dealers, confirmed by botanist and literature[6]. Other materials used were, electrical pH meter PHS-25 (Life Care, England) and Lab23A spectrophotometer.

METHODS

Preparation of the Wood Sample

The wood sample was grounded into fine powder using angle grinder (Siemens Germany). The ground sample was kept in air- tight polyurethane bag, until required.

Determination of Physical Properties/Constituents of Wood Samples

pH Determination

The hydrogen ion concentration (pH) of the powdered wood was determined using these reported work[7,8].

Determination of Specific Gravity

The specific gravity was determined gravimetrically by measuring the oven-dried wood powder sample using specific gravity bottle.

Determination of Charring Temperature

The charring temperature was determined by placing 0.50g of the wood powder sample inside an ignition tube which contained a thermometer (0-360^{o}C). The combustion tube was then clamped, heated on a heating mantle and regulated at constant heating point. As the material were heated to char point, the exact char temperature was recorded.

Determination of Porosity Index

One gram of cold water starch was prepared with 5cm^3 of water. The starch which serves as an adhesive was mixed with 1.03g of the wood powder. The mixture was moulded into ring shape and allowed to dry on exposure to air for 15hours. The moulded dry wood sample was weighed using an electronic weighing balance, Model B218. The dry wood sample was soaked in 75cm^3 paraffin oil for 24hours. The soaked dry wood sample was weighed and the weight noted before the porosity index was calculated.

Determination of Colour

The colours of the wood powder were determined using physical visual identification compared with a colour chart. The colours of the wood samples were matched with Chemistry Colour Chart and respective colours were obtained (http://www.rfs.org.uk/learning/what-wood).

Qualitative Analysis of the Phytochemicals of wood the Sample

Tannin, Saponin, Flavonoids, Terpenoids, Steroids, Alkaloids, Glycosideof the wood sample were determined qualitatively using the standard methods as described by [9-13].

Quantitative Determination of the phytochemical Constituents of the Wood Sample

Tannin, saponin, flavonoids, terpenoids, steroids, alkaloids, cyanogenic glycoside, of the wood sample were determined quantitatively using standard methods as described [7].

Determination of Proximate Analysis

The proximate analysis (Carbohydrate, Protein, Moisture content, Ash content,Crude Fibre) were carried out using standard methods[14,15].

Chemical constituents

The chemical constituents (total acidity, lipids, total lignin content, hemicellulose, cellulose, crude fibre, carbohydrate) were carried out using standard methods as described by[16--20].

RESULTS AND DISCUSSION

Table 1 shows the physical properties of *Kaempferiagalanga*. Result shows that the pH of the wood sample was found to be 6.54. Its specific gravity was 0.23 and the charring temperature was between 93 - 113°C with porosity index of 1.19%. These characteristics are remarkable variables inwoods and often provide the first clue to identify a particular wood species.

Table 1: Physical Properties of *KaempferiaGalanga*

Wood Sample (Botanical name)	Physical Properties	Values
KaempferiaGalanga (Soft wood)	pH Values	6.54
	Specific Gravity	0.23
	Charring Temperature (^{0}C)	93-113℃
	Porosity index (%)	1.19%
	Colour	Lemon Chiffon

Proximate Analysis of *Kaempferiagalanga*

Table 2 contains data on the proximate analysis of the wood sample. Carbohydrate was calculated to be 2mg/g, protein 2.8%, fibre 2%, ash content 3.4% and lipids 7.7%. This issimilar to the experimental values of seedy crude fibre[21].

Table 2: Proximate Analysis of *KaempferiaGalanga*

Proximate Analysis	Values	Units
Moisture content	27.0	%
carbohydrate	2	mg/g
protein	2.8	%
Fiber	2	%
Ash Content	3.4	%
Lipids	7.7	%

Chemical Constituents of the Wood Sample and their Concentration

Table 3 represents the chemical constituents of *KaempferiaGalanga*. Results reveals that 3.8% flavonoids 3.6% alkaloids 6.2% saponins, 20.5% lignin, 32% of hemicellulose, 680 mg/100g of glycoside, and 43% cellulose was present in the wood sample. The phenol value was found to be 1.8 mg/g (Table 3). This is in contrast to a reported work [23] whose phenol value was 0.000147 mg/g.

Table 3: Quantitative Determination of the Chemical Constituents of *KaempferiaGalanga*

Chemical Constituents	Units	Results
Flavonoids	%	3.8
Alkaloids	%	3.6
saponins	%	6.2
Lignin	%	20.5
Phenol	mg/g	1.8
Hemicellulose	%	32.0
Glycosides	mg/g	680/100
Cellulose	%	43

Table 4 presents the qualitative analysis of the phytochemicals in the wood sample.Result shows the highest precipitation of saponins in the wood sample (*KaempferiaGalanga*).

Table 4 Qualitative analysis of the phytochemicals

Phytochemicals	Interference
Flavonoids	+
Alkaloids	+
saponins	+++
Glycosides	++
Steroids	-

Heavily present +++ Slightly present ++ Present + Absent -

CONCLUSION

Wood timber contains chemical constituents which are essential for human consumption, in pharmaceutical industries as well as other industries.

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Quality Assessment of some Vegetable Oil Products sold in Ekwulobia, Aguata Metropolis, Anambra State, Nigeria. *O.G. Okoye and V.E. Mmuo

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Abstract

The physicochemical properties of different brands of commonly consumed vegetable oils sold within Ekwulobia in Aguata LGA were determined to ascertain their quality. Five vegetable oils namely; Chido, Muchel, Sous, Baron and Envoy were randomly selected for the study and characterized for specific gravity, refractive index, viscosity, free fatty acid, iodine value, saponification value, peroxide value, cholesterol content and color using standard methods. The specific gravity of all the oils range from 1.41 – 1.55g/m which is above the standard range of 0.10 – 0.91 approved by Standard Organisations of Nigeria. Envoy oil has the highest viscosity value of 6239.40Pa.s with Baron having the lowest value of 1410.90Pa.s. The value of free fatty acids was highest (3.09mgKOH/g) in chido oil compared with other brands and falls within the stipulated CODEX standard of 4.0 mgKOH/g. The highest and lowest saponification value was obtained 164.09mg/kg for chido oil and 81.35mg/kg for sous respectively. Envoy recorded the least peroxide value of 15.00mleq/kg while muchel has the highest value of 29.60mleq/kg. All the oil contains varying concentrations of cholesterol ranging from 53.38mg/l to 98.88mg/l. Highest iodine value (5.46mg/kg) was observed in envoy and lowest (2.09mg/kg) in muchel though both fall within the NAFDAC standard range of 4.51 – 7.16mg/kg.. The results revealed the quality status of the different oil brands, as it will aid in selection for usage.

Keyword: Vegetable oil, specific gravity, refractive index, viscosity, free fatty acid, iodine value, saponification value, peroxide value, cholesterol.

INTRODUCTION

Oils have always been an integral part of human existence. They play important roles in the development of different areas of chemical products, pharmaceuticals, cosmetics and foods[1,2]. From chemical point of view, they are products of esterification reaction between a triol(glycerol) and three molecules of fatty acids.[3] Edible oils are vital constituents of our daily diet which provide energy, essential fatty acids and serve as a carrier of fat soluble vitamins [4]. Oils are basically obtained from two sources, namely; animal and plant sources.[5]Oils obtained from plant source are termed vegetables oil which remain in the liquid form at room temperature. Vegetable oils had made an important contribution to the diet in many countries, serving as good source of protein, lipid and fatty acids for human nutrition, the repair of worn-out tissues, new cells formation as well as a useful source of energy.[6] Despite their importance, one major drawback in their handling and utilization is their ease of oxidative deterioration, which renders them less acceptable to consumers or for industrial use especially as food ingredients.[7] Two common practices that render vegetable oils in most commercial centers prone to oxidative deterioration are packaging in transparent containers (plastic bottles and sachets) and displaying oils either under direct sunlight or artificial lights.[7]Some of the oxygenated decomposition products are implicated in degenerative diseases such as aging, membrane damage, heart disease and cancer; as a result the study of lipid oxidation has received great attention recently.[8]Cholesterol found in the cell membrane of all cells, has been of great importance in recent years, because its high level in the body has been associated with coronary heart disease (CHD).[9] Nigeria is one of the countries of the world with a variety of vegetable oils. The importance of analyzing for the physicochemical properties of these oils cannot be overemphasized to ascertain their quality for better utilization and application.

Materials and Methods

Five different brands of edible refined vegetable oils (envoy,chido, sous,muchel and baron) were purchased from the popular Eke Ekwulobia market in Aguata LGA, Anambra State, Nigeria.

The specific gravity, refractive index, viscosity, free fatty acid, iodine value, saponification value, peroxide value, cholesterol content and color were determined using AOAC (2000) methods.[10]

Determination of cholesterol content: 1mL of each sample and standard cholesterol were put into a beaker and dissolved in chloroform in the ratio 1:10. This was evaporated to dryness in a water bath at 50ºC. 2mL of glacial acetic acid and 2mL of sulphuric acid were added to each sample and the standards, then shaken vigorously to dissolve and cooled for 30minutes at room temperature. Absorbance of standard and samples were read at 560nm using SP65 Apel UV-visible Spectrophotometer.

Results and Discussion

Table 1: Physicochemical properties of the vegetable oil

Parameters	Baron	Envoy	Muchel	Chido	Sous
Colour	Light yellow	Light brown	Pale yellow	Dark brown	Orange
Viscosity (Pa.s)	1410.90	6239.40	1474.90	7131.10	1453.60
Specific Gravity (g/mL)	1.55	1.54	1.41	1.54	1.55
Refractive Index	0.01	0.01	0.01	0.01	0.02
Saponification Value (mg/kg)	137.45	122.02	131.84	164.09	81.35
Iodine Value (mg/kg)	5.20	5.46	2.09	3.87	2.67
Peroxide Value (mleq/kg)	17.60	15.00	29.60	18.40	28.20
Free Fatty Acids (mgKOH/g)	1.68	1.96	1.26	3.09	2.24
Cholesterol content (mg/L)	53.38	67.25	98.88	59.13	75.88

The results of the physicochemical analysis of the oil samples are presented in the table 1 above. The results obtained for the oil samples shows that there was no significant difference in the refractive index. The refractive index ranges from 0.01 to 0.02 with Envoy having the highest value. These values are in line with the study by Angaye et al.[8] The specific gravity of the different brands of vegetables oils have values that are above the standard range of 0.10 – 0.91 approved by Standard Organisations of Nigeria (2000).[11] This could be attributed to the oil being contaminated.

Saponification value provides the information of the average chain length and hence the molecular weight of the fatty acid in the oil. The shorter the average chain length of the fatty acids, the higher the saponification value and the lower the average molecular weight of the fatty acids and vice-versa.[12] The result of the Saponification value showed that Sous oil have the lowest value (81.35mg/kg) while Chido oil contains the highest value (164.09mg/kg). Studies showed that high saponification values indicate that the oil are normal triglycerides and will be useful for industrial purposes as it has no nutritional value.[13]

The iodine values of the vegetable oil samples analysed showed that the values of Envoy oil (5.46mg/kg) and Baron Oil (5.20mg/kg) fell within the NAFDAC standard range of 4.51 – 7.16mg/kg. Iodine value is an indicator of double bonds in the molecular structure which influences the long term stability properties of the oil. The greater the iodine value, the more the unsaturation and the higher the susceptibility to oxidation.[14]It has been reported that lowering the iodine value improves the stability and good yield of the liquid oil.

The free fatty acid values for the oil ranged from 1.26 – 3.09mgKOH/g which is within the stipulated CODEX standard of 4.0 mgKOH/g maximum. Muchel oil has the least free fatty acid value(1.26 mgKOH/g) and Chido has the highest value (3.09mgKOH/g). This is an indication that the extent of hydrolytic rancidity in these oils is appreciable.

The peroxide value is an indicator of deterioration of oils. This ranged from 15.00mleq/kg to 28.20mleq/kg. The successive increase in peroxide value indicates the rancidity of oils due to relative higher oxidation in oils.

The colors of the oil samples determined by visual comparison showed light yellow, dark brown and orange.

All the brands of oil have varying concentrations of cholesterol. Amongst, Muchel oil has maximum cholesterol content of 98.88mg/L and Baron has the lowest cholesterol content of 53.38mg/L. Findings from the study refutes the claims by some producers of vegetable oil on the oil labels (No Cholesterol).

Conclusion

The results revealed the quality of the various brands of vegetable oil consumed in the study location. It is also evident that all the five samples tested have different concentrations of cholesterol, thus predisposing the consumers to associated diseases.

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Phytochemical And Antimicrobial Analysis Of Plantain (Musa Parasidiaca) Pseudostem

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Abstract

This work examined the preliminary phytochemical screening of ethanol, butanol and ethyl acetate extracts of plantain pseudo stem and the determination of their antimicrobial activities using standard procedures. The phytochemical screening and analysis of the pseudo stem indicated the presence of alkaloid (1%), flavonoids (3.6%), saponin (0.34%), phenol (2.73%), tannin (24.1%) and glycoside (142%) except steroid which was found to be absent. Butanol and Ethylacetate extract had the highest amount of phytochemicals, than the ethanol extract. This implies that the butanol and ethylacetate are better solvents to be used in the extraction of phytochemicals from the plantain pseudostem. The efficacy of the antimicrobial activities of the pseudostem were carried out against different fungi (*Aspergillus niger* and *Candida albican*), and gram-positive (*Streptococcus spp and Staphylococcus aureus*) and gram negative (*Escherichia Coli and Pseudostem aeruginosa*) bacteria strains. The control drugs used for the antibacterial (gram-positive, gram-negative) and antifungal screening were Gentamycin and Ketoconazole respectively. Some of the extracts indicated antimicrobial activities for the pseudostem as well. The pseudostem extracts and control drugs had zone of inhibition ranging from 0-5mm and 0-19mm respectively depending on the test organisms and extract in question. The ethylacetate extract notably had a high ZID (zone of inhibition diameter) of 5mm to Bacillus SPP and ethanol extract had ZID (zone of inhibition diameter) of 4mm on Bacillus SPP and Candida Albican (fungi). The results obtained shows that the banana pseudostem has significant antimicrobial activities which can be put to various therapeutic effects.

Keywords: Phytochemical,Antimicrobial,Bioassay,Alkaloid,Flavonoid**.**

Introduction

Nature has been a source of medicinal agents for thousands of years and generally produces many secondary metabolites which constitute important leads for the development of new environmentally friendly microbicides, pesticides, herbicides and many pharmaceutical drugs[1] Traditional societies in Africa and elsewhere have always used plants to promote healing [2] and about 80 % of the world's population depends on the use of traditional medicine for health care. Therefore, such plants should be investigated to better understand their properties, safety and efficacy [3].

As science advanced, however, it became possible to determine rigorously the active components of these extracts through painstaking and laborious chemical methods. This rational approach to the discovery of drugs inaugurated an era of bio-prospecting that is, raiding nature's storehouses of plant and microbiological life. Bio-Prospecting literally involves exploring the forests, diving in the oceans and digging in the dirt to obtain environmental samples. The study of the compounds discovered by these methods has become a major area of research in organic chemistry, biological science and has led to the isolation and identification of thousands of different structures, mostly extracted from plants and more recently from micro- organisms, with the animal kingdom contributing rather sparsely to the total [4]. Over the last few decades, the biological and pharmacological potentials of organic substances from many indigenous plants have been well understood. For instance, phenolic compounds have been associated with antimicrobial [5], anti-inflammatory, antiviral, and cytotoxic activities [6]. However, the bioactive constituents conferring these properties on many plant species have also been implicated in allelopathy [7]. Allelopathy has been defined as the effect(s) of one plant on other plants through the release of chemical compounds in the environment [8]. Different plant

parts, including flowers, leaves, leaf litter and leaf mulch, stems, bark, roots, soil and soil leachates and their derived compounds, can have allelopathic activity that varies over growing seasons [9].

It has been indicated that phenolic acids are the most commonly occurring natural products noted for allelopathic activities [10, 11] have also included alkaloids, coumarin, flavonoids, saponnins and volatile constituents of the essential oils as being allelopathic agents. Generally, the presence of different phytochemicals in crude plant extracts has been linked to the detrimental effects of leachates, root exudates or decomposing residues of such plants on the other vegetation or succeeding crops [12]. Phytochemical analysis of several species of medicinal plants and allelopathic activities of the crude chemical compounds on crops and plants has yielded positive results [13]. Of the different plant families studied [14] indicated that members of the Asteraceae family have great potential for inhibitory activities. Thus, the increased interest in the isolation and identification of the chemical compositions of organic products associated with biological activities with particular emphasis on germination, growth and yield of crops has stimulated research on plants having both medicinal and allelopathic properties [15].

All members of the genus *Musa* are indigenous to the tropical regions of Southeast Asia and Oceania, including the Malay Archipelago (modern Indonesia, Malaysia, Brunei and the Philippines) and Northern Australia. Africa is considered a secondary centre of diversity of *Musa* cultivars: West Africa for plantains and the central highlands for East African Highland bananas (*Musa* AAA-EAHB, also known as *matooke* or matoke in Uganda), most of which are cooked although some are primarily used to produce beer.

Phytochemical Method

Sample collection

Figure 2: plantain stem

Mature plantain pseudo stem was obtained from Enugu (Ugwuaji) Enugu State on 20th September 2015 (rainy season)

Sample preparation The plantain pseudo stem was washed under running tap to remove debris. It was sliced and then dried at room temperature for three weeks. After drying, the sample was ground into powder using special grinding machine to avoid contamination and then kept ready for analysis.

Phytochemical screening The different phytochemical tests were carried out using standard laboratory techniques as detailed below. Phytochemical test were carried out on the ground plantain pseudo stem.

a) Qualitative Test

Qualitattive analysis

10g each of the ground sample was weighed into three labeled conical flask (250ml). 100ml each of the three (3) different solvents (Ethylacetate, Buthanol and Ethanol) were poured into the three different conical flasks to extract the phytochemicals. After 24 h, the mixtures were filtered using Whatman filter paper (No. 1) into conical flasks. The filtrates were concentrated by placing the flasks into water bath at 100 ^{0}C. The resulting filtrate were cooled to room temperature, qualitative test was then carried out on the cool solution using [16] to ascertain the presence of different phytochemicals in plantain pseudo stem.

Alkaloid test

1 ml of 1% HCl was added to 3 ml each of the extract in a test tube.The mixtures were heated for 20 min in a water bath and shook continuously on heating. They were cooled and filtered. 1 ml of each filtrate from above was added to 0.5 ml of **Meyers Reagent.**

Observation: Creamy colour change confirmed the presence of alkaloid

1 ml of each filterate from above was added to 0.5 ml of **Wagners Reagent.**

Observation: Brown ppt.

Test for saponins

a. **Frothing test**

3 ml of each extract was diluted with 2ml of distilled water in a test tube and the mixture was shook vigorously.

Observation: A persistent frothing movement confirmed the presence of saponins.

b. **Emulsion test**

3 ml of each extract was added to 5 drops of olive oil in a test tube and the content were vigorously shaken.
Observation: Emulsification confirmed the presence of saponins.

Test for flavonoids

3 ml of each extract added to 10 ml of distilled water, and the mixture is shaken.1 ml of 10% NaOH solution was added to the mixture.
Observation: Yellow Colouration confirmed the presence of flavonoids.

Test for steroids (Salkowski test)

2 ml chloroform was added to 1 ml of each extract in a separate test tube. Five drops of Conc. H_2so_4 was added to the mixture.
Observation: A reddish brown colour at the interface confirmed the presence of steroids.

Test for tannin

3 ml each of the extract was taken in a test tube and diluted with chloroform.
1 ml of acetic anhydride was added carefully by the side of test tube to the solution.
Observation: A green colouration confirmed the presence of tannin.

Test for glycoside (keller killiani's test)

1 ml of each extract was dissolved in 1 ml of glacial acetic acid containing one drop of ferric chloride solution. 1 ml of conc sulphuric acid was added to each test tube.
Observation: A brown ring obtained at the interface indicates the presence of deoxy-sugar characteristics of cardenolides.

Test for phenol (Ferric chloride test) 3

to 4 drops of dilute ferric chloride solution was added to 1ml of the extract in water. Observation: Red, Blue, Green or Purple coloration indicates the presence of phenols.

NB: Where sample is insoluble in water, it may be dissolved in dichloromethane with a small amount of pyridine.

Results for qualitative phytochemical analysis

Solvents	**Ethanol**	**Butanol**	**Ethylacetate**
Parameters			
1. Alkaloids	Meyer's Wagner's ++ ++	Meyer's Wagner's ++ ++	Meyer's Wagner's ++ ++
2. Saphonins	Emulsion Frothing ++ ++	Emulsion Frothing ++ ++	Emulsion Frothing - -
3. Flavonoids	+	++	++
4. Steroids	-	-	-
5. Tanins	-	+	+
6. Phenols	+	+	+
7. Glycoside	++	++	++
	Keys ++ Present + Mildly Present Absent		

The qualitative phytochemical estimation of different plantain pseudo stem extracts has shown the presence of alkaloids, phenols, flavonoids, tannins, saponins and glycosides as shown in the table above.

Generally, the butanol and ethyl acetate extracts fared better with respect to the presence of phytochemicals in the pseudo stem except for steroids which were absent in all the extracts.

The presence of virtually all the pytochemical except steroid in butanol and ethyl acetate extracts shows that the two solvents can serve as main solvent for the extraction of these phytochemicals for the rapeuti purposes. Worthy of note also is the efficiency of butanol compared to ethanol and ethyl acetate extracts as it gave the best in all the parameters tested.

Conclusion

In this study, an attempt has been made to identify the phytochemicals and antimicrobial properties of plantain pseudo stem. The entire butanol, ethanol, and ethyl acetate extracts of pseudo stem have been found to contain significant amount of phytochemicals which includes alkaloids, phenols, flavonoids, tannins, saponins and glycosides. The study also indicated that the butanol extracts were effective against some of bacterial organisms hence zone of inhibition was observed and this is attributed to the presence of those phytochemicals contained in the extracts. The results of the study suggest that the plantain pseudo stem may be used for the preparation of various antibacterial formulations in pharmaceutical industries.

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Teaching And Learning Of Biomass Energy In Secondary Schools: Implications For Chemistry Education

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Abstract

The continuous increase of energy consumption has generally improved the standard of living but it has also caused serious environmental problems. Burning of fossil fuels to generate energy has resulted to many negative environmental problems on the climate, natural environment and the society at large. There is the need o change to other renewable and environmental free sources of energy. Biomass is a biological material that has been recovered from once living organisms. It is a renewable energy source used to reduce our overdependence on fossil fuels and to help reduce air pollution. Secondary school chemistry students need to be taught about this biomass and biomass energy at the early stage of their educational pursuit even as they are the future scientists. .Thus this paper discussed teaching and learning of biomass energy in secondary schools and its implication for chemistry education. The paper examined the concept of biomass and biomass energy, conversion of biomass to energy, advantages of biomass energy over fossil fuels and the implication of teaching biomass and biomass energy to secondary school chemistry students .

Introduction

Developing new energy sources to produce reliable, affordable energy, while respecting the environment is one of today's most compelling needs. Of late, much attention is being focused on identifying suitable alternative and renewable energy sources, which can provide high-energy outputs, to replace conventional fossil fuel energy sources [1]. The total energy stored in terrestrial biomass is not only enormous but is also highly available and renewable. Biomass if properly harnessed can form a substantial part of future energy sources which will reduce the pressures on the global energy crises[1] The quest for biofuels in Nigeria is no doubt, a reasonable ambition. This is so because the focus on biofuel production has assumed a global dimension, and the benefits that may accrue from such effort may turn out to be enormous if the preconditions are adequately satisfied.[2] As a member of the global community, it has become necessary for Nigeria to explore other potential means of bettering her economy[2] . Biomass refers to the diverse materials obtained from plants and animals, which can be used as raw materials for the creation of useful energy in various forms and for diverse purposes

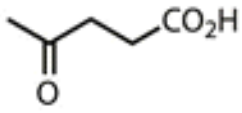
Biomass is fuel that is developed from organic materials, a renewable and sustainable source of energy used to create electricity or other forms of power[3]. Some examples of materials that make up biomass fuels are: scrap lumber; forest debris; certain crops manure; and some types of waste residues. With a constant supply of waste – from construction and demolition activities, to wood not used in papermaking, to municipal solid wastes , bioenergy production can continue indefinitely[3] Biomass is a renewable source of fuel to produce energy because: waste residues will always exist – in terms of scrap wood, mill residuals and forest resources. Also, properly managed forests will always have more trees These trees will always have crops and the residual biological matter from those crops will also continue to exist.. . Biomass fuels come from things that once lived: wood products, dried vegetation, crop residues, aquatic plants and even garbage. It is known as *'Natural Materials'*. Plants used up a lot of the sun's energy to make their own food through photosynthesis. They stored the foods in the plants in the form of chemical energy. As the plants died, the energy is trapped in the residue. This trapped energy is usually released by burning and can be converted into biomass energy..[4]

Biomass is such a widely utilized source of energy, probably due to its low cost and indigenous nature, that it accounts for almost 15% of the world's total energy supply and as much as 35% in developing countries, mostly for cooking and heating [4]. In biomass power plants, wood waste or other waste is burned to produce steam that runs a turbine to make electricity, or that provides heat to industries and homes. Fortunately, new technologies — including pollution control and combustion engineering — have advanced to the point that any emissions from burning biomass in industrial facilities are generally less than emissions produced when using fossil fuels (coal, natural gas, oil).

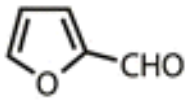
Advancements in technology have allowed biomass energy to be used in a wide variety of applications, including liquids and gases used for biofuels to power transport. **Biofuel** is any fuel that is derived from biomass—that is, plant or algae material or animal waste [7]. Biofuels are either liquid or gaseous fuel. They can be produced from any source that can be replenished rapidly, e.g. plants, agricultural crops and municipal waste. Current biofuels are produced from sugar and starch crops such as wheat and sugar cane, which are also part of the food chain**.** One of the key targets for energy researchers is a sustainable route to biofuels from non-edible lignocellulosic (plant) biomass, such as agricultural wastes, forestry residues or purpose grown energy grasses[5]. These are examples of so-called advanced biofuels.

Current biofuels, such as ethanol, have a lower energy content (volumetric energy density) compared with conventional hydrocarbon fuels, petroleum and natural gas. The aim is to produce fuels that have a high carbon contentt /and therefore have higher volumetric energy density. This can be achieved by chemical reactions that remove oxygen atoms from biofuel chemical compounds. This process produces a so called 'drop-in biofuel', i.e. a fuel that can be blended directly with existing hydrocarbon fuels that have similar combustion propertiess

Efficient synthesis of renewable fuels remains a challenging and important line of research.



Levulinic Acid



Furfural

Levulinic acid and furfural are examples of potential 'platform molecules', *i.e.* molecules that can be produced from biomass and converted into biofuels. Levulinic acid can be produced in high yield (>70%) from inedible hexose bio-polymers such as cellulose, which is a polymer of glucose and the most common organic compound on Earth while Furfural has been produced industrially for many years from pentose-rich agricultural wastes and can also act as a platform molecule Recent reports have highlighted the use of organic chemistry to convert platform molecules like levulinic acid and furfural into potential advanced biofuels by specifically, changing parts of the molecules that are responsible for their structure and function. This process is called 'functional group interconversion and is part of the basic toolkit of organic chemistry[5]

Organic chemistry is the study of the structure , properties, composition, reactions, and preparation of carbon-containing compounds, which include not only hydrocarbons but also compounds with any number of other elements, including hydrogen , nitrogen, oxygen, halogens, phosphorus, silicon, and sulfur [6]. The range of application of organic compounds is enormous and also includes, but is not limited to, pharmaceuticals, petrochemicals, food, explosives, paints, and cosmetics. Organic chemistry is a highly creative science in which chemists create new molecules and explore the properties of existing compounds. It is a popular field of study for chemists. Organic compounds are all around us. They are central to the economic growth of many counties in the areas of rubber, plastics, fuel, pharmaceutical, cosmetics, detergent, coatings, dyestuff, and agrichemical industries, to name a few.

Considering the importance of knowledge of organic chemistry in the production of biofuels, it is expedient that chemistry teachers should in the process of teaching this branch of chemistry to secondary school students try to incorporate the concept of biomass as an important source of energy. They should also link the study of organic compounds to include those chemical compounds found in biomass. The different methods of obtaining bioenergy from biomass, the main advantages of bioenergy over fossil fuels should also be taught to students. This paper therefore examines the teaching and learning of biomass and biomass energy in secondary schools and its implication to chemistry education.

Concept of Biomass and Bioenergy

The term "biomass" refers to organic matter that has stored energy through the process of photosynthesis. It exists in one form as plants and may be transferred through the food chain to animals' bodies and their wastes, all of which can be converted for everyday human use through processes such as combustion, which releases the carbon dioxide stored in the plant materia1 [7]. Many of the biomass fuels used today come in the form of wood products, dried vegetation, crop residues, and aquatic plants. Biomass has become one of the most commonly used renewable sources of energy in the last two decades, second only to hydropower in the generation of electricity. It is such a widely utilized source of energy, probably due to its low cost and indigenous nature, that it accounts for almost 15% of the world's total energy supply and as much as 35% in developing countries, mostly for cooking and heating [7].

Biomass can be used as a source of biofuels (a renewable energy source) to reduce our dependence on fossil fuels, and to help reduce air pollution Panwar et al. [8] considered biomass as carbon neutral, due to the fact that the amount of carbon released is equivalent to the amount absorbed during its life time. Biomass has been widely considered as major source of energy in both developed and developing nations Examples of biomass and their uses for energy include:

- Wood and wood processing wastes—burned to heat buildings, to produce process heat in industry, and to generate electricity
- Agricultural crops and waste materials—burned as a fuel or converted to liquid biofuels
- Food, yard, and wood waste in garbage—burned to generate electricity in power plants or converted to biogas in landfills
- Animal manure and human sewage—converted to biogas, which can be burned as a fuel

Biomass energy is a relatively clean, renewable energy source involving the use of organic matter which collected energy from the Sun and converted it into chemical energy when it was alive [9]. It is a renewable source as this matter is continually growing and absorbing the Sun's energy, particularly where biomass crops are farmed. Most biomass energy is sourced from plants which have gathered energy from the Sun through the process of photosynthesis. This form of energy has been used by humans for thousands of years, since humans began to burn wood for heat. Advancements in technology have allowed biomass energy to be used in a wide variety of applications, including liquids and gases used for biofuels to power transport. The main uses of biomass energy today are for producing electricity through driving turbines and providing biofuel for transportation such as biodiesel and ethanol[10]. The acceptance of bioenergy has increased together with many modern ways to utilize it, especially in many of the industrialiszed countries. The use of bioenergy instead of fossil fuels is an attractive option to reduce fossil-fuel dependency and mitigate global climate change.. Biofuels are either liquid or gaseous fuel. They can be produced from any source that can be replenished rapidly, e.g. plants, agricultural crops and municipal waste [3]. Current biofuels are produced from sugar and starch crops such as wheat and sugar cane, which is also part of the food chain The continuous increase of energy consumption has generally improved the standard of living but it has also caused serious environmental problems. Since the beginning of the pre-industrial revolution, burning fossil fuels during energy production processes has been the main source of anthropogenic emissions of greenhouse gases (GHGs), causing global climate change with potential

negative impacts on climatic systems, natural environment, and human society [11,12]. In this context, renewable energies (REs) have emerged as sustainable and environmental friendly sources of energy, which can satisfy our current and future socio-economic needs [8].

Three main categories of bioenergy resources globally used are forestry biomass, agricultural biomass and wastes biomass. There exist forest and agriculture-based highly developed bioenergy sectors in countries such as Finland, Sweden, Germany, Austria, Brazil, and the United States of America [13]. The spread of the modern bioenergy sector is, however, still in its primary stages in many of the developed and developing countries in the world.

Converting Biomass to Energy

Solid biomass, such as wood and garbage, can be burned directly to produce heat. Biomass can also be converted into a gas called biogas or into liquid biofuels such as ethanol and biodiesel. These fuels can then be burned for energy.[7]

Biogas forms when paper, food scraps, and yard waste decompose in landfills, and it can be produced by processing sewage and animal manure in special vessels called digesters. Ethanol is made from crops such as corn and sugar cane that are fermented to produce fuel ethanol for use in vehicles. Biodiesel is produced from vegetable oils and animal fats and can be used in vehicles and as heating oil. . Some examples of biofuels and how they can be pricesssed is shown Table 1 [source [7]

	Processing	**Use**
Biogas	Bacteria break down sewage in a digester	The methane in biogas can be used as a fuel for heating homes
Bioethanol	Yeast breaks down the sugar in sugar cane to produce alcohol	Bioethanol is used in Brazil to fuel cars
Fast-growing timber	Trees such as willow can be burned in power stations	Electricity is generated using renewable biomass instead of fossil fuels

The methods involved in the conversion of biomass to energy include the following [4]

1. 1)Burning**:**

This is a very common way of converting organic matter into energy. Burning stuff like wood, waste and other plant matter releases stored chemical energy in the form of heat, which can be used to turn shafts to produce electricity. A simple illustration of how biomass is used to generate electricity. is shown as follows:

2. Energy from the sun is transferred and stored in plants. When the plants are cut or die, wood chips, straw and other plant matter is delivered to the bunker
3. This is burned to heat water in a boiler to release heat energy (steam).
4. The energy/power from the steam is directed to turbines with pipes
5. The steam turns a number of blades in the turbine and generators, which are made of coils and magnets.
6. The charged magnetic fields produce electricity, which is sent to homes by cables.
   Other ways in which organic matter can be converted into energy include:

2)Decomposition:

Things that can rot, like garbage, human and animal waste, dead animals and the like can be left to rot, releasing a gas called biogas (also known as methane gas or landfill gas). Methane can be captured by a

machine called Microturbine and converted into electricity. Sometimes, animal waste (poop) can also be converted into methane by a machine called 'Anaerobic Digester'

3)Fermentation:

Ethanol can be produced from crops with lots of sugars, like corn and sugarcane. The process used to produce ethanol is called gasification

Advantages of biomass energy over fossil fuels

A major advantage of biomass energy is that it produces a smaller amount of harmful greenhouse gases than fossil fuel alternatives produce. Bioenergy derived from biomass is a promising energy alternative that can reduce the greenhouse gas emissions generated from non-renewable fuels. Renewable energy from biomass is environmentally friendly when compared to fossil fuel resources; hence they are agent of sustainable development. Levels of the greenhouse gases methane and carbon (iv)oxide could be reduced through the use of biomass energy sources as these gases are produced by organic matter if left to decay without being used for a purpose such as this. Another environmental benefit of biomass energy is that it produces lower levels of sulfur (ivi) oxide which is a major component of acid rain. Biomass energy is easily sustainable if crops are farmed and managed effectively and is available wherever plants can be grown. Biomass energy can also be used for a range of different purposes, including heat production, fuel for cars and the production of electricity. Biofuels play an essential role in reducing the carbon emissions from transportation. The development of 'drop in' fuels produced from lignocellulosic raw materials will increase both the availability of biofuels and the sustainability of the biofuel industry".

Biomass is a renewable source of fuel to produce energy because:

- waste residues will always exist – in terms of scrap wood, mill residuals and forest resources; and
- properly managed forests will always have more trees, and we will always have crops and the residual biological matter from those crops..

Biomass power is carbon neutral electricity generated from renewable organic waste that would otherwise be dumped in landfills, openly burned, or left as fodder for forest fire. The environmental benefits of biomass power generation – using biomass fuel – are clear. By using waste material for fuel in our green energy plants, we prevent that waste from burdening our landfills even more, or being left to decay on the forest floor or urban lot.

Implication of Teaching Biomass and Biomass Energy to Secondary School Chemistry Students

The importance of chemistry in national development cannot be ruled out. Chemistry has generally led to improved living conditions of mankind. Research has shown that the teacher's mode of presentation of various science concepts in the class affects performance and achievement [14]. The role of the teacher in chemistry teaching is to provide varied opportunities for students to engage in activities that will enable them make sense of the world around them, make new discoveries, solve problems and develop skills that are sustainability driven [15]

The concept of organic chemistry is introduced to secondary school students at the senior secondary II(SS2) class where they are taught coal, hydrocarbons petroleum and petroleum products. In their SS3, they are also taught organic chemistry II which includes alkanols, alkanoic acids, esters carbohydrates, amines, amino acids etc [16].In teaching the concept of organic chemistry to SS2 students, teachers should not limit it to studying about coal, petroleum ,and petroleum products as if they are the only sources of fuels to the country.The teacher should include several activities to teach the students the concept of biomass and biomass energy as a renewable source of fuel and energy, differences between fossil fuels and bioenergy resources and how the depletion of fossil fuel is a serious global issue The teaching of alkanols, carbohydrates, cellulose etc should also incorporate the

current methods of obtaining biofuels like bioethanol ,biogas(methane) from biomass and also their uses as bioenergy sources. The teaching of chemical industries in SS3 should include biofuel industries –their products and the hazardous effects. Through these delivery approaches, the chemistry teacher will help to create more awareness of the concept of biomass and biomass energy to chemistry students. When the students are best informed about these concepts at the early stage of their education and they develop interest in learning them, they will be in a better position to enhance their utilization as good sources of renewable energy in future as they are the hope of the nation as future scientists.

Conclusion

The purpose of teaching is learning. When a lesson is prepared, we need to understand what the students want to know and what they need to know in order to encourage them to become lifelong learners. Teaching the concept of biomass and biomass energy to secondary school chemistry students will go a long way to change the students' perception positively towards the utilization of biomass as a renewable energy resource in Nigeria considering the depletion of fossils and the serious environmental hazards associated with their use as energy resources.

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Synthesis, Characterisation And Antimicrobial Studies Of Cerium(Iii) And Lanthanum(Iii) Complexes Of Schiff Base Derived From Salicylaldehyde And 2-Aminophenol

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Abstract

The Schiff base ligand, Salicylidene-2-aminophenol was synthesized by the condensation of salicylaldehyde with 2-aminophenol. The Cerium (iii) and Lanthanum (iii) complexes of the Schiff base were synthesized and characterized on the basis of melting point, solubility test, Infra-red spectroscopy, UV-visible spectroscopy and molar conductance. The IR spectra revealed that the metal ions coordinated through the azomethine nitrogen and the two phenolic oxygen depicting the tridentate nature of the ligand. The antimicrobial analysis of the prepared ligand and complexes were carried out against some fungi, gram positive and gram negative bacteria and the results compared with some known antibiotics. It was found that the complexes were effective against some tested organisms compared with the ligand.

Keywords: 2-Aminophenol, Salicylaldehyde, Antimicrobial studies, Complexes

Introduction

The coordination chemistry of nitrogen-oxygen donor ligands is an interesting area of research and considerable attention has been paid to it. Chelating ligands containing N and O donor atoms showed broad biological activity and are of special interest because of the ways in which they are bonded to metal ions[1]. Schiff bases are condensation products of primary amines with carbonyl compounds. The synthesis, structural investigation and reaction of Schiff base complexes have received a special attention because of their biological activities as antibacterial, antifungal, antimalaria, antiproliferative, anti-inflammatory, antiviral and antipyretic properties [2,3]. Mononuclear as well as polynuclear complexes can be formed when Schiff base ligands coordinate to one or more lanthanide ions. The Schiffbase ligands can act as tridentate ligands when they coordinate through the azomethine nitrogen and two phenolic oxygen. Lanthanide complexes have been studied recently due to their biological activities. Lanthanide ions possess the properties of antibacterial, antitumor and antivirus. Salicyladehyde is an important intermediate in the chemical industry, medical and pharmaceutical companies. It is used in perfumes, dyes, fragrances, pharmaceuticals etc. [4,5]. Salicylaldehyde is a key precursor to a variety of chelating agents [6]. In this paper, we present the synthesis and characterization of Cerium (iii) and Lanthanum (iii) complexes of salicylidene-2-aminophenol. The antimicrobial properties of the ligand and complexes were also reported.

Experimental

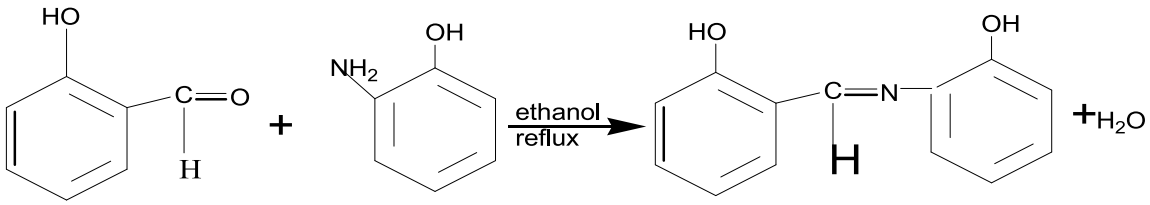
Chemicals and Instrumentation

2-Aminophenol, salicylaldehyde, lanthanum (iii) nitrate hexahydrate, cerium (iii) nitrate hexahydrate were procured from Sigma-Aldrich and supplied by Bristol Scientific Company Limited. The absolute ethanol was obtained from JHD. Infra-red spectra were carried out using Shimadzu FTIR-8400S Fourier Transform Infrared Spectrophotometer using KBr pellets. UV-Vis spectra were obtained using UV-2500PC series model spectrophotometer. The conductivity test was checked using DDS-22C high accuracy digital conductivity meter.

Synthesis of Salicylidene-2-aminophenol (HBAP)

HBAP was synthesized by literature procedure [7,8]. 1.5ml (10mmol) of salicylaldehyde in 20ml of ethanol was added drop wise to 20ml ethanolic solution of 2-aminophenol (1.09g,10mmol).

The reaction mixture was then refluxed for three hours. After cooling, the solid product obtained was collected by filtration and recrystallized from ethanol to obtain orange crystals.



Salicyaldehye 2-aminophenol Salicyaldehyde-2-aminophenol

Scheme 1: Synthesis of salicyaldehyde-2-aminophenol

Synthesis of Complexes

The complexes were also synthesized by literature procedure[9].The metal salts (0.5 mmol) in 20 ml of ethanol was added dropwise to ethanolic solution (20 ml) containing 1mmol of the Schiff base. The mixture was refluxed for three hours, concentrated by evaporation and allowed to cool. The crystals formed were purified by recrystallization using ethanol.

Antimicrobial Analysis

The ligand and complexes were screened for their antimicrobial activity against four bacteria (*staphylococcus aureus, streptococcus species, escherichia coli and pseudomonas species*) and two fungi (*aspergillusniger and candida albicans*) using Agar well diffusion technique [10]. Augumentinand ketoconazole were used as bacteria and fungi control respectively. The antimicrobial analysis was carried out at the Department of Microbiology, Imo State University, Owerri.

Results and Discussion

Physical properties of the synthesized compounds

The ligand and complexes were coloured, non-hygroscopic and stable solids. They are soluble organic solvents such as ethanol, acetone, methanol, DMSO and diethylether but insoluble in water and benzene. The low conductivity values of the ligand and complexes indicate that they are non-electrolytic in nature [11].

Table 1: Physical data of the ligand and complexes

Compound	Colour	Yield (%)		Conductivity
[HBAP]	Orange-red	92	162	2.1
[Ce(iii)complex]	Black	58	158	2.7
[La(iii)complex]	Brick-red	42	152	2.5

Table 2: Solubility test

Compound	Ethanol	Acetone	Methanol	DMSO	Benzene	Diethylether	water
[HBAP]	S	S	S	S	IS	S	IS
[Ce(iii)complex]	S	S	S	S	IS	S	IS
[La(iii)complex]	S	S	S	S	IS	S	IS

S= Soluble IS =Insoluble

Electronic spectra

Table 3: Electronic spectra data

Compound	Band (nm)	Assigned transition
[HBAP]	332.50	n➡π*
[Ce(iii)complex]	366.50	n➡π*
[La(iii)complex]	349.00	n➡π*

In the electronic spectra of the Schiff base ligand (HBAP), the bands which appeared at 332.50nm was assigned to n ➡π* which is due to the azomethine transitions. In the Ce(iii) and La(iii) complexes, the n ➡π * transitions had a red shift to 366.50nm and 349nm respectively. This shows coordination of the nitrogen atom of the azomethine group to the metal ion.

Infrared spectra

Table 4: IR spectra data

Compound	ν(O-H)	ν(OH)	ν(C=N)	ν(C=O)
[HBAP]	3695	-	1452.45	1193.98
[Ce(iii)complex]	-	3315.74	1500.67	1246.06
[La(iii)complex]	-	3400.62	1494.88	1234.48

The IR spectra of the ligand and complexes are similar but with slight differences. This indicates that the complexes derived from the same ligand possess similar structure. In the Ce(iii) and La(iii) complexes, the appearance of a broad band in the region of 3315 -3400cm^{-1} indicates the presence of coordinated water molecules. The disappearance of (OH) phenolic band around 3695cm^{-1} which was in the ligand can be attributed to the coordination through the phenolic oxygen after deprotonating. The band due to ν(C=O) vibration was observed at 1193cm^{-1} in the ligand but was observed in the range of 1234 - 1246cm^{-1} in the complexes. This observation may be attributed to the formation of the M-O bond after the deprotonating of the hydroxyl group in the phenyl ring. The azomethine bond vibration ν(C=N) of the ligand was observed at 1452.45cm^{-1}. There was a red shift in the Ce(iii) and La(iii) complexes to 1500cm^{-1} and 1494cm^{-1} respectively. This confirms the coordination through azomethine nitrogen in all the complexes.

Antimicrobial Analysis

Table 5: Antimicribial activity data of the Schiff base

Zone of Inhibition (DZI)mm

Conc(ppm)	*Pseud.*	*E.Coli*	*Staph*	*Strep.*	*Asperg.*	*Candida*
1000	2	15	20	25	-	-
500	-	8	15	14	-	-
250	-	5	8	9	-	-
100	-	5	6	5	-	-

Table 6: Antimicrobial activity data of the Ce(III) complex

Zone of Inhibition (DZI)mm

Conc(ppm)	*Pseud.*	*E.Coli*	*Staph*	*Strep.*	*Asperg.*	*Candida*
1000	15	15	15	15	8	-
500	15	13	13	9	5	-
250	13	8	10	5	5	-
100	12	6	7	4	4	-

Table 7: Antimicrobial activity data of the La (III) complex

Zone of Inhibition (DZI)mm

Conc(ppm)	*Pseud.*	*E.Coli*	*Staph*	*Strep.*	*Asperg.*	*Candida*
1000	20	16	28	13	5	21
500	13	6	13	5	5	17
250	6	5	12	4	4	11
100	3	-	11	2	-	9

The results of the zone of inhibition diameter (DZI) shown in the table 5,6,7 showed that the complexes were more active than the ligand on some organisms especially the fungi. The organisms are *pseudomonas, aspergillusniger and candida albicans.* The La (III) complex was more active on the two fungi compared to the Ce(III) complex..This observation is due to the overtones and chelation theory [12].

Tconclusion

The Ce(iii) and La (iii) complexes of salicylidene-2-aminophenol were synthesized and characterized by physical and spectroscopic techniques. The ligand acts as a tridentate species coordinating through to the metal ions through the oxygen of the phenolic group and the nitrogen in the azomethine group. The antimicrobial analysis showed that the complexes exhibited antimicrobial activity than the ligand on the fungi and one bacterium.

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The Kinetics of The Oxidation of [N-(2-hydroxy-ethyl)ethlyenediamine- N, N', N'-triacetatocobalt (II)] by Copper (II) Ion.

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ABSTRACT

The kinetics of the oxidation of N-(2-hydroxy-ethyl) ethylenediaminetriacetatocobaltate (II) ions by Cu(II) in aqueous perchloric acid medium was studied under pseudo- first order conditions of large excess of Cu(II) at T =28^{0}C, 1=0.05moldm^{-3} ($NaClO_4$), [H^+]= 5x10^{-3} moldm^{-3}. The stoichiometric studies showed that for every mole of [CoHEDTA(H_2O)], one mole of Cu(II) was consumed. The rate data for the oxidation of [CoHEDTA(H_2O)] by Cu(II) were obtained as a decrease in absorbance of the resulting mixture at 510nm.

The kinetic curves obtained under this conditions were exponential and the rate constant were obtained from the logarithmic plot of absorbance difference log(A_t -A∞) against time (t). pseudo- first order rate constants were determined from the slope of the plot, based on the following equation:

$(A\infty - A_t) - (A\infty - A_0)e^{-kobs.t}$

The logarithmic plot of the difference in absorbance of the reacting solution at 510nm against time was linear and the k_{obs} increased with [Cu(II)]. The plot of logk_{obs} versus log[CU^{2+}] at constant [H^+] and constant ionic strength was linear with negligible intercept and a slope of 0.5 indicating half-order dependence of rate with respect to [Cu(II)]. The reaction showed positive acid dependence, negative Bronsted-Dye primary salt effect. The plausible rate of constant acid concentration is given as:

$$\frac{-d[Cu^{2+}]}{dt} = \{c + d[H^+]\}[Co^{II}HEDTA(H_2O)][Cu^{2+}]$$

INTRODUCTION

Copper is one of the transition elements frequently found at the active site of proteins[1-3]. The copper-containing enzymes and proteins constitute an important class of biologically active compounds (Mukherjee, 2003).[4] The biological functions of copper proteins/enzymes include electron transfer[5], dioxygen transport[6], oxygenation, oxidation, reduction and disproportionation[7-9]

In nature, a variety of copper proteins are essential constituents of aerobic organisms[10], including hemocyanins (arthropodal and molluskan O_2 carriers) and enzymes that "activate" O_2, promoting oxygen atom incorporation into biological substrates (Holm etal, 1996)[11].

The latter include tyrosinase (a monooxygenase, incorporating one oxygen atom to the substrate and reducing the other to water)[12] and dopamine β -hydroxylase (a monooxygenase). "Blue" multicopper oxidases [e.g.. laccase (phenol and diamine oxidation)[13], ascorbate oxidase (oxidation of l- ascorbate) and ceruloplasmin] promote substrate one- electron oxidation while reducing O_2 to water[14].

EXPERIMENTAL

All reagents used were of analar grade. The stock solutions of [$CoHEDTAOH_2$] were prepared according to the method of Mansour (2003)[15-17], Copper (II) tetraoxosulphate (VI) was prepared by

dissolving accurate weighed amount of the salt in a known volume of distilled water. The $_{max}$(510nm) was determined by running the electronic spectrum of the solution of [$CoHEDTAOH_2$] in the wavelength range of 340 -700nm, and plotting a graph of the absorbance against wavelength.

A stock solution of perchloric acid was made by diluting analar grade acid (70%, specific gravity 1.67) and standardizing titrimetrically. Analar grade sodium perchlorate ($NaClO_4$) was used to maintain the ionic strength.

KINETICS

The wavelength of maximum absorption, $_{max}$ of $[CoHEDTAOH_2]^-$ was 510nm using spectrum lab 330 – 1000 spectronic 23_A spectrophotometer. The rate of the reaction of $[CoHEDTAOH_2]^-$ with Cu^{2+} ion was studied at this $_{max}$ by observing the change in absorbance of $[CoHEDTAOH_2]^-$ at 28°C and 0.05moldm^{-3} ($NaClO_4$) ionic strength.

The plots of log (A_t - A∞) versus time were made. From the gradient, the pseudo – first order rate constants k, were determined as given by the equation.

$$\text{Log}(A_t - A\infty) = \frac{K_1 t}{2.303} + \log(A_O - A\infty) \quad \text{(I).}$$

Where A∞, A_t are the absorbances of the reaction mixture at time infinity, and t, respectively. The second order rate constants (k_2) were obtained from k, as $k_1/[Cu^{2+}]$. The results are presented in table 1.

RESULTS/ DISCUSSION

STOICHIOMETRY

The stoichiometry of the $[CoHEDTAOH_2]^-$ with Cu^{2+} reaction was determined by spectrophotometric titration using the mole ratio method. The concentration of the $[CoHEDTAOH_2]^-$ was kept constant at 1x 10^{-4}moldm^{-3}, while that of Cu^{2+} was varied from 1.5 x 10^{-5} - 1x10^{-4}moldm^{-3} at ionic strength, 1= 0.05moldm^{-3} ($NaClO_4$) and $[H^+]$ = 5x 10^{-3}moldm^{-3}.

The reactions were allowed to go to completion and the absorbances of the solutions were taken at 510nm. The stoichiometry was determined from the plot of absorbance versus mole ratio $[Cu^{2+}]$ / $[CoHEDTAOH_2]^-$.[18]

On the basis of the stoichiometry, final absorbances at completion of reaction were plotted against mole ratio. The result indicated that one mole of $[CoHEDTAOH_2]$ reacted with one mole of $[Cu^{2+}]$. The stoichiometric equation for reaction is presented as equation 2.

$$/[CoHEDTAOH_2]^{-+} Cu^{II} \qquad [CoHEDTAOH_2] + Cu^{I} \quad \text{.....(2).}$$

ORDER OF REACTION

The pseudo – first order plots of log (A_t - A∞) versus time were linear to greater than 75% extent of the reaction respectively.

The linearity of the plot indicates that the reaction is first order with respect to $[CoHEDTAOH_2]$[19-20].

From the slopes of the plots, the pseudo – first order rate constants (k_{obs}) were obtained. Analysis of the plot of log kobs versus log $[Cu^{2+}]$(fig 2), gave a slope of 0.46, indicating half – order dependence of rate on $[Cu^{2+}]$.

The reaction is (1½) order overall. The rate law at constant $[H^+]$ is represented as in equation 3.

$$\frac{d[CoHEDTAOH_2]}{at} = k_{obs}[CoHEDTAOH_2][Cu^{2+}] \quad \ldots\ldots\ldots\ldots (3).$$

The effect of $[H^+]$ on the rate of the reaction was investigated using perchloric acid in the range $3.0 \leq H^+ \leq 11.0 \times 10^{-3}$ moldm^{-3}, while the $[CoHEDTAOH_2]^-$ and $[CU^{2+}]$ were kept constant. The reaction was carried out at 28^{0}C and 1 = 0.05moldm^{-3} ($NaClO_4$).

The results are presented in table 1. The results shows that the rate of reaction increased with increase in $[H^+]$ in the range investigated. The plot of k_2 versus $[H^+]$ was linear with intercept on the k_2 axis as shown in figure 3.

The acid dependent rate constant is represented as in equation 4.

$$k_2 = c + d[H^+]. \qquad (4).$$

The overall rate equation in the acid range investigated is

$$\frac{-d[Cu^{2+}]}{dt} = (c+d[H^+])[CoHEDTAOH_2][Cu^{2+}] \qquad (5).$$

Acid dependence of this type shows that there are two parallel reaction pathways; one which is acid dependent and the other that is acid – independent[16,17,21.]

The effect of ionic strength on the rate of the reaction was investigated in the range 1 = 0.02 – 0.06moldm^{-3} ($NaClO_4$), while the concentration of other reagents was kept constant. The results are presented in table 1. Ionic strength dependence studies show a general trend of decrease in reaction rate with increase in ionic strength of the reaction medium, as shown in table 1. Decrease in reaction rate with increase in ionic strength of the medium is noted to occur in reaction that involves oppositely charged ions[17,22–24]. The plot of logk_2 versus √1 was linear.

Table 1: Pseudo-first order rate constant for the reaction of [$Co^{II}HEDTA(H_2O)$] and [Cu^{II}] at [$Co^{II}HEDTA(H_2O)$]=1×10^{-4} ($moldm^{-3}$), T=29±1°C and ^max =510nm.

10^3 [Cu^{2+}] ($moldm^{-3}$)	10^3 [H^+] ($moldm^{-3}$)	I, $NaClO_4$($moldm^{-3}$)	10^3 k_{obs} (S^{-1})	K_2 $dm^3mol^{-1}(S^{-1})$
2.0	5.0	0.05	4.2989	21.4945
5.0	5.0	0.05	4.606	9.212
7.0	5.0	0.05	5.066	7.237
10.0	5.0	0.05	6.333	63.33
12.0	5.0	0.05	8.0605	67.171
7.0	3.0	0.05	5.389	7.699
7.0	5.0	0.05	6.275	8.964
7.0	7.0	0.05	6.37	9.1
7.0	9.0	0.05	7.860	11.229
7.0	11.0	0.05	9.81	14.014
6.0	5.0	0.02	10.608	17.68
6.0	5.0	0.03	7.64696	12.743
6.0	5.0	0.04	5.47485	9.125
6.0	5.0	0.05	3.915	6.525
6.0	5.0	0.06	3.247	5.412

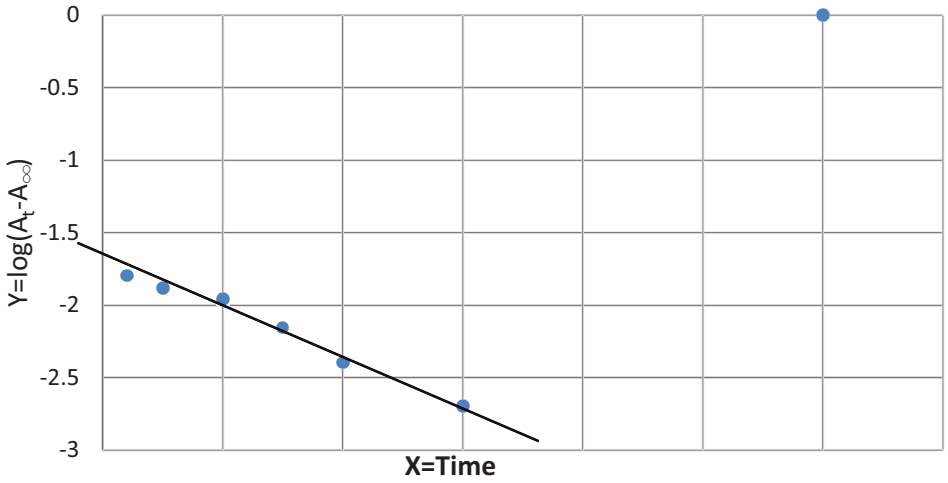

Fig. 1: Graph of log (A_t-A_∞) versus time

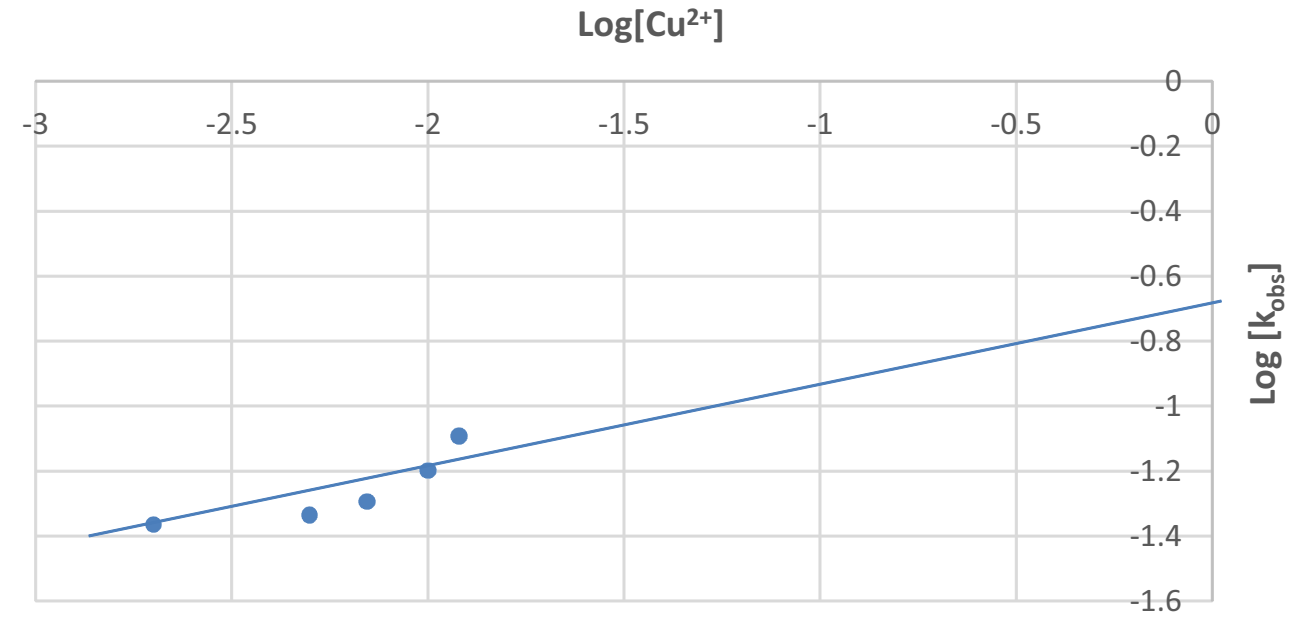

Fig. 2: Plot of log k_{obs} versus log $[Cu^{2+}]$ at constant $[H^+]$ and constant ionic strength.

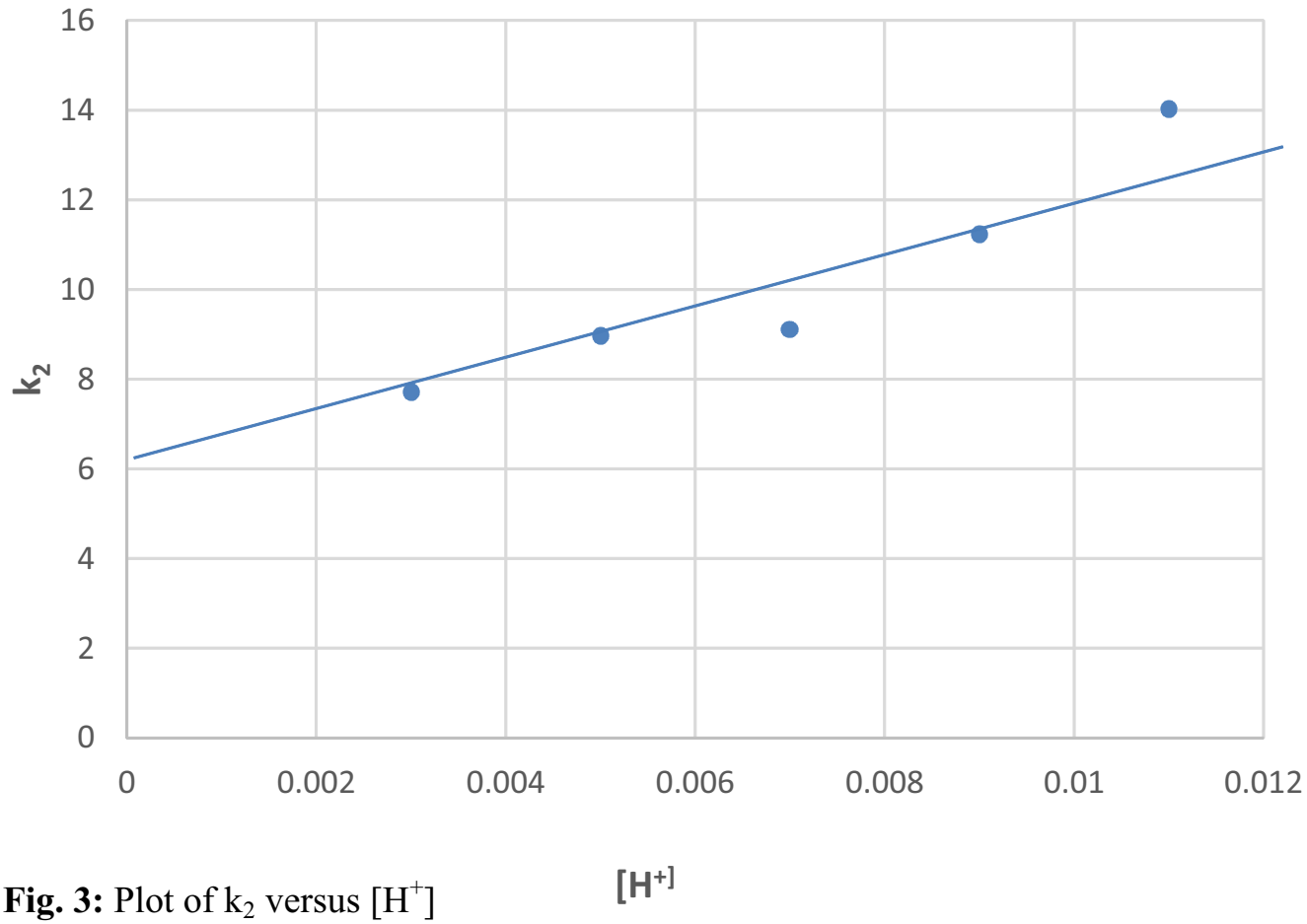

Fig. 3: Plot of k_2 versus $[H^+]$

CONCLUSION

We are preparing to investigate the effect of other interesting parameters like dielectric constant (D), activation parameters and some other things such as catalysis and the presence of free radicals in this reacting system. This will enable us to formulate plausible mechanism for this reaction. If we are able to do this, then we are fulfilling our ultimate aim which is to avail more understanding in the numerous important reactions that are going on in biomolecules (copper protein/enzymes).

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Hydrocarbon distributions in a vegetated and un-Vegetated spent engine oil polluted soil

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ABSTRACT

The aim of this study was to determine the total petroleum hydrocarbon distribution in un-vegetated and vegetated (*Senna alata)* soil polluted by spent engine oil (SEO) during rainy and dry seasons. One hundred polythene bags filled with 20 kg of soil were separated into two groups; A (50) and B (50). Group A contained *S. alata*plant while Group B had no plant. They were set up in completely randomized design. Both parts were polluted with different concentrations (0.5% v/w, 1.5% v/w and 2.5% v/w) of SEO 60 days after planting (DAP). The hydrocarbon distributions of the vegetated and un-vegetated soil samples in the rainy season and dry season were analyzed ninety days after pollution. Results showed that percentage of total hydrocarbons degraded/removed from 0.5% v/w, 1.5% v/w and 2.5% v/w vegetated soils with *S. alata* were 99.95%, 99.68% and 99.28%, respectively. *S. alata*removed 0.06%, 0.18% and 8.05% hydrocarbons for the same pollution concentrations, respectively. The study gives positive remediation potentials for *S.alata.* Hence, the plant is an ideal plant for the removal of hydrocarbons in SEO contaminated soil. *S.alata* can be regarded as a hyper accumulator for some polycyclic aromatic hydrocarbons.

INTRODUCTION

Spent engine oil contains complex mixtures of paraffinic, naphthalenic and aromatic petroleum hydrocarbons and various contaminants that may contain one or more of the following: carbon deposits, sludge, wear metals and metallic salt, aromatic and non aromatic solvents, water (as water in-oil emulsion), glycols, silicon based antifoaming compounds, fuel, polycyclic aromatic hydrocarbons (PAHs) and miscellaneous lubricating oil additive materials (Ayoola and Akaeze, 2012). Engine oil becomes contaminated as a result of physical and chemical reactions. Metals from engine from time to time erode into the engine oil forming impurities. Oxidation of hydrocarbon chains bond together to form sludge due to high temperature. Incombustible gasoline up to about 5% wt often leak from fuel injector line, contaminating the oil (Fedak, 2001). Some additives such as multiple sulfur-based detergents which keep materials from depositing on the engine piston often begin to break down as sludge and accumulate in motor oil (Fedak, 2001).

Used motor oils are also characterized by high concentrations of PAHs. Dominguez-Rosado and Pichtel (2003) found that the PAHs content of used motor oil was often between 34 and 90 times higher than new oil. PAHs belong to a group of over 100 hazardous substances of organic pollutants consisting of two or more fused-benzene aromatic rings (Obini*et al.,* 2013). In nature, PAHs may be formed by high temperature pyrolysis of organic materials or low to moderate temperature diagenesis of sedimentary organic materials to form fossil fuel or direct biosynthesis by microbes and plants (GFEA, 2012 and USGS, 2014). Sources of PAHs can be both natural and anthropogenic. Natural sources include forest and grass fire, oil seeps, volcanoes, chlorophyllous plants, fungi and bacteria. Anthropogenic sources include petroleum, power generation, refuse incineration, home heating, internal combustion engine etc. (GFEA, 2012 and USGS, 2014).

Polycyclic aromatic hydrocarbons (PAHs) have low solubility in water and are highly lipophilic. In water or when adsorbed on particulate matter, PAHs can undergo photodecomposition in the presence

of ultraviolet light from solar radiation (Obinet et al 2003) Heavy PAHs (C_{16}-C_{50}) are more stable and toxic than the light PAHs (C_6-C_{16}) (ATSDR, 1995). According to Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) list of hazardous substances, PAHs ranked 7th in 2005 biennial ranking of chemicals deemed to pose the greatest possible risk to human health. Some PAHs have been demonstrated to be mutagenic and carcinogenic in humans and those that have not been found to be carcinogenic may, however, synergistically increase the carcinogenicity of other PAHs (Obini*et al.,* 2013).

The process of reduction, containment, and/or alteration of soil and water contamination by plants is called phytoremediation () The significance of phytoremediation is not just in cleaning toxins from the soil and ground water, but also to prevent the contaminated solution (leachate) from migrating and spreading beyond the boundaries of a polluted site.

Current public concern and rising costs regarding the conventional clean-up procedures demonstrates the need for a less expensive bioremediation option such as phytoremediation. Bioremediation is largely considered a promising technology for the tropics because climatic conditions favor microbial growth and activity. One of the first steps in the selection of species for phytoremediation in the tropics is the screening of plant species for their capability to grow and establish in contaminated soil, followed by the evaluation of their influence on the degradation of petroleum hydrocarbons in soil (Merkl*et al.,* 2004).

Objective of the study

The aim of this study was to determine the total petroleum hydrocarbon distributions in a vegetated and un-vegetated spent engine oil polluted soil.

MATERIALS AND METHODS

Sample collection

The soil samples were collected from different locations within MgbukaObosi market in Obosi, Idemili North LGA, Anambra state. Samples were obtained by hand auger at drainage collection points, in the direction of natural drainage (South-East to North-West). Samples were collected simultaneously, usually wrapped in an aluminium foil and then put in polyethylene bags for onward transportation to the laboratory.*Senaalata*used in the study were collected from Anyafulugu botanical garden Awka, Anambra state Nigeria and were identified by the botanist from Dept. of Science Laboratory Technology, Federal Polytechnic Oko, Anambra State.

Experimental design

The experiment were carried out in dry season and rainy season. In both experiment, One hundred polythene bags were filled with 20 kg of soil each, separated into two groups A (50) and B (50). Group A contained *S. alata*plant, while Group B had no plant. Both parts were polluted with different concentrations (0.5% v/w, 1.5% v/w and 2.5% v/w) of SEO 60 days after planting (DAP). The bags were well labeled and displayed in a completely randomized design under the sun in the open space.

Procedure for phytoremediation experiment

The *S. alata* of equal length (10cm each) with circumference of 5 to 6 cm was sown in 50 soil bags. The remaining 50 bags left un-vegetated. Vegetated and un-vegetated bags were watered sparingly when necessary.

- 14 days after pollution, both vegetated and un-vegetated soils were randomly collected from the different treatments separately in amber bottles and labeled accordingly. The stems, leaves and roots of plants treated with different concentrations of spent engine oil (as well as the control) were also randomly collected and labeled accordingly.
- All samples were subjected to Gas Liquid Chromatography (GLC) to determine the Total

Petroleum Hydrocarbons (TPH) degraded by the plants and those left in the soil. The un-spent engine oil were also analysed to determine the TPH composition which serves as the reference standard.This were repeated until 90 days after pollution.

Data analysis

The data were presented as mean ± standard error using SPSS version 16. Analysis of variance (ANOVA)was carried out and P values of < 0.05 wereconsidered statistically significant.

RESULTS AND DISCUSSION

Physicochemical characteristics of soil such as pH, organic matter (OM), electrical conductivity, nitrogen contents and other mineral content are known to influence the interactions and dynamics of PAHs within the soil matrix. The results of the physicochemical characteristics of the soils investigated during rainy and dry seasons are summarized in Table 1 and 2. From the tables, it was observed that the polluted soil was higher in total nitrogen content, pH, exchangeable base and micronutrient content than the unpolluted soil.

Polycyclic aromatic hydrocarbons (PAHs) of various concentrations were detected in unused SEO and range from C_{14}-C_{22} (Table 3 and 4). The concentrations of total hydrocarbons in the un-vegetated and vegetated soils increased with increase in the concentrations of SEO applied among the treatments. Un-vegetated soils had more concentrations of total hydrocarbons than those of the vegetated soils (Table 3 and 4).

Table 1: Physiochemical properties of vegetated and un-vegetated soil during dry season

SOIL PROPERTIES	SOIL SAMPLE	SEOZERO	SEO50	SEO150	SEO250
pH	*S alata* Vegetated	6.740 ± 0.0208	6.450 ± 0.0289	6.300 ± 0.0577	6.033 ± 0.0333
	Un-vegetated soil	6.633 ± 0.0167	13.333 ± 0.1764	15.033 ± 0.1453	15.233 ± 0.1453
EC	*S alata* Vegetated	13.210 ± 0.0058	16.000 ± 0.5774	17.000 ± 4.155	15.667 ± 0.3333
	Un-vegetated soil	11.420 ± 0.0153	13.333 ± 0.1764	15.033 ± 0.1453	15.233 ± 0.1453
Organic matter	*S alata* Vegetated	2.887 ± 0.0088	4.167 ± 0.0882	6.000 ± 0.5774	5.900 ± 0.0577
	Un-vegetated soil	3.050 ± 0.0289	5.750 ± 0.0115	5.767 ± 0.0333	5.977 ± 0.0233
C	*S alata* Vegetated	1.680 ± 0.0116	2.417 ± 0.0186	4.167 ± 0.0882	3.533 ± 0.0667
	Un-vegetated soil	1.790 ± 0.0058	3.387 ± 0.0567	3.373 ± 0.0088	3.490 ± 0.0058
N	*S alata* Vegetated	0.243 ± 0.0088	0.160 ± 0.0058	0.160 ± 0.0058	0.157 ± 0.0033
	Un-vegetated soil	0.237 ± 0.0067	0.150 ± 0.0115	0.150 ± 0.0058	0.153 ± 0.0033
P	*S alata* Vegetated	36.667 ± 0.8812	30.757 ± 0.0285	30.757 ± 0.0285	25.480 ± 0.2894
	Un-vegetated soil	37.250 ± 0.1277	0.150 ± 0.5774	30.533 ± 0.2334	22.933 ± 0.6350
K	*S alata* Vegetated	0.233 ± 0.0203	0.200 ± 0.0000	0.200 ± 0.0000	0.287 ± 0.0067
	Un-vegetated soil	0.243 ± 0.0176	0.180 ± 0.0379	0.260 ± 0.0058	0.260 ± 0.0058
	S alata Vegetated	2.843 ± 0.0233	3.233 ± 0.1453	3.233 ± 0.1453	1.367 ± 0.1856
	Un-vegetated soil	2.733 ± 0.1202	2.833 ± 0.0333	54.0404 ± 52.4800	1.843 ± 0.0296
Magnesium	*S alata* Vegetated	0.927 ± 0.0371	4.134 ± 0.08882	1.133 ± 0.0882	1.833 ± 0.1667
	Un-vegetated soil	1.797 ± 0.0088	2.783 ± 0.1642	1.600 ± 0.1155	1.380 ± 0.0116
Na	*S alata* Vegetated	0.983 ± 0.0088	0.800 ± 0.0577	0.800 ± 0.0578	0.217 ± 0.0441
	Un-vegetated soil	1.227 ± 0.0120	0.680 ± 0.0116	1.047 ± 0.0291	1.227 ± 0.3712

Table 2: Physiochemical properties of vegetated and un-vegetated soil during rainy season

SOIL PROPERTIES		SEO ZERO	SEO 50	SEO 150	SEO 250
pH	***S alata* Vegetated**	6.140 ± 0.0702	6.416 ± 0.0167	6.800 ± 0.1000	7.067 ± 0.0667
	Un-vegetated soil	6.140 ± 0.0702	6.416 ± 0.0167	6.800 ± 0.1000	7.067 ± 0.0667
Electrical Conductivity	***S alata* Vegetated**	39.203 ± 0.1155	41.633 ± 0.8762	57.333 ± 1.4530	73.100 ± 0.1000
	Un-vegetated soil	39.203 ± 0.1155	41.633 ± 0.8762	57.333 ± 1.4530	73.100 ± 0.1000
Organic Matter	***S alata* Vegetated**	4.030 ± 0.0300	4.267 ± 0.0333	5.967 ± 0.0333	5.967 ± 0.0333
	Un-vegetated soil	4.030 ± 0.0300	4.267 ± 0.0333	5.967 ± 0.0333	5.967 ± 0.0333
Carbon	***S alata* Vegetated**	1.870 ± 0.0208	3.533 ± 0.1333	3.933 ± 0.0667	3.933 ± 0.1202
	Un-vegetated soil	1.870 ± 0.0208	3.533 ± 0.1333	3.933 ± 0.0667	3.933 ± 0.1202
Nitrogen	***S alata* Vegetated**	0.260 ± 0.0173	0.173 ± 0.0088	0.140 ± 0.0153	0.1400 ± 0.0208
	Un-vegetated soil	0.260 ± 0.0173	0.173 ± 0.0088	0.140 ± 0.0153	0.110 ± 0.0203
Phosphorous	***S alata* Vegetated**	30.417 ± 0.0167	30.000 ± 0.0000	27.923 ± 0.5124	27.923 ± 0.3333
	Un-vegetated soil	30.4160 ± 0.0167	30.000 ± 0.0000	27.923 ± 0.5124	25.333 ± 0.3333
Potassium	***S alata* Vegetated**	0.250 ± 0.0153	0.230 ± 0.0208	0.207 ± 0.0217	0.208 ± 0.0058
	Un-vegetated soil	0.250 ± 0.0153	0.230 ± 0.0208	0.208 ± 0.0217	0.290 ± 0.0058
Calcium	***S alata* Vegetated**	3.283 ± 0.1424	3.600 ± 0.2887	2.717 ± 0.1093	2.717 ± 0.3333
	Un-vegetated soil	3.283 ± 0.1424	5.000 ± 0.2887	2.717 ± 0.1093	2.033 ± 0.3333
Magnesium	***S alata* Vegetated**	1.800 ± 0.0333	1.567 ± 0.0667	1.483 ± 0.0441	1.483 ± 0.1528
	Un-vegetated soil	1.567 ± 0.3333	1.567 ± 0.0667	1.483 ± 0.0441	1.200 ± 0.15218
Sodium	***S alata* Vegetated**	1.800 ± 0.0577	1.100 ± 0.2517	1.143 ± 0.3285	1.143 ± 0.5525
	Un-vegetated soil	1.800 ± 0.0577	1.100 ± 0.2517	1.143 ± 0.3285	1.217 ± 0.5525

Table 3: Total petroleum hydrocarbon distribution (mg/ml) in soil vegetated and un - vegetated, polluted with different concentrations of spent engine oil in dry season

HYDROCARBON	SEOunused	Treatment	SEOzero	SEO 50	SEO 150	SEO 250
Naphthalene (C_{10} H_8)	1090.00 ± 0.0000	*S alata* Vegetated	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
		Un-vegetated soil	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
Acenaphthylene (C_{12} H_8)	0.00 ± 0.00	*S alata* Vegetated	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
		Un-vegetated soil	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Acenaphthylene (C_{12} H_{10})	1959.333 ±29.6722	*S alata* Vegetated	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
		Un-vegetated soil	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	334.000 ± 2.0817
Fluorene (C_{13} H_{10})	0.000 ± 0.5774	*S alata* Vegetated	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.267 ± 0.0203
		Un-vegetated soil	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.267 ± 0.0203
Phenanthrene (C_{14} H_{10})	1201.667 ± 1.6667	*S alata* Vegetated	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
		Un-vegetated soil	0.000 ± 0.0000	0.000 ± 0.0000	0.347 ± 0.0291	0.563 ± 0.0186
Anthracene (C_{14} H_{10})	0.000 ± 0.0000	*S alata* Vegetated	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
		Un-vegetated soil	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
Pyrene (C_{16} H_{10})	0.000 ± 0.0000	*S alata* Vegetated	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
		Un-vegetated soil	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
Fluoranthene (C_{16} H_{10})	455.667 ± 2.9627	*S alata* Vegetated	0.000 ± 0.0000	12.023 ± 0.0233	17.467 ± 0.3643	27.330 ± 0.1670
		Un-vegetated soil	0.000 ± 0.0000	21.10 ± 0.0000	33.88 ± 0.0000	56.12 ± 0.0000
Chysene (C_{18} H_{10})	0.000 ± 0.0000	*S alata* Vegetated	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
		Un-vegetated soil	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
Benzo (b) Fluroanthene (C_{20} H_{12})	0.000 ± 0.0000	*S alata* Vegetated	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
		Un-vegetated soil	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
Benzo (k) Fluroanthene (C_{20} H_{12})	0.000 ± 0.0000	*S alata* Vegetated	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
		Un-vegetated soil	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000

Benzo (a) Pyrene (C_{20} H_{12})	0.000 ± 0.0000	***S alata* Vegetated**	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
		Un-vegetated soil	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
Indeno (1,2,3, -cd) Pyrene (C_{22} H_{12})	6.293 ± 0.3550	***S alata* Vegetated**	0.000 ± 0.0000	0.263 ± 0.0445	1.263 ± 0.1200	2.000 ± 0.00002
		Un-vegetated soil	0.000 ± 0.0000	10.733 ± 0.0208	12.000 ± 0.0000	.21.00 ± 0.1054
Benzo (ghi) Perylene (C_{22} H_{12})	0.00 ± 0.0333	***S alata* Vegetated**	0.000 ± 0.0000	3.133 ± 0.0882	7.153 ± 0.2598	11.443 ± 0.7279
		Un-vegetated soil	0.000 ± 0.0000	10.000 ± 0.0000	20.697 ± 0.0203	21.097 ± 0.0524
Dibenzia (h) Anthracene (C_{22} H_{12})	0.000 ± 0.0000	***S alata* Vegetated**	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
		Un-vegetated soil	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000

Table 4: Total petroleum hydrocarbon distribution (mg/ml) in soil vegetated and un - vegetated, polluted with different concentrations of spent engine oil in dry season

HYDROCARBONS	**SEO UNSED**	**Treatment**	**SEO ZERO**	**SEO 50**	**SEO 150**	**SEO 250**
Naphthalene (C_{10} H_8)	**0.000 ± 0.0000**	***S alata* Vegetated**	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
		Un-vegetated soil	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
Acenaphthylene (C_{12} H_8)	**7004.333 ± 0.8819**	***S alata* Vegetated**	0.000 ± 0.0000	13.167 ± 0.2186	24.477 ± 0.7487	26.000 ± 1.5275
		Un-vegetated soil	0.000 ± 0.0000	15.333± 0.1764	25.143 ± 0.1433	54.067 ± 0.9684
Acenaphthylene (C_{12} H_{10})	**1959.000 ± 0.5774**	***S alata* Vegetated**	0.000 ± 0.0000	9.633 ± 0.3180	32.000 ± 1.1547	77.667 ± 0.3333
		Un-vegetated soil	0.000 ± 0.0000	18.933 ± 0.5812	33.733 ± 0.8969	181.433 ± 136.2833
Fluorene (C_{13} H_{10})	**1270.233 ± 0.0667**	***S alata* Vegetated**	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
		Un-vegetated soil	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
Phenanthrene (C_{14} H_1)	**1200.967 ± 0.5484**	***S alata* Vegetated**	0.000 ± 0.0000	10.000 ± 0.1732	42.333 ± 1.2028	65.100 ± 0.1000
		Un-vegetated soil	0.000 ± 0.0000	15.000 ± 2.0817	56.000 ± 1.5275	75.000 ± 1.1547
Anthracene (C_{14} H_{10})	**0.000 ± 0.0000**	***S alata* Vegetated**	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
		Un-vegetated soil	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
Pyrene (C_{16} H_{10})	**0.000 ± 0.0000**	***S alata* Vegetated**	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
		Un-vegetated soil	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000

Fluoranthene (C_{16} H_{10})	**455.500 ± 0.2517**	***S alata* Vegetated**	0.000 ± 0.0000	2.083 ± 0.0426	22.467 ± 0.7424	42.333 ± 1.4530
		Un-vegetated soil	0.000 ± 0.0000	12.167 ± 0.1202	28.000 ± 1.5275	46.333 ± 1.8559
Chysene (C_{18} H_{10})	**0.000 ± 0.0000**	***S alata* Vegetated**	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
		Un-vegetated soil	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
Benz (a) anthacene (C_{18} H_{12})	**1208.610 ± 0.1986**	***S alata* Vegetated**	0.000 ± 0.0000	2.436 ± 0.0318	30.400 ± 2.0881	56.800 ± 0.6110
		Un-vegetated soil	0.000 ± 0.0000	12.467 ± 0.0333	38.667 ± 4.1767	70.000 ± 2.0817
Benzo (b) Fluroanthene (C_{20} H_{12})	**0.000 ± 0.0000**	***S alata* Vegetated**	0.000 ± 0.0000	22.233 ± 1.0806	30.000 ± 0.0000	99.800 ± 1.3317
		Un-vegetated soil	0.000 ± 0.0000	24.867 ± 1.0729	40.333 ± 4.0552	106.000 ± 2.0817
Benzo (k) Fluroanthene (C_{20} H_{12})	**0.000 ± 0.0000**	***S alata* Vegetated**	0.000 ± 0.0000	24.500 ± 0.2887	70.333 ± 1.4530	61.267 ± 4.1478
		Un-vegetated soil	0.000 ± 0.0000	25.333 ± 0.3333	73.667 ± 2.3333	63.000 ± 3.0000
Benzo (a) Pyrene (C_{20} H_{12})	**0.000 ± 0.0000**	***S alata* Vegetated**	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
		Un-vegetated soil	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
Indeno (1,2,3, -cd) Pyrene (C_{22} H_{12})	**6.291 ± 0.0049**	***S alata* Vegetated**	0.000 ± 0.0000	1.763 ± 0.2934	12.667 ± 0.6667	18.467 ± 0.1856
		Un-vegetated soil	0.000 ± 0.0000	11.667 ± 0.3333	22.733 ± 0.6360	28.333 ± 0.3333
Benzo (ghi) Perylene (C_{22} H_{12})	**7.960 ± 0.0153**	***S alata* Vegetated**	0.000 ± 0.0000	24.500 ± 0.2887	70.333 ± 1.4530	61.267 ± 4.1478
		Un-vegetated soil	0.000 ± 0.0000	26.333 ± 0.6667	72.607 ± 1.4530	63.333 ± 2.6034
Dibenzia (h) Anthracene (C_{22} H_{12})	**0.000 ± 0.0000**	***S alata* Vegetated**	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000
		Un-vegetated soil	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000	0.000 ± 0.0000

Analysis of the unused spent engine oil (SEO) showed that polycyclic aromatic hydrocarbons(PAHs) were the only hydrocarbons present. This suggests that vehicles in Nigeria overuse theircrankcase oil between each change with the result that more toxic and lethal pollutants were oftenproduced. This agreed with the findings of Dominguez-Rosado and Pichtel (2013) who reported thatthe PAHs content of used motor oil was often between 34 and 90 times higher than new oil.Hydrocarbons such as Acenaphthylene, Benzo (k) flouranthene, Flourene, Benzo (b) flouranthene,Naphthalene, Pyrene, Chrysene, Anthracene and Benzo(g-h-i) perylene were not detected in unusedSEO but were detected in polluted vegetated and un-vegetated soils. Their formation might perhaps beas a result of microbial and plant activities acting on the SEO. This agreed with the findings of GFEA and (USGC 2014) who reported that PAHs may be formed by microorganisms and plantsthrough biosynthesis. Hence, SEO pollution enhanced the production of more hydrocarbons bycombined activities of microbes and *Sennaalata*.

Though, biosynthesis of PAHs by microbes andplants is still a controversial issue but this result has also pointed to the possibility of biosynthesis ofPAHs by these organisms. However, plants enhance microbial synthesis and degradation of organicpollutants. reported that plant roots can supply readily availablecarbon sources for microorganisms and so, influence the soil microbial community by increasingmicrobial numbers in the rhizosphere. In the present work, percentage hydrocarbons left un-degradedin the vegetated soil were less than those of the un-vegetated soil. This expressed the plant's ability toremediate SEO polluted soil. Hence, *S. alata*has the ability to remove hydrocarbons from the soil. Thisagreed with the findings of who reported that Clover plant(*Trifolium*) sown in the soil treated with used motor oil in green house, removed all the oil after 150days. Nwadinigwe and Obi-Amadi (2014) also observed that *Pennisetumglaucum*significantly reduced the percentage of hydrocarbons in spent engine oil polluted soil.

CONCLUSION

This work has proved that *Sennaalata* is an ideal plant for the phytoremediation of soil polluted with spent engine oil. This was done by phytodegradation and phytoaccumulation. Spent engine oil did not have any adverse effect on the plant rather the plant degraded it andutilized it for growth. With the spate of oil pollution goingon in many parts of Nigeria especially in oil producing states and mechanic workshops, planting of thisperennial shrub will not only decontaminate these pollutants but also aerate the environment duringphotosynthesis. Bioaccumulation of hydrocarbons along food chain should be curbedthrough recycling of SEO rather than indiscriminate pouring into the environment.

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Characterization Of Oil Bean Seed (*Pentaclethra Macrophylla*) Oil And Its Fatty Acid Profile

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Abstract

In this work, "the physiochemical properties and the fatty acid composition of African oil bean seed (Pentaclethra macrophylla) oil extract were analyzed to establish the nutritional, chemical and pharmaceutical values. The iodine and saponification values of the oil extract were determined using AOACs Cd 1-25 and 3-25 of (1993) respectively, while the fatty acid profile was determined using Gas chromatograhic method. The physicochemical properties showed that the oil is light brown in colour and the percentage oil yield was 36, its iodine value was (95.01mgl_2/100g), saponification value (136.6mgKOH/g) and specific gravity (0.8738). The fatty acid composition showed presence of omega-3 fatty acid (Eicosapentaenoic acid 7.0953 ppm, docosahexanoic acid 0.0071ppm, linolenic acid 0.0079ppm), and omega-6 and 9 fatty acid (oleic acid 19.7617ppm, linoleic acid 0.0071ppm, arachidnic acid 0.0079ppm). The iodine value obtained shows that the oil is a semi drying oil ranging between 85-130 mgl_2/50g and the fatty acid profile shows that the oil contains mono-unsaturated fatty acid implicated in the cure of cardiovascular diseases such as arteriscleosis, stroke, cancer and also reduces high level of cholesterol and therefore recommended as an edible , pharmaceutical and other clinically useful oil.

Key words: Physicochemical, arteriosclerosis, cardiovascular, arachidonic, cholesterol

Introduction

The demand for vegetable oil with diversified chemical and nutrient industrial values has dominated present research in Nigeria as industrialist rely mostly on the popular oil such as palm oil, palm kernel oil, groundnut oil, and coconut oil for their various applications [1]. The chemical nature of various fats and the fatty acids determine their demand for both domestic and industrial purposes by the teaming population. In this country there is scientific evidence that all fats are not equivalent with regard to consumer's health and applications [2]. Fatty acids are graded into saturated fatty acid (SAF), mono unsaturated fatty acid (MUFA). Scientists from public health and food research institutes have recommended daily amount for each grade of fatty acids, saturated fatty acids(SFA), mono unsaturated fatty acid(MUFA), poly unsaturated fatty acids (PUFA), trans-fatty acid (TFA), as well as specific fatty acids such as linolenic acid (LLA) and long chain poly unsaturated fatty acid (LPUFA) [3]. Nigeria being a tropical country has wide variety of domestic plant seed from which oil can be extracted. The plant seeds range from largely known soybean, palm kernel, groundnut and African oil bean to underutilized ones like walnut, locust bean, castor oil bean, grape seed, rice bran and oil bean seed produced by the plant, consumed in different Nigeria menu in some parts of the country like Imo, Abia , Ebonyi, Enugu and part of Anambra with name 'ugba' source. [4]. It imparts good flavor and provides a very rich source of protein [5]. Despite the utilization as food, little has been studied about the seed oil, fatty acid content of the oil and possible industrial and health benefits of the oil. Oil processing has been around for quite a while; however, little work has been done to exploit the usefulness of the oil. A study of the oil bean seed oil will go a long way to reveal the physicochemical characteristic and fatty acid profile of the oil.

The source of nutritionally and industrially valuable oil from available data is diminishing and this has led to a growing need for more resources from this end [6]. Oil bean tree (*Pentaclethra macrophylla*), that bears the seed is a tropical tree that thrive well in the rainforest zone and bears many long pods containing 10-15 big seeds enough for both domestic and industrial uses.

This research was carried out to analyze the physicochemical properties of oil bean seed extract with a view to finding it's suitability as alternative oil and also to determine the fatty acid group content in order to meet the nutritional and industrial demand of the Nigeria teaming population.

Most of these unsaturated fatty acids are present in the semidrying oils which are rich in omega 3, 6 and 9 known to have clinical implications on the reduction of cholesterol build up and arterial diseases and therefore are used by food and pharmaceutical industries [7]. The (PUFA) and (LCPUFA) are used in many cosmetic, transmission fluid and biodiesel industries [8]. Omega -3 components include; ecosapentaenoic acid, a twenty carbon chain fatty acid with the double bond at carbon *y* (5) represented as (C20 :5), docosahexaenoic acid (C22:6) and linolenic acid (C18:3).

Omega-6 and 9 are represented by oleic acid (C18:1), linoleic acid (C18:2) and arachidonic acid (C20:4). These fatty acids are present in semidrying oils in various concentrations and their ratio in a given oil indicate the grade and the usefulness of the oil [9, 10, 11].

Materials and Methods

All equipment's used were of analytical grade, Soxhlet extractor (GallenKamp), Blender (Philips), Tripod stand, Electric oven (gen, lab England), Weighing balance, Gle (400 cerics, bulk scientific)

All the reagents were of analytical grade; N-hexane, Hydrochloric acid, phenolphthalein, 95% ethanol magnesium silicate, potassium hydroxide, carbon tetrachloride and sodium thiosulphate.

Oil extraction

500g of oil bean seeds were cracked, crushed and the oil extracted with n-hexane using soxhlet extractor. The oil was analyzed for physicochemical properties, (color, iodine value, saponification value and specific gravity (S.G), while fatty acid profile was analyzed using gas chromatography.

Percentage oil value yield was calculated using the formula;

$$\text{Oil yield} = \frac{\text{wt. of oil}}{\text{Wt. of seed}} \times \frac{100}{1}$$

Iodine value: Iodine value was obtained by titrimetry using AOAC Cd 1-25 method [12]: An iodine solution of the oil was titrated using 0.IN sodium thiosulphate solution and the iodine value was calculated using the formula below;

$$\text{Iodine value} = \frac{(b - a) \times 0.1269 \times 100 \times f}{\text{wt (g)}}$$

Where;

b=blank burette reading

a= burette reading against sample oil solution

f = factor of standard thiosulphate solution used

wt. = weight of sample oil

Saponification value

The saponification value was obtained using: (AOAC Cd 3-25method [12].

2g of oil was dissolved in 25ml alcoholic potassium hydroxide and refluxed for 1hr; 1ml of phenolythalein was added and the excess alkali was titrated with 0.5M HCl and a blank titration was also carried out. The saponification value was calculated using the formula below;

$$\text{Saponification value} = \frac{(b-a) \times 28.05}{\text{wt(g)}}$$

Where;

b=bank titration

a= sample titration (excess alkali against 0.5ml HCl)

wt. = weight of sample.

Specific Gravity (S.G)

This was done using specific gravity/ pyrometer bottle

Specific gravity was calculated using the formula below;

$$\text{Specific gravity (S.G)} = \frac{\text{weight of xml oil}}{\text{wt. of xml water}}$$

Fatty acid profile of Omega 3, 6 and 9 was determined using gas chromatography.

0.5g n-hexane solution of the oil extract was prepared and was placed in the gas chromatography for fatty acid analysis and the result was read.

Results

Table 1; shows some physicochemical properties of the oil extract of African oil bean seed while Tables 2a and 2b shows the fatty acid profile of the oil extract.

Table 1: Physicochemical properties of oil

Parameter	values
Oil yield (%)	36
Iodine value ($mgI_2/100g$)	95.0
Saponification value (mgKOH/g)	136.6

Table 2a: Omega 3 fatty acid concentrations of oil bean seed oil

Component	Name	Concentration (ppm)	Conc.%
C20:5	Ticosapentaenocic acid	7.0953	67.612
C22:6	Docosahexaenoic acid	0.0071	0.00676
C18:3	Linolenic acid	0.0079	0.0752
Total		7.1103	

Table 2b: Omega 6and 9 fatty acid concentration in the sample oil

Component	Name	Concretion(ppm)	% conc.
C18:1	Oleic acid	19.7617	83.314
C18:2	Linoleic acid	0.0071	0.0299
C20:4	Arachidonic acid	0.0079	0.0333
Total		19.7767	

Discussion

This study showed the physicochemical properties and fatty acid profile of P. *macrophylla.* The seed oil extract is a light brown liquid with an oil yield of 36% which is higher than 12% yield obtained by Akubogwo and Ugbogu [13] at room temperature. The difference in oil yield may have depended on location and source of the seed. The iodine value obtained was 95.01mgl2/100g which is higher than the value obtained in C. *albidin* seed oil. (50.76mgl_2/100g) [6].

These values indicate that the oil contains appropriate level of unsaturated bonds and is a semi-drying oil. Semi-drying oils are known to contain unsaturated fatty acids omega 3,6 and 9 with their clinical effects on reduction of arterial diseases and reduction of cholesterol [14]. The high saponification value recorded for *P. macrophylla* seed oil (136.6mg KOH/100g, is an indication that it has potentials for use in the pharmaceutical industry [15]. The specific gravity of *P. macrophylla* was found to be 0.87 and this value is within the range of specific gravities reported for other fats and waxes in the group of semi-drying oil [16]. Tables 2a and 2b shows the fatty acid profile of *P. macrophylla* oil. Oils from vegetables are complex mixtures that contain several compounds and are made up of free fatty acid (FFA), triglycerols, glycolipids, diacylglycerols, phospholipids, and other minor components (Wu, 2007, Adewuyi *et al.,* 2010). Vegetable oil usage is largely centered on the type of fatty acids present in the oil and this fatty acids fall into various lipid categories saturated and unsaturated [17, 18]. The oil contains high amount of Eicosapentaenoic acid (7.0953) and Heptadecanoic acid (3.0096) which showed that the African oil bean seed oil is the semi-drying oil. Semi-drying oils are triglycerides whose fatty acid constituents are mostly mono-unsaturated bonds [19]. Oleic acid was the predominant fatty acid in the oil, the low fatty acids in the oils were with docosahexaenoic acid C22:6, linolenic acid C18:3, linoleic acid C18:2, Arachidonic acid C20:4. The fatty acid profile of *P. macrophylla* was 68% and 83% respectively as shown in Tables 2a and 2b. Oleic acid was the major fatty acid and the acid with the highest concentration (19.76 ppm) and this result compares with the studies of Enujiugha [19] who found out that an omega-9 fatty acid, oleic acid was the major unsaturated fatty acid in the seed oil (20.05 ppm). Odoemelam [9] stated that 4-47% of the oil from *P. macrophylla* contained oleic acid. It is well known that dietary fat that is abundant in linoleic and oleic acid helps to prevent high blood pressure and cardiovascular diseases, such as atherosclerosis and coronary heart diseases [20]. This shows that the oil of *P. macrophylla* offers potential benefit to health.

Conclusion

From the analysis conducted, results showed that oil bean has a good yield of oil and it contains mono-unsaturated fatty acids, omega-3,6 and 9 fatty acids which is implicated in reducing bad cholesterol level in the blood thereby lowering the risk of cardiovascular diseases such as arteriosclerosis and cancer.

Recommendation

A much more detailed work is required to study the functional properties of African oil bean seed oil and its uses for various traditional dishes

Dietitians should be encouraged to incorporate oil bean seed oil into infants feeds so as to boost their protein content.

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Thermal Analyses Of Briquette Fuels Produced From Coal Dust And Groundnut Husk

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Abstract

This study involved the production and thermal characterization of biomass briquettes produced by blending a major agricultural waste with coal dust. In the work, nine different compositions of coal dust/groundnut husk briquettes were produced using starch as the binder while $Ca(OH_2)$ was the de-sulphurizing agent. The ash content, volatile matter, fixed carbon, moisture content, compressive strength, ignition time, calorific value, water boiling test and burning rate were carried to determine the physical, mechanical and thermal properties of the briquettes. The results show that moisture content values are in the range 2.43 - 6.44%, compressive strength (7.72 - 10.85 N/mm^3), ash content (24.18 - 29.15 %), calorific value (21714.17 - 25027.18 kJ/kg), fixed carbon (16.77-53.22%), ignition time (22.23-45.20 s), water boiling test (1.50-4.99 min) and burning rate (16.10-28.32 g/min). The results showed that the agro-wastes briquettes are beneficial for heating purposes rather than open incineration of the wastes.

Keywords: Agro-waste, binder, biomass, briquette, coal dust.

Introduction

Bio-coal briquettes have wide range of Industrial and domestic applications, with very lower ash content, long shelf-life and no danger of fire or explosion of the fuel. It saves the trees as the practice of cutting of trees for fuel is replaced by these better quality briquettes. The briquettes are bio-degradable fuel that does not leave residues and cause contamination of the water and soil. It also acts as a good substitute for polluting fuels as well as costly renewable energy resource and they can be produced in almost every part of the world where bio-mass is easily available [1].

Solid waste management is one of the major problems in Nigeria. This is not only found in the urban areas but also at the rural areas. The major waste generated at the rural areas is agricultural waste or residue (crop by-product). Despite this level of waste generation, the rural dwellers still rely on wood fuel and charcoal fuel for heating, cooking and other purposes [2].

Bio-coal briquette is a type of solid fuel prepared by compacting pulverized coal, biomass, binder and sulphur fixation agent. The high pressure involved in the process ensures that the coal and the biomass particles are sandwiched and adhere together, as a result they do not separate during transportation, storage and combustion. During combustion, the co-combustion of the coal and the biomass gives a better combustion performance and reduces pollutant emission [3]. Bio-coal briquette has a favourable ignition, better thermal efficiency, emits less dust and soot [4]. The mechanism behind this is that, since the biomass component of the briquette ignites at low temperature compared to the coal, this ensures that the volatile matter in the coal which would have otherwise be liberated as smoke at low combustion temperature combusts completely. Furthermore, the presence of sulphur fixation agent otherwise known as desulfurizing agent ensures that most of the sulphur content of the coal is fixed into the ash instead of being liberated into the atmosphere as SO_2 [5].

The use of briquetting for conversion of agricultural residues is comparatively recent, however,

and has only been taken up in developing countries in the last few years. Main agricultural residues that are produced are rice husk, coconut dregs, hay, groundnut husk, jatropha husk, palm nut shell, corn cob and cotton stem. Beside the problem of transportation, storage and operation, open burning of this bio wastes with traditional style without control can cause critical air pollution.

Methods

Preparation of the samples

The coal sample was sun dried to reduce its moisture content, broken into smaller sizes, ground in an electric milling machine to pass through 1mm sieve and stored. The groundnut husk was collected, sun dried, ground and sieved through 1mm sieve and stored.

Preparation of the briquette samples

The briquettes were produced using a manual hydraulic briquetting machine with three cylindrical mould. Briquettes of coal and groundnut husk of different compositions were produced with a specific amount of $Ca(OH)_2$ added as desulphurizing agent based on the quantity of coal added. Starch was added as binder. Specific quantity of water was added and mixed properly. The pressure and compression force of 276.36 N and 31.67 N/m^2 was maintained for 20 min before the briquettes were extruded. After production, the briquettes were sun dried for 7 days before analysis [6].

Characterization of the briquette blends

Combustion analysis

The combustion analysis was carried out to determine the combustion characteristics of the briquette blends.

Ignition time (s): The different samples were ignited at the edge of their bases with a burnsen burner. The time taken for each briquette to catch fire was recorded as the ignition time using a stopwatch [7].

Burning time (min): The time taken for each briquette blend to burn completely to ashes. Subtracting the time is turned to ashes completely from the ignition time gives the burning time [7].

Burning time = Ashing time – Ignition time ..... (1)

Water boiling test (min): This was carried out to compare the cooking efficiency of the briquettes. It measures the time taken for each set of briquettes to boil an equal volume of water under similar conditions. During the process, 100 g of each briquette sample was used to boil 250 ml of water using small stainless cups and domestic briquette stove [7].

Calorific value: The calorific values of the briquettes were determined using Oxygen Bomb Calorimeter of model-OSK 100A. The calorific value (kJ/kg) of the samples under test was calculated from the temperature rise in the calorimeter vessel and the mean effective heat capacity of the system [8]. This was done using the formula below;

$VI = (Ee + W1)\,TR - C)/S \times 4.1868$ ..... (2)

Where;

Ee is the water equivalent of the calorimeter (581 g),

W_1 = quantity of water in the vessel

TR = Temperature rise ^oC

C = correction factor from ignition 154 Cal

S = weight of sample in grams (g)

Proximate Analyses

Moisture content: The moisture contents of the briquettes were determined. A portion 2 g each of the

samples was weighed out in a wash glass. The samples were placed in an oven for 2 hours at 105 ºC. The moisture content was determined according to ASTM standard [9].

$$MC = \frac{W_1 - W_2}{W_1} \times 100 \quad \ldots\ldots (3)$$

Where;

W_1 = Initial weight,

W_2 = Weight after drying

Ash content: In the determination of the ash contents of the briquettes, a portion 2 g were placed in a pre-weighed porcelain crucible and transferred into a preheated muffle furnace set at a temperature of 600 ºC for 1 hour after which the crucible and its contents were transferred to a desiccator and allowed to cool. The crucible and its content were reweighed and the new weight noted. The percentage ash content was calculated using ASTM standard [9].

$$AC = \frac{W_2}{W_1} \times 100 \quad \ldots\ldots (4)$$

W_1 = Original weight of dry sample

W_2 = Weight of ash after cooling

Volatile matter: The volatile matter of the briquettes was also determined. A portion 2 g of the sample was heated to about 300 ºC for 10 minutes in a partially closed crucible in a muffle furnace. The crucible and its content were retrieved and cooled in a desiccator. The difference in weight was recorded and the volatile matter was calculated using ASTM standard [9].

$$VM = \frac{W_1 - W_2}{W_1} \times 100 \quad \ldots\ldots (5)$$

Where;

W_1 = Original weight of the sample.

W_2 = Weight of sample after cooling.

Fixed carbon: The fixed carbon of the briquettes was also determined using the formula according to ASTM standard (1992).

$$FC = 100 - (\%VM + \%AC + \%MC) \quad \ldots\ldots (6)$$

Where VM = Volatile matter

AC = Ash content

MC = Moisture content

Determination of the Compressive Strength: Compressive strength in cleft of briquettes was determined in accordance with ASTM, [10] using an Instron Universal Strength testing machine with load cell capacity of 100 kN. The cross-head speed was 0.305 mm/min. A sample of briquette to be tested was placed horizontally in the compression test fixture and a load was applied at a constant rate of 0.305 mm/min until the briquette failed by cracking. The compressive strength in cleft was then computed as follows;

$$\text{Compressive strength in cleft (N/mm)} = \frac{3 \times \text{The load at fracture point(N)}}{[l_1(mm) + l_2(mm) + l_3(mm)]}, \quad \ldots\ldots (7)$$

Where l_1, l_2 and l_3 were lengths of briquettes at points one, two and three, respectively in (mm).

Results

Table 1. Physico-thermal Parameters of Bio-Coal Briquettes of Groundnut Husk

Samples	Ash content (%)	Volatile matter (%)	Fixed carbon (%)	Moisture content (%)	Calorific value (kJ/kg)	Ignition time (s)	Burning rate (g/min)	Water boiling test (min)	Compressive strength (N/mm^3)
0%	24.18	20.17	53.22	2.43	25027.18	45.20	28.32	1.50	7.72
10%	24.75	23.28	49.49	2.48	24788.28	42.76	26.54	1.77	8.43
20%	25.08	26.72	44.83	3.37	24340.63	39.12	25.12	2.21	9.47
30%	25.65	28.15	42.29	3.91	23947.58	36.21	23.41	2.66	9.91
40%	26.25	31.77	37.83	4.15	23628.88	35.76	22.56	2.89	10.85
50%	27.02	34.82	33.68	4.48	23225.69	33.16	21.51	3.41	10.36
60%	27.52	36.95	30.51	5.02	22850.80	32.73	19.50	3.80	10.21
70%	28.00	39.52	27.04	5.44	22520.30	31.31	19.15	3.98	9.83
80%	28.43	42.01	26.30	5.86	22765.31	29.20	18.33	4.35	8.76
90%	28.85	44.15	20.88	6.12	21956.25	25.91	16.65	4.65	8.21
100%	29.15	47.64	16.77	6.44	21714.17	22.23	16.10	4.99	7.84

Discussion

The results of the physico-thermal properties of the briquette blends are presented in table 1. The ash content of the coal briquette is the amount of ash that remains after the briquette is incinerated. The results showed a rise in ash content as the biomass was blended with coal dust, in the range of (24.75-29.15 %). According to Loo and Koppejan [11], the higher the fuel's ash content, the lower the calorific value, this was witnessed in the reduction of the calorific values as the biomass was blended with the coal dust from (25027.18- 21714.17 kJ/kg). The fixed carbon of a fuel is the percentage of carbon available for char formation during combustion [12]. The table showed that briquette blends produced from coal dust and groundnut husk by varying their compositions resulted in briquettes with reduced fixed carbon content values from (53.22-16.77 %). The findings compared favourably with

the work of Ajueyitsi and Adegoke [13].

The calorific (heating) value is the standard measure of the energy content of a fuel. The differences in values for sample (0 %) with calorific value (25027.18 %) and samples (30-40 %) with calorific values (23628.88-23947.58 %) are not significantly high, as such biomass can be blended with coal to produce fuel source. The results showed that the briquette blends ignited faster as the biomass were introduced thereby reducing the ignition time from 45.20-22.23 s. The briquettes with more composition of groundnut husk ignited faster because of the porous nature of the briquette blends that allowed enough supply of oxygen which supported combustion.

The data presented showed that briquettes consisting only of coal had a lower compressive strength value than briquettes with additional biomass. The use of biomass therefore increased the material strength of briquettes. Analysis of the data showed that the maximum strength value of compression is somewhat higher for briquettes with a 40 % biomass (10.85 N/mm^3) and lowers down though using the same pressure strength in the press. The findings are corroborated by the work of Borowski [14].

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Evaluation Of Desulphurization Potentials Of Barium Chloride, Calcium Hydroxide, Sodium Hydroxide And Hydrochloric Acid On Diesel, Kerosene And Gasoline

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ABSTRACT

In view of the menace of sulphur in crude oil or its refined products, there has been continued search for simple, efficient and cost-effective methods of removing it from crude oil or its product. The sulphur content in refined petroleum products namely diesel, kerosene and gasoline obtained from a commercial filling Station in Abakaliki, Ebonyi State, Nigeria were determined before and after desulphurization with aqueous ionic solutions of sodium hydroxide (NaOH), barium chloride ($BaCl_2$), calcium hydroxide [$Ca(OH)_2$] and hydrochloric acid (HCl) using a single beam UV-Vis spectrophotometer. Results obtained showed that the amount of sulphur contained in the samples before desulphurization were 1.457, 0.992 and 1.294 mg/L in diesel, kerosene and gasoline respectively. The highest sulphur recovery of 0.676 mg/L (46.4 %), 0.315 mg/L (31.8 %), and 0.555 mg/L (42.9%) in diesel, gasoline and kerosene respectively were obtained with a 10 % w/v solution of NaOH after desulphurization. This was followed by a 10 % w/v solution of $BaCl_2$ which gave 35.0 % and 23.0 % desulphurization in diesel and gasoline respectively. The performance of aqueous solutions of $Ca(OH)_2$ and HCl in desulphurizing kerosene and gasoline were relatively low (3.2 %,11.4 % and 15 %, 4.6 % respectively) compared with aqueous NaOH. The results showed appreciable reduction in sulphur content of the samples with NaOH exhibiting the highest desulphurization potential in all the samples assayed. Thus these ionic solutions possess the potential to extract sulphur compounds in petroleum products.

Keywords: Petroleum products, sulphur content, ionic liquids, corrosion, emission, desulphurization

INTRODUCTION

Sulphur removal from transportation fuels has become an increasing challenge in refineries. The presence of sulphur or its compounds in petroleum reduces the functional properties of its products and is always objectionable due to their environmental problems. Sulphur present in transportation fuel leads to sulphur oxide (SO_x) emissions into the atmosphere and also inhibits the performance of vehicles pollution control equipment[1,2]. Sulphur can occur in many forms in petroleum such as free elemental sulphur, H_2S, thiols or marcaptans (RSH), sulphides (S^{2-}) disulphide, (RS-SR) and thiophenes or thiofurans (C_4H_4S). Sulphur in crude oil can exist as "active" or "inactive" based on their reactivity with metals. The active sulphur can react directly with a metal, examples include elemental sulphur, H_2S and mercaptans (RSH), while the inactive sulphur cannot react directly with metals examples are sulphides, carbon disulphide (CS_2) and thiophenes[3]. These compounds both the active and inactive forms are undesirable in petroleum because of their potential corrosion problems in refining process. It is generally necessary to remove these sulphur bearing compounds from petroleum products in order to improve calorific value of petroleum products, preserve public health, reduce corrosion in oil pipelines and to control odour in these petroleum products[1]. Keeping in view the hazardous effects of sulphur in petroleum, much attention is being paid to desulphurization in recent years to protect not just the environment and the refinery equipment but also the automobiles and other stationary engines which are powered with the various refined fractions of petroleum.

Doctor sweetening and oxidation of thioles to sulphides and disulphides were some of the primitive techniques used to eliminate corrosion and obnoxious odour effects of elemental sulphur but not the sulphur compound itself[4]. Later, hydro-desulphurization (HDS) process which uses large amount of hydrogen was practiced and can still be used to obtain low sulphur fuels from hydro-cracking processes in the petroleum industry[5]. The HDS process had other operational problems such as requiring expensive cobalt-molybdenum catalyst and very high operating temperature and pressure[5]. In view of the foregoing, it became pertinent that a more economical and less reaction condition demanding methods of desulphurization in petroleum be sought as alternative in providing potential solutions for sulphur-free clean fuels. Among the new methods under investigation, desulphurization of petroleum products using simple ionic solutions is gaining importance in view of the fact that there is no hydrogen consumption and much less severe operating conditions are required. These ionic solutions are easy to handle because they are non-volatile, non-flammable and possesses high thermal stability[4]. However, this process is limited to desulphurization of lighter petroleum products such as those examined in this study. For high boiling fractions, only a small degree of desulphurization may be achieved due to increased viscosity which can alter the interfacial properties and solubility of these ionic liquids[6]. In this work, comparative study of the capabilities of NaOH, $BaCl_2$, $Ca(OH)_2$ and HCl solutions to extract sulphur as H_2S from refined petroleum products namely, kerosene (DPK), diesel (AGO) and gasoline (PMS) was carried out.

MATERIALS AND METHODS

Sample Collection and Reagents

Samples used in this study include diesel, kerosene and gasoline. The samples were bought from a commercial petroleum marketer in Abakaliki, Ebonyi State and used without further processing. The aqueous ionic solutions used (10 % w/v each of NaOH, $Ca(OH)_2$, $BaCl_2$ and 1.0 M HCl) were prepared from analytical grade reagent chemicals. The 10 % w/v NaOH solution, 10 % w/v $Ca(OH)_2$ and 10 % w/v $BaCl_2$ solutions were prepared by dissolving 10 g of solid NaOH pellets, 10 g of anhydrous $Ca(OH)_2$ and 10 g anhydrous $BaCl_2$ respectively in distilled water and made up to 100 cm^3 mark in a volumetric flask. The 1.0 M HCl solution was prepared by carefully transferring 41.75 cm^3 of 36 % concentrated HCl into a 200 cm^3 of distilled water in a 500 cm^3 volumetric flask and made up to the mark with distilled water.

Extraction of Sulphur (Liquid-liquid Extraction)

A 20 cm^3 of each petroleum sample was measured into different conical flasks and 20 cm^3 of a particular aqueous ionic solution was added into the flask. The mixtures in the flasks were placed on a rotary shaker (Remi RS-12 model with inbuilt digital timer) set at 300 rpm for 5 minutes to ensure thorough interfacial interaction before it was transferred into a separating funnel and kept for 10 minutes to allow for complete separation. Thereafter, two layers were formed in each case. The lower layer containing ionic extractant was carefully drained off from the separating funnel and the upper layer containing the petroleum sample was subsequently analyzed to determine the quantity of sulphur in it.

Preparation of Calibration Standard

The calibration standard (10 % w/v NaS_2 stock solution) was prepared by dissolving 10 g of NaS_2 in 100 cm^3 of distilled water. This stock solution contained 0.53 % sulphide. Serial dilutions were made from the stock solution to prepare 1 %, 2 %, 4 % and 5 % from which a calibration curve was obtained by measuring their absorbance at 228 nm.

Determination of Sulphur Content in the Raw and Desulphurized Samples

The amount of sulphur in the raw samples (PMS, DPK and AGO) prior to desulphurization and the amount left after desulphurization were determined using UV-visible spectrophotometer (Genesys 10S, Thermo Scientific). This was done by measuring their various absorbance at 228 nm to minimize interference from other species especially hydrocarbons[7].

RESULTS AND DISCUSSION

The sulphur content of the raw samples (Figure 1) and after desulphurization (Figure 2) with the various aqueous ionic solutions are presented in Table 1, while Table 2 shows the amount of sulphur removed from the samples by each of the ionic solutions after the desulphurization process. Table 3 shows the percentage equivalents of the sulphur removed from the samples. Comparatively, the desulphurization abilities of these ionic solutions on each of the sample are discussed.

Table 1: Sulphur content of oil samples before and after desulphurization

Samples	**Sulphur content of raw samples (mg/L)**	**Sulphur content of the samples after Desulphurization (mg/L)**			
		NaOH (10 %)	$Ca(OH)_2$ (10 %)	HCl (1.0M)	$BaCl_2$ (10 %)
Diesel	1.457	0.781	0.956	1.188	0.947
Kerosene	0.992	0.677	0.960	0.855	0.903
Gasoline	1.294	0.739	1.146	1.234	0.995

Table 2: Sulphur depletion in the oil samples

Concentration of Extractants	**Sulphur Depletion (mg/L)**		
	Diesel	**Kerosene**	**Gasoline**
NaOH (10%)	0.676	0.315	0.555
$Ca(OH)_2$ (10%)	0.501	0.032	0.148
HCl (1.0M)	0.269	0.137	0.060
$BaCl_2$ (10%)	0.510	0.089	0.299

Table 3: Percentage of sulphur desulphurized

Concentration of Extractants	% Desulphurization		
	Diesel	Kerosene	Gasoline
NaOH (10%)	46.4	31.8	42.9
$Ca(OH)_2$ (10%)	34.4	3.2	11.4
HCl (1.0M)	18.5	15.0	4.6
$BaCl_2$ (10%)	35.0	9.0	23.0

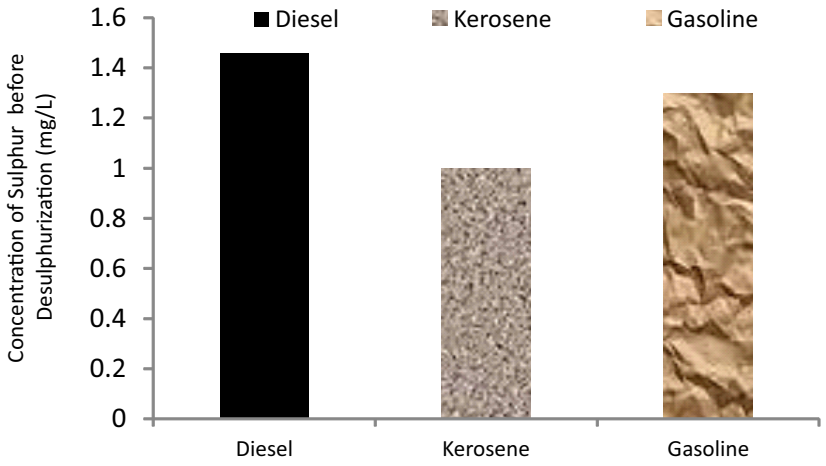

Fig. 1: Sulphur Content of the Samples before Desulphurization

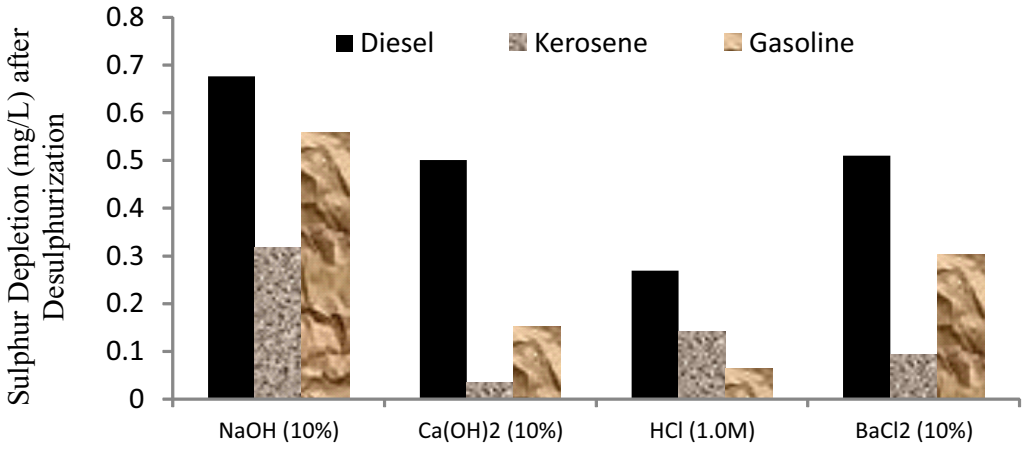

Fig 2: Sulphur Depletion after Desulphurization

Desulphurization of Diesel Sample

In the diesel sample, the highest desulphurization yield of 46.4 % (0.676 mg/L sulphur reduction) was achieved with 10 % w/v solution of NaOH (Table 2, Fig. 2), this was followed by $BaCl_2$(10%), which gave 35.0 %, and $Ca(OH)_2$ (10%) which gave 34.4 %. Ionic solution of HCl (1.0M) gave the least percentage desulphurization (Table 3). The high percentage yield of NaOH solution which is suggestive of its high desulphurization potential may be attributed to the high alkaline nature of NaOH and greater affinity for the sulphur compounds, hence its use in sweetening processes. In the aqueous alkaline layer, the highly acidic sulphur compounds such as mercaptans which are contained in diesel oil readily distributes into the alkaline layer of NaOH and converts to sulphides which enhances its extraction. The slight decrease in the percentage of sulphur removed with $Ca(OH)_2$ may be explained on the basis of the low basic character of $Ca(OH)_2$ when compared with NaOH and the fact that $Ca(OH)_2$ is largely insoluble in water at room temperature, thus affecting the ability of $Ca(OH)_2$ to extract more of the sulphur compounds. On the other hand, the poor desulphurization yields with HCl showed that it is not the best alternative in the extraction of sulphur either as sulphides or thiophenes from organic phase because of the low electronegativity of sulphur (2.58) compared with chlorine (3.0), which implies that sulphur cannot displace chlorine in HCl in order to be extracted[8].

Desulphurization of Kerosene Sample

For the kerosene sample, the highest desulphurization yield of 31.8 % was achieved with 10 % w/v solution of NaOH. This was followed by HCl (1.0 M), which gave 15.0 %. $BaCl_2$ and $Ca(OH)_2$ solutions gave very poor percentage desulphurization of 9.0 and 3.2 % respectively. The results obtained regarding the abilities of these ionic solutions to remove sulphur from kerosene show the same trend to those obtained in the case of diesel oil for the same reasons.

Desulphurization of Gasoline Sample

In the gasoline sample, NaOH solution equally gave the highest percentage desulphurization of 42.9 % when compared with the other aqueous ionic solutions. This was followed by $BaCl_2$ which gave 23.0 %. $Ca(OH)_2$ and HCl had very low sulphur recovery of 11.4 % and 4.6 % respectively.

CONCLUSION

The results obtained in this study showed that simple and cheap ionic solutions of NaOH, $BaCl_2$ and $Ca(OH)_2$ has the potential to reduce the amount of sulphur in petroleum products significantly through liquid-liquid extraction method. It was also observed that 10 % w/v solutions of NaOH and $BaCl_2$ gave the highest percentage sulphur recovery from all the samples at 28 $^{\circ}$C under extraction time of 5 minutes. However, the amount of sulphur removed depends on their relative chemical reactivity with the predominant species of sulphur compound in the petroleum product and the conditions upon which the extraction was done.

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Antimicrobial Activity And Phytochemical Analysis Of Methanolic Extract Of Psidium Guajava (Guava) Leaf

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ABSTRACT

Methanolic extract of the leaves of *Psidium guajava* (Guava) was screened for its phytochemicals and antimicrobial activities on some clinical bacterial isolates, such as *Pseudomonas Spp, Staphylococcus aureus, Proteus mirabilis, Escherichia coli,* and *Vibrio cholera.* The agar diffusion technique was used to assay the growth inhibition against the five bacterial isolates. The antibacterial effect of the methanolic extract of *Psidium guajava* was compared to that of some selected commercially available antibiotics. The inhibitory effect of the methanolic extract was comparable at all concentrations tested to the commercially available antibiotics indicating the high potency of the methanolic extract of *Psidium guajava*. Results obtained showed the extract inhibits the growth of the test isolates with diameter of zones of inhibition of 5.2 to 19.0 mm for *Vibrio cholera*, *Eschericha coli*, *Proteus mirabilis*, *Staphylococcus aureus* and *Pseudomonas Spp*. The Broth microdilution assay gave minimal inhibitory concentration values ranging from 12.5 to 16 ug/ml.the result of the phytochemical revealed the presence of saponins, alkaloids, carbohydrates, etc.

Keywords: Guava, Phytochemical, Antimicrobial, Oko

INTRODUCTION

Plant leaves have been used as herbal medicine for their healing properties since ancient times.[1] The medicinal activities of various plant materials and extracts have been recognized since the beginning of the 6th century.[2] Some bioactive compounds within these plants are responsible for their medicinal value.[3] The most prominent of these bioactive compounds are alkaloids, saponins, tannin, flavonoid, phenolic compounds and carbohydrates.[4] Their concentrations may vary in different plants which result in unique medicinal properties for a specific plant.[5] Leaves and bark of the guava plant are well recognized for the treatment of gastrointestinal disorders, diarrhea, colds, tooth aches and inflammations.[1]

The use of plant whether herbs, shrubs or tree, in parts or whole in the treatment and management of diseases dated back to pre-historic times. Plants extracts have been used in folk medicinal practices for the treatment of different types of ailments since antiquity.[6] In spite of the great advances achieved in modern medicine, plant still make an important contribution to health care. This is due to the recognition of the value of traditional medicinal systems.[7]

Constant consumption of guava leaves is considered to provide protection against lung, esophagus, pancreas, liver, breast, colon and skin cancers induced by chemical carcinogens.[4] Guava leaves are capable of preventing hepatitis and controlling diabetes. And it is also known to be highly effective in healing of burns and bruises.[8]

Medicinal plants are of great importance to the health of individuals and communities.[3] During the last few decades, the global interest has increased rapidly due to their antibacterial and antioxidant activities, low toxicity and the potential to be a cheaper alternative to costly synthetic drugs.[9] The determination of antibacterial activities of different medicinal plants is of special interest these days

due to the current global issue of increasing antibiotic resistance of microorganisms.[1] It is assumed that the drug resistance in pathogenic microorganisms is developing due to indiscriminate use of commercial antimicrobial drugs.[10] Antimicrobial resistance threatens the prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi.[5] Therefore, it is highly imperative to determine compounds which can be used to develop novel medicines with higher antimicrobial properties.[9]

Psidium guajava is indigenous to India but grown widely in West Africa including Nigeria. Recent studies on *Psidium guajava* proved it to be a useful medication for people living with upper respiration tract and pneumonia.[7] This study was conducted to determine the antimicrobial properties in *Psidium guajava* (Guava) leaves available in Nigeria. The primary objective of the study was to aid to the progressive research works related to the antimicrobial activity of plants.

MATERIALS AND METHODS

Sample Collection

Guava (Psidium guajava) leaves collected from the farmland at Nodu Village, Okpuno, Awka South L.G.A. Anambra State, Nigeria.

Source of test bacterial isolate

The clinical isolates of *Pseudomonas Spp, Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli* and *Vibrio choleriae* were obtained from the Diagnostic and Bacteriology Laboratory, 133 Zik Avenue, Awka, Anambra State, Nigeria. Pure cultures of each of the bacterial isolates were obtained by culturing the isolates on their selective media. The biochemical and physiological tests were performed to re-identity and confirm the purity of the isolates.

Preparation of plant extract

The fresh leaves of Guava (psidium guajava) were harvested and washed carefully in running tap water and then rinsed in distilled water. The leaves were air dried at room temperature (25 ^{0}C) before grinding into fine powder using mortar and pestle. The provided powder obtained was store in airtight glass containers protected from sunlight until required for analysis.

Crude-Extract preparation

For the preparation of crude extract 500 g of grinded materials of *Psidium guajava* leaves was soaked in methanol taken in round bottom flask for about 24 hours. Then the extracts in solvent form filtered (by watt's man filter paper) to a new round bottom flask. The process of filtration was recurring 3 days using supplementary concentration of methanol (300, 200 and 100 ml) earlier the filtration process. The crude (dried out) plant extracts was achieved when evaporation of water via water bath done.

Fractionation of crude extract

Ten grams of crude plant extract was kept back inside round bottom flask. Different concentration of distilled water (200, 150 and 100 ml) was poured to the extract contained in flask for three times. Then through separating funnel, filtration was done. For concentration of water extract, process of rotary evaporation was performed under reduced temperature between 30to 50 ^{0}C.

Phytochemical Screening

Methanol extract were subjected to find the phytochemical such as alkaloids, saponins, flavonoids,

phenol, glycosides, terpenoids, steroids, reducing sugar, tannin, emodin, fatty acid, anthocyanin, coumarin, starch and protein by using standard procedures. Chemical tests were carried out on the extract to ascertain the presence of the bioactive components present in *Psidium guajava*. The presence of phytochemicals were determined as described by Sofowora[11] and Edeoja, et al (2005).

Test for antibacterial property of *Psidium guajava*

Susceptibility tests were carried out using the modified agar diffusion method of Garrod, et al (1981) and Irobi (1992). Commercial antibiotics were used as positive reference standard to determine the sensitivity of the isolates.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined using the method of Rusell and Fur (1977). The methanolic extract at concentration of 45, 85, 145 and 155 mg/ml were added to molten sterile nutrient agar (oxoid) aseptically and thoroughly mixed together in a sterile petri dish. This was then allowed to set, the surface of the agar was allowed to dry properly before streaking with the appropriate bacterial isolate. The plates were then incubated at 37 ^{0}C for 72 hrs. The lowest concentration preventing all visible growth was taken as the minimal inhibiting concentration.

RESULTS

The methanolic extract of *Psidium guajava* showed antimicrobial activity against all the test organisms with the highest activity on *Escherichia Coli* and the least with *Pseudomonas Spp* (Table 1). Partial purification of the crude extract by TLC showed two components with Rf values: 0.85 and 0.88cm (Table 3) respectively. The phytochemical screening of the crude extract revealed the presence of alkaloids, saponins, carbohydrates, etc. (Table 2). The presence of these phytochemicals/bioactive compounds is a confirmation of the importance of *Psidium guajava*, serving a good purpose on the treatment of diarrhea and dysentery.

Table 1: Susceptibility of test organism to the crude extract and the standard reference antibiotics

Test Organisms/ Crude Extract/ and Antibiotics	Diameter Zone of Inhibition (mm)				
	Pseudomonas Spp	**Staphylococcus Aureus**	**Proteus Mirabilis**	**Escherichia Coli**	**Vibro Cholerae**
Methanolic Crude extract	5.2	8.50	11.0	12.0	6.0
Ampicillin	6.3	17.0	8.80	9.50	18.0
Oxacillin	17.0	10.0	-	10.0	-
Nitrofurentin	16.0	-	-	11.0	15.0
Tetracycline	15.0	14.0	-	-	-
Vancomycin	17.0	-	-	12	-
Piperacillin	-	-	19.0	-	-

Key:- = no zone of inhibition

Table 2 : Phytochemical composition of *Psidium guajava* extract

Phytochemical compounds	Status
Flavonoids	+
Phenolic Compounds	-
Carbohydrates	+
Tannin	+
Saponins	+
Alkaloids	+
Reducing Group	-

Table 3: Purified spots of the two components the Rf Factor

Spot	R_f – Factor
C_1	0.85
C_2	0.88

DISCUSSION

Plants extracts have been used in folk and even modern medical practices for the treatment of different ailments, most of which are due to the bioactive components of plants and its microbial activities.[1] Naturally occurring substance of plant origin have been reported to inhibit the growth of microorganisms. A bacterial infection seems especially controllable due to the availability of effective antimicrobial drugs. The inevitable consequence of the application of antibacterial drugs is a development of resistance to antibiotics.[4]

Recently, synthetic drugs have come into use unlike the many years ago when medicine depended exclusively on leaves, flowers and barks of plants.[5] In traditional medicine, a plant is simply eaten raw, cooked or infused in water or even prepared as food while in orthodox medicine, a plant may be subjected to several chemical processes before its active ingredients are extracted.[2] The results presented in this paper shows the cruse extracts of *Psidium guajava* possesses antimicrobial activity against the common gram-negative and gram-positive organisms, thus confirming the use of the plant in the treatment of diarrhea and dysentery. Several studies have revealed that extracts from medicinal plants possess antimicrobial activity against bacterial.[7] The revealed antimicrobial affects of the methanolic extracts of *Psidium guajava* on the bacterial isolates used, though in vitro, appear interesting and promising.

The antimicrobial and phytochemical screening and quantitative estimation of crude extracts of the chemical constituents of *Psidium guajava* studied showed that the extract was rich, in alkaloids, saponins and carbohydrates. *Psidium guajava* is a potential source of useful drug.

CONCLUSION

All the extracts of *Psidium guajava* are rich in various phytoconstituents. The methanol extract can act as standard drug against bacterial strains as it showed more inhabitation zone than the standard drug Piperacillin. The test gave validity to the traditional use as a natural antimicrobial.

It is therefore, recommended that further studies should be carried out on the efficacy of the crude plant extract to enhance the primary health care delivery systems in the developing countries.

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Osmotic Dehydration Of Fluted Pumpkin (*Telfairia Occidentalis*) Leaf: A Value-Added Technique For Preservation Of Vegetables

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ABSTRACT

Fluted pumpkin (*Telfairia occidentalis*) leaf was subjected to dehydration using three different osmotic solutions (sucrose, corn syrup, and sodium chloride). A portion (control) of the leaf was not treated. The osmo-dehydrated leaves and untreated were furthered dried and the effects of these osmotic agents on the nutritional qualities of fluted pumpkin leaves were investigated using standard protocols. Generally, there were significant differences in the proximate compositions in the entire samples. With the exception of phosphorus and zinc, among the elements determined, the concentrations of other elements were detected at higher concentrations in the treated samples when compared to the untreated sample. The pH of the samples indicated that they were slightly acidic (5.20 – 5.60), but no significant difference in the pH of the entire samples statistically at ($p \leq 0.05$) in the percentage composition of ascorbic acid, except in the sample treated with sodium chloride. Sucrose and corn syrup treated samples with values of 37.50 and 27.46 % respectively, had high percentage of sugar content compared to sodium treated (12.94 %) and untreated samples (13.55 %). Overall, the high proportion of beneficial elements such as calcium (2.67 – 4.56 mg/kg), magnesium (95.00 – 460 mg/kg), phosphorus (0.82 – 0.88 mg/kg), potassium (0.23 -0.44 mg/kg); moderate presence of physicochemical parameters, and appreciable chemical compositions of the osmo-treated samples compared to untreated sample bared credence to the fact that osmotic dehydration is a value-added technique for preservation of vegetables.

Keywords: *Telfairia occidentalis*, preservation, vegetables, osmotic-dehydration

INTRODUCTION

The preservation of fruits and vegetables through sun and solar drying techniques dates back many centuries. However, the contamination and poor quality of the products of these ancient practices led to the development of alternative decent drying techniques (Bezyma and Kutovoy, 2005). The fundamental purpose of food dehydration is to lower the water content in order to minimize the rates of chemical reactions and to facilitate distribution and storage (Tortoe, 2010). At low water activity, most of the chemical reactions which deteriorate the food, the growth, and toxin production by micro-organisms are ceased. The most applicable methods of preservation such as freezing, vacuum drying, cabinet or tray dying, micro wave drying, and combination thereof have been reported to be less efficient and present some limitations (George *et al*., 2004). Nevertheless, osmotic dehydration has recently proved more successful for drying and preservation of fruits and vegetables.

Osmotic dehydration is the diffusion of a solvent (removal of water) through a semi permeable membrane from a dilute to a more concentrated solute solution resulting in the equilibrium condition in both sides of the membrane (Tiwari, 2005). This technique is preferred over other methods due to its retention value in colour, aroma, nutritional constituents and flavour of compounds. The process is less energy intensive compared to air or vacuum drying process as no phase transition takes place during the moisture removal from the substrate (Sutar and Sutar, 2013). This technique can remove 30 to 40 % moisture from the product; the amount of water loss taking place during initial period is high, and as dehydration period increases, the rate of water loss decreases and the rate of solute gain increases (Sutar and Sutar, 2013; Chwastek, 2014).

The process is carried out by immersing the raw material in a hypertonic solution. The solutes used are generally sugar syrup with fruit slices or cubes and salt or brine with vegetables (Phisut, 2012). In this process (multi-component diffusion), water flow from fruits or vegetables to solution and along with water some components of fruits and vegetables such as minerals, vitamins, and fruit acids also move towards solution. In addition, sugar and salt migrate towards the fruits and vegetables (Yadav and Singh, 2012; Chwastek, 2014; Ramya and Jain, 2016). During this process, the most important processing parameters that influence diffusion of molecules are time, temperature, and the type of osmo-active substance used (Phisut, 2012; Chandra and Kumari, 2015). Higher temperature causes a loss of nutrients, changes in the structure, destruction of cell membranes, loss of selectivity, and increase in the amount of osmotic substances penetrating the interior of the tissue (Phisut, 2012; Chwastek, 2014).

In Sub-Sahara Africa, Nigeria inclusive, preservation of vegetables by drying provides livelihood opportunities for people including producers of raw materials, commodity traders, food processors, vendors, and exporters alike. In spite of developments in new food manufacturing processes and designs, the potential for sustainable increases in income is jeopardised by market constraints related to perceived problems of product safety and quality. This calls for the implementation of improved drying techniques such as osmotic dehydration. Most vegetables have a definite harvesting time and limited shelf-life. Most harvested vegetables during peak season quickly deteriorate (due to microbial and biological activities) prior to reaching the final consumer due to lack of preservation and storage facilities. Different preservative methods are associated with one problem or the other during storage of the products. However, osmotic dehydration has been proved to be a better alternative because there is retention and improvement in nutritional qualities of osmo-dried products during storage (Naknean *et al.*, 2013; Chandra and Kumari, 2015).

Most researches in the literatures focused on weight gained, water lost, functional properties, and sensory evaluation of osmo-dried products; this trial was designed to evaluate the nutritional differences between the osmo-dried vegetables using different osmotic solutions and finally compare the results with those of untreated product with a view to ascertaining the technique food quality enrichment.

EXPERIMENTAL

Sample Collection and Pre-treatment

Telfairia occidentalis leaf was purchased in Eke market in Afikpo, Ebonyi State, Nigeria from five different sellers. They were pooled together and random sample was selected. The selected part was sorted, hand plucked, washed thoroughly, and finally cut into pieces using wooden knife. This was divided into four groups: osmo-treated leaf with 50°*Brix* sucrose (Suc-T), osmo-treated leaf with 50°*Brix* cornsyrup (Syr-T), osmo-treated leaf with 10 % sodium chloride (Sod-T), non- treated leaf (Unt-T)

Production of Osmo-dried Fluted Pumpkin

Based on mass transfer in accordance with past researches elsewhere, the conditions of osmotic dehydration process selected were osmotic solution of 50°*Brix* for sucrose and corn syrup, and 10 % for sodium chloride. The impregnation process took 2 hr while the temperature of the osmotic solution was maintained at 40°C Osmo-dried fluted pumpkin products were prepared per the standard methods (Bahadur and Hathan, 2016; Naknean *et al.*, 2013) with slight modifications. Three osmotic agents were selected as dehydration solutions. Prior to dehydration, a water solution of each osmotic agent at 50°*Brix* (sucrose and corn syrup) and 10 % NaCl was prepared and equilibrated to 40°C. Each of the three of the four groups was impregnated by soaking in each osmotic solution prepared. The forth portion served as control, that is, it was not treated with any osmotic solution. The proportion of the vegetable and each osmotic solution was in ratio 1:4 (w/w), and impregnation was performed for 2 hr in beakers with occasional shaking. After osmotic dehydration, the vegetables were drained and rinsed with cold ultra-pure water. The osmo-dehydrated and untreated samples were dried using conventional oven drying method. After drying, they were grinded and stored separately prior for analyses.

Analyses

Contents of moisture, ash, total protein, ether extract, and crude fire were determined as recommended (AOAC, 1990; Onwuka, 2005), carbohydrate was determined by difference while energy value was calculated from carbohydrate, protein, and fat results (Onwuka, 2005)).pH was measured with Orion pH meter model 420. Titratable acidity of the samples was determined using the method described by Pearson (1970). Vitamin C was determined according to the method of Naknean *et al.* (2013). Total sugar and reducing sugar content were quantified by the Lane and Eynon Volumetric method as described by Naknean *et al.*, (2013).Mineral elements of the samples (calcium, magnesium, iron, zinc, copper, manganese, potassium, and sodium) were analysed with the aid of atomic absorption spectrophotometer, Buck Scientific (210VG) after digestion, while phosphorus was determined using ammonium molybdate method as described by Onwuka (2005).

Data Analysis

Data were presented as *mean concentration* $\pm$ *standard deviation* of three replicates. The difference between experimental groups was established using one-way analysis of variance. Means were compared by the Duncan' multiple range tests and a probability of $p \leq 0.05$ was considered statistically significant using SPSS 2008, version 15.0 package.

Table 1 presented the proximate composition of fluted pumpkin leaf osmo-dehydrated with different osmotic agents and untreated leaf. The results obtained shown that there were no significant difference in the percentage composition of ash for sucrose treated and untreated samples. The moisture content varied from 9.93 ± 0.06 (Unt-T) to 13.33 ± 0.23 (sod-T). Percentage fat and carbohydrate indicated that there was significant difference in the values obtained for all the samples. As far as percentage fibre and protein were concerned, there were no significant difference ($p \leq 0.05$) between the values obtained for Suc-T and Syr-T.

Table 1: Proximate Compositions of different Osmo-dried *Telfairia occidentalis* Leaf

Parameter	Suc-T	Syr-T	Sod-T	Unt-T
Ash (%)	4.99 ± 0.01^{c}	5.49 ± 0.06^{b}	9.92 ± 0.01^{a}	4.98 ± 0.15^{c}
Moisture (%)	10.80 ± 0.69^{b}	11.10 ± 0.26^{b}	13.33 ± 0.23^{a}	9.93 ± 0.06^{c}
Ether Extract (%)	3.20 ± 0.20^{c}	3.80 ± 0.30^{b}	4.47 ± 0.25^{a}	2.50 ± 0.10^{d}
Fibre (%)	18.00 ± 0.80^{b}	20.06 ± 0.24^{b}	25.70 ± 2.16^{a}	14.20 ± 0.20^{c}
Protein (%)	15.38 ± 0.37^{b}	14.95 ± 0.40^{b}	16.70 ± 0.80^{a}	11.76 ± 0.50^{c}
Carbohydrate (%)	47.63 ± 2.18^{b}	44.16 ± 0.84^{c}	29.82 ± 2.74^{d}	56.63 ± 0.29^{a}
Gross Energy (kcal)	139.81	140.04	138.97	127.60

Mean values in the same row with different letters are significantly different ($p \leq 0.05$), Mean ± standard deviation (n = 3).

Physicochemical properties of *T. occidentalis* leaf as analysed in the samples revealed that there was no statistical difference at p = 0.05 relative to the pH of the samples (Fig. 1). With exception of Sod-T, there was no different in concentrations with respect to ascorbic acid (Fig. 2). Titratable acidity (Fig. 3) and total sugar (Fig. 4) in the samples ranged from 0.23 ± 0.05 to 0.36 ± 0.04 and 12.94 ± 0.34 to 37.50 ± 0.63, respectively.

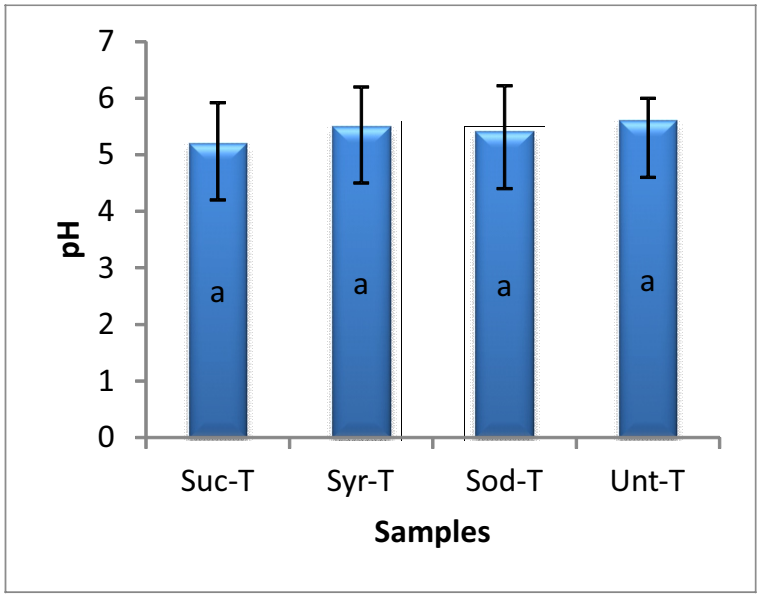

Fig. 1: pH of osmo-dried and untreated *Telfairia occidentalis*.

Mean values with same letter are not significantly different (⩽0.05).

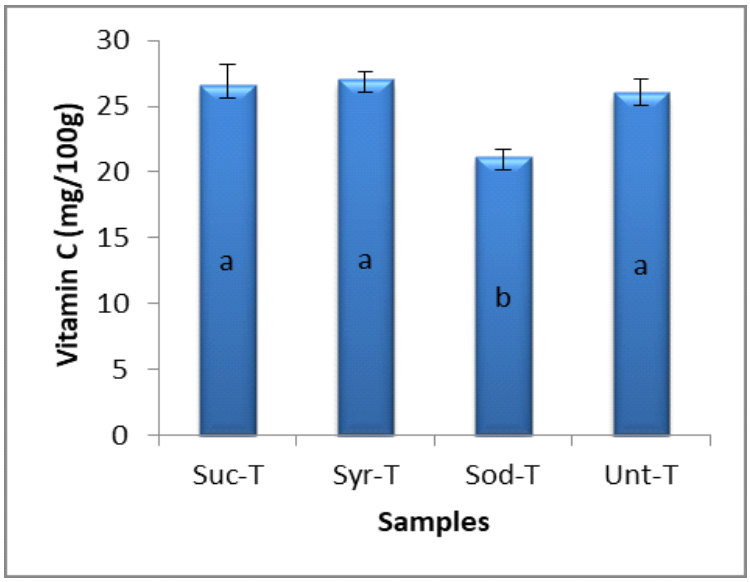

Fig. 2: % Ascorbic acid content of osmo-dried and untreated *Telfairia occidentalis*. Mean values with different letter are significantly different (⩽0.05).

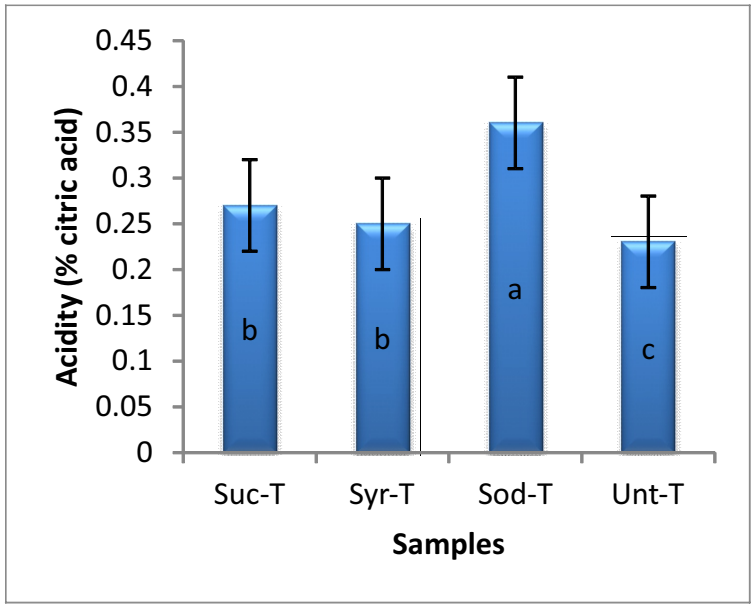

Fig. 3: Acidity in % citric acid of osmo-dried and untreated *Telfairia occidentalis*. Mean values with different letter are significantly different (⩽0.05).

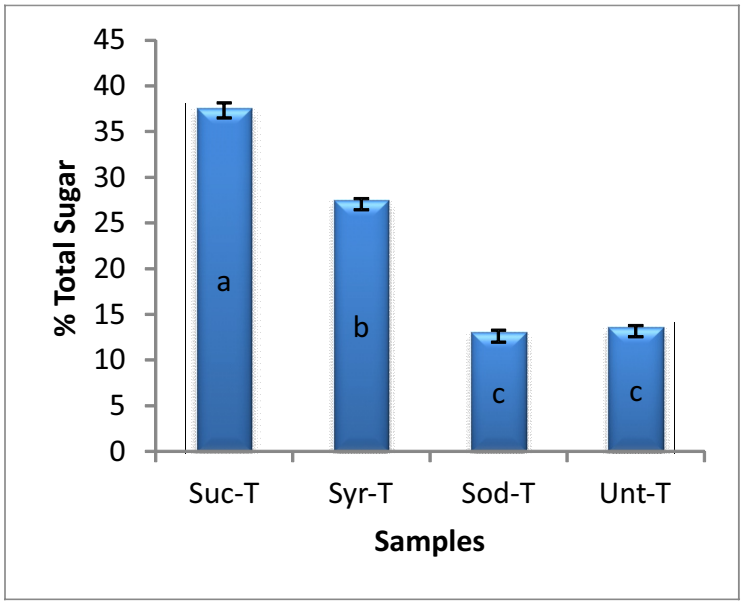

Fig. 4: % Total sugar of osmo-dried and untreated *Telfairia occidentalis*.

Mean values with different letter are significantly different ($\leqslant 0.05$).

Concentrations of mineral elements determined in the various samples were presented in the Table 2. Calcium concentration ranged from 0.61 ± 0.30 to 4.56 ± 0.35. There was no difference statistically between the concentrations of phosphorus in all the samples. The concentration of sodium in Sod-T was far higher than in other samples. There were remarkable variations in the concentrations of other elements analysed in all the samples.

Table 2: Concentration (mg/kg) of elemental compositions of different osmo-dried *Telfairia occidentalis* leaf.

Parameter	Suc-T	Syr-T	Sod-T	Unt-T
Ca	4.56 ± 0.35^{a}	4.49 ± 0.22^{a}	2.67 ± 0.54^{b}	0.61 ± 0.30^{c}
P	0.84 ± 0.18^{a}	0.88 ± 0.14^{a}	0.82 ± 0.06^{a}	0.94 ± 0.14^{a}
Na	25.60 ± 1.94^{b}	22.10 ± 0.33^{c}	40.00 ± 1.05^{a}	7.70 ± 0.17^{d}
K	34.04 ± 1.03^{ab}	44.93 ± 0.18^{a}	23.33 ± 0.21^{bc}	12.62 ± 0.12^{c}
Mg	373.00 ± 5.00^{b}	460.00 ± 3.00^{a}	95.00 ± 1.30^{c}	33.50 ± 1.52^{d}
Fe	0.34 ± 0.01^{b}	0.74 ± 0.06^{a}	0.80 ± 0.03^{a}	0.30 ± 0.00^{b}
Zn	0.12 ± 0.01^{a}	0.30 ± 0.00^{b}	0.13 ± 0.03^{a}	0.40 ± 0.01^{b}
Cu	0.30 ± 0.04^{c}	0.50 ± 0.05^{a}	0.40 ± 0.03^{b}	0.10 ± 0.02^{d}
Mn	0.21 ± 0.01^{a}	0.17 ± 0.04^{a}	0.18 ± 0.02^{a}	0.01 ± 0.00^{b}

Mean values in the same row with different letters are significantly different ($p \leq 0.05$), Mean ± standard deviation (n = 3).

DISCUSSION

Table 1 presented the proximate compositions of the untreated and osmo-treated fluted pumpkin leaves. The proportion of proximate compositions of the samples showed that the moisture contents of the *T. occidentalis* leaf ranged from 9.93 ± 0.06 to 13.33 ± 0.23%. These values were lower compared to 14.11 ± 0.58 to 14.95 ± 1.12 determined by Naknean *et al.* (2013) in osmo-dried cantaloupe and that obtained by Nishadh and Mathai (2014) in osmotic dehydration of radish. The low value reduces the tendency of microbial attack since water is a good medium for microbial growth (Oyeleke *et al.*, 2006). Statistically, there was significant difference between the results obtained for the fat content of *T. occidentalis* impregnated in different osmotic solution. Generally, the results are acceptable since the tendency of having obesity and arteriosclerosis in human being is reduced (Lee, 2005). Sod-T had highest fat content while Unt-T has least.

Ash content represents the inorganic residue remaining after burning the organic matter. It may not necessarily be equivalent to mineral matter as some losses may occur due to volatilization (Nishadh and Mathai, 2014). Sod-T had the highest value. This is expected since the osmotic solution (sodium chloride) added to the mineral present in the sample. The ash contents as obtained in this research were within the results (3.8 to 7.2 %) obtained by Nishadh and Mathai (2014) in osmotic dehydrated radish. The crude protein content of Sod-T (16.70 %) recorded the highest value followed by Suc-T (15.38 %), while Unt-T (11.76) had the least value. The crude fibre content of the samples varied from 14.20 % (Unt-T) to 25.70 % (Sod-T) and since fibre determines the degree of digestibility of food in animals, these values would help in human digestion (Oyeleke *et al.*, 2006). The carbohydrate contents were calculated to be 56.63 (Unt-T), 47.63 (Suc-T), 44.16 (Syr-T), and 29.82 (Sod-T).

The results of pH, ascorbic acid, titratable acidity, and total sugar of the samples were presented in Figures 1 to 4. Generally, there was no significant difference ($p \leq 0.05$) in the pH as determined in the entire samples. The pHs of the samples were in consonant with the range 5.82 ± 0.03 to 6.02 ± 0.05 reported for osmo-dried cantaloupe by Naknean *et al.* (2013). Vitamin C is essential in prevention of scurvy in infants. Apart from Sod-T with low ascorbic acid, there was no statistical variance in other samples. The values obtained in this study (21.21 to 27.03 mg/100g) were higher than that of raw and osmotic dehydrated radish (17.6 to 21.6 mg/100g) by Nishadh and Mathai (2014) and compared favourably for data obtained for osmo-dried cantaloupe by Naknean *et al.* (2013) which varied from 21.15 to 28.45 ± 0.31 mg/100g.

The results of total sugar content (Fig. 4) revealed the inclusion of sugars in Suc-T and Syr-T. There was no difference in the total sugar content of the NaCl-treated and untreated samples. The percentage total sugar obtained for Suc-T and Syr-T reflected the use of sugars as osmotic agents in these samples. The values (37.50 % and 27.46 %) were in agreement with the range obtained for osmo-dried cantaloupe (24.66 ± 0.01 % to 40.05 ± 0.07 %). NaCl-treated and untreated samples had low percentage sugar content of 12.94 % and 13.55 % respectively. The titratable acidity varied from 0.23 to 0.36 %. These values were higher than what was obtained by Naknean *et al.* (2013) in osmo-dried cantaloupe which ranged from 0.07 ± 0.01 to 0.11 ± 0.02. The higher values may prevent the growth of microorganisms, hence increase shelf life.

The results of some mineral elements compositions determined in the samples were shown in Table 2. Generally, the samples contained appreciable concentrations of all the elements analysed, therefore could easily serve as good sources of both micro and macro elements that are required in man's diet. There was no significant difference between calcium concentrations determined in Suc-T and Syr-T. Calcium functions as a constituent of bone and teeth, and regulation of nerve and muscle. The value obtained in this study was high enough to activate the conversion of prothrombin to thrombin (in blood coagulation) and also takes part in milk clotting (Soetan *et al*., 2010). There was a statistical difference in the entire concentrations obtained for sodium in the samples. Sod-T recorded the highest value (40.00 mg/kg) of sodium widely followed by Suc-T with a value of 25.60 mg/kg, while the least values of 7.70 mg/kg was associated with Unt-T. The high concentration of sodium in Sod-T could be attributed to the osmotic agent (sodium chloride) used. There was no significant difference at $p \leq 0.05$ in the concentrations of phosphorus determined in the entire samples. Phosphorus is vitally concerns with many metabolic processes, including those involving the buffers in body fluids (Soetan *et al.,* 2010). It functions as a constituent of bones and teeth, adenosine triphosphate (ATP), phosphorylated metabolic intermediates and nucleic acids.

The values obtained for potassium in the samples were low considering its importance in human diet. The values ranged from 0.12 mg/kg (Unt-T) to 0.44 mg/kg (Syr-T). This mineral element balances acid-base content of the body, regulates osmotic pressure and conducts nerve impulse, muscle contraction particularly, the cardiac muscle, and cell membrane (Soetan *et al.*, 2010). Magnesium is an active component of several

enzyme systems in which thymine pyrophosphate is a cofactor. Oxidative phosphorylation is greatly reduced in the absence of magnesium (Murray *et al.,* 2000). Sty-T had the highest concentration with a value of 460 mg/kg while 33.5 mg/kg was determined in Unt-T. This an indication that there was an increase in the magnesium concentration in all the treated samples compare to untreated (control).

Copper is an essential micronutrient necessary for the growth and formation of bone, formation of myelin sheaths in the nervous systems, helps in the incorporation of iron in haemoglobin, assists in the absorption of iron from the gastrointestinal tract (GIT) and in the transfer of iron from tissues to the plasma (Murray *et al.,* 2000). This trace element had its concentration to be in the range of 0.10 mg/kg and 0.50 mg/kg. Iron was determined and varied from 0.30 mg/kg to 0.80 mg/kg. It functions as haemoglobin in the transport of oxygen. There was no significant difference between the concentrations of manganese in the treated samples, while its concentration was minimal in the control sample. Manganese is a cofactor of hydrolase, decarboxylase, and transferase enzymes (Murray *et al.*, 2000). The highest concentration of zinc was implicated in the Unt-T.

CONCLUSIONS

This trial has proved that osmotic dehydration technique if properly and effectively applied will not only reduce wastage experienced as a result of vegetable deterioration during peak harvest period but also improve the quality of the dried and preserved products. The direct implication of this process is that there is quality improvement, no chemical treatment required, product stability, and retention of nutrients during storage. Sodium chloride proved to be the best osmotic agent among the tested solutions but care must be taken because of the health implication associated with high intake of sodium. This process could be used on small scale for development of self-entrepreneurs and home scale industries. Consumption of such nutritional and valued products could be popularized through exhibition and media in order to make this product available round the year.

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Assessment Of Pollution Status Of Unwana River Through Trace Metals In Sediments

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Abstract

The trace metals in the sediment of Unwana River were assessed using Atomic Absorption Spectrophotometer (AAS). The trace metals analyzed were Cd, Cr, Cu, Pb, Fe, Zn and Co. the mean concentrations of Cd were 0.00±0.00mg/kg, 0.05±0.002mg/kg, and 0.00±0.01mg/kg for Locations A, B and C, respectively. The mean concentrations of Cr were 0.0±0.0mg/kg, 0.003±0.001mg/kg and 0.0±0.0mg/kg for Locations A, B and C respectively. The mean concentrations of Cu were 0.06±0.000mg/kg, 0.08±0.02mg/kg and 0.09±0.32mg/kg for Locations A, B and C respectively. The mean concentrations of Pb were 0.32±0.01mg/kg, 0.508±0.04mg/kg and 0.64±0.01mg/kg for Locations A, B and C respectively. The mean concentrations of Fe were 0.913±0.15mg/kg, 1.23±0.03mg/kg and 2.94±0.018 mg/kg for Locations A, B and C respectively. The mean concentrations of Zn were 4.21±0.95mg/kg, 2.94±0.04mg/kg and 5.74±1.24mg/kg for Locations A, B and C respectively. The mean concentrations of Co were 0.0±0.0mg/kg, 0.0±0.0mg/kg and 0.15±0.02mg/kg for Locations A, B and C respectively. The results of the analysis showed that the concentrations of Cd, Pb and Zn were very high in the three Locations sampled and could pose danger to the biota. Cr and Co were detected only in samples B and C respectively unlike the other metals, which were detected in all the samples. Continuous monitoring of the sediments is recommended.
Keywords: Trace metals, spectrophotometer, concentration, monitoring and sediments

Introduction

Pollution of aquatic environment could be associated with growth in human population, increasing industrial development, agricultural practices, urbanization, and heavy metals from motor vehicles and inadequate consideration for its impact in the environment [1]. Again, the desires of most people for a higher material standard of living are resulting in worldwide pollution on a massive scale. Environmental pollution can be divided among the categories of water, air and land pollution. These three categories of environmental pollution are linked. For example, some gases emitted to the atmosphere can be converted to strong acids by atmospheric chemical processes, fall to the earth as acid rain and pollute water with acidity. Improperly discarded hazardous wastes can leach into groundwater that is eventually released as polluted water into streams [2]. Pollution brings about undesirable consequences in the environment, which in turn affects the compositions of an ecosystem. Aquatic ecosystem is greatly affected by stressors which deplete biodiversity [3].

It has been suggested that water pollution is the leading cause of death and diseases and that it accounts for the death of more than 14,000 people daily [4], [5]. Water is typically referred to as polluted when it is impaired by anthropogenic contaminants and either does not support human use, such as drinking or undergoes a marked shift in its ability to support its constituent biotic communities, such as fish. Natural phenomena such as volcanoes, algae blooms, storms and earthquakes also cause major changes in water quality and the ecological status of water.

The indiscriminate release of organic and inorganic substances change the physico-chemical properties of water which are deleterious to flora (plants) and fauna (animals) in aquatic environment and to man as well [6]. Aquatic organisms accumulate contaminants from the environment and therefore it is evident that aquatic environment may be polluted [7]. Contaminants often remain in solution or in suspension, and finally tend to settle down at the bottom of aquatic environments or are

ingested by benthos in aquatic environments. The easy accessibility of rivers for the disposal of domestic and industrial wastes has made them very susceptible to pollution, especially by anthropogenic activities [8].

Trace metal is a term that refers to those metals that occur at very low level of a few part per million or less in a given system [2]. They are one of the major water pollutants. Ingestion of trace metals or exposure to excessive quantities can be toxic. Trace metals are needed by living organisms to function properly and are depleted through the use of energy by various metabolic organisms. They are replenished in animals through diet as well as environmental exposure and in plant through the up-take of nutrients from the soil in which the plant grows [9], [10].

This study is to assess the pollution status of Unwana River through trace metals in samples of sediment.

Materials and Method

Sample Collection

The sediment samples were collected at three different points from inside Unwana River at interval of 50 to 100m with non-metallic materials (cans), which has been properly washed and dried. Then, each of the cans was covered.

Sample Pretreatment

Each sample was air dried at room temperature under a ceiling fan. A mortar and pestle were used to crush each of the samples one at a time to obtain homogeneous sediment samples. Exactly 1g of each sample was placed in a 250ml beaker. Then, 20ml of freshly prepared aqua regia was added. The beakers were covered, and the contents heated on a hot plate. The mixtures were allowed to cool, and then each of them was filtered through a Whatman No. 42 filter paper into different100ml standard volumetric flasks, each filtrate was diluted to 100ml with distilled water. They served as the digested samples that were used for the trace metal analysis using Atomic Absorption Spectrophotometer (AAS).

Statistical Analysis

Results will be expressed as standard error of mean. More so, the mean value will be compared with the World Health Organization (WHO) permissible limit.

Results and Discussion

The results for the concentrations of the trace metals (cadmium, chromium, copper, lead, iron, zinc and cobalt) that were determined in the sediments from Unwana River are represented in the summary Table 4 and Figures 1 and 2.

Table 1: Statistical Analysis of the Trace Metals in Sediments of Unwana River at Point A

Parameter	1st	2nd	3rd	Mean	STDev	SE
Cd	0.000	0.010	0.000	0.003	0.005	0.003
Cr	0.000	0.000	0.000	0.000	0.000	0.000
Cu	0.057	0.059	0.058	0.058	0.001	0.000
Pb	0.467	0.039	0.400	0.302	0.188	0.108
Fe	0.563	1.176	1.000	0.913	0.258	0.149
Zn	6.100	2.104	4.430	4.211	1.639	0.946
Co	0.000	0.000	0.000	0.000	0.000	0.000

Table 2: Statistical Analysis of the Trace Metals in Sediments of Unwana River at Point B

Parameter	1st	2nd	3rd	Mean	STDev	SE
Cd	0.056	0.057	0.050	0.054	0.003	0.002
Cr	0.005	0.000	0.003	0.003	0.002	0.001
Cu	0.107	0.044	0.100	0.084	0.028	0.016
Pb	0.590	0.435	0.500	0.508	0.064	0.037
Fe	1.179	1.300	1.220	1.233	0.050	0.028
Zn	1.070	5.359	2.400	2.943	1.793	1.035
Co	0.000	0.000	0.000	0.000	0.000	0.000

Table 3: Statistical Analysis of the Trace Metal s in Sediments of Unwana River at Point C

Parameter	1st	2nd	3rd	Mean	STDev	SE
Cd	0.026	0.053	0.034	0.038	0.011	0.007
Cr	0.000	0.000	0.000	0.000	0.000	0.000
Cu	0.029	0.165	0.084	0.093	0.056	0.032
Pb	0.674	0.632	0.620	0.642	0.023	0.013
Fe	2.968	2.960	2.900	2.943	0.030	0.018
Zn	2.732	7.632	6.860	5.741	2.151	1.242
Co	0.195	0.100	0.164	0.153	0.040	0.023

Table 4: Mean Concentrations of the Trace Metals in Sediments Of Unwana River

Parameters	A	B	C
Cd	0.003±0.002	0.054±0.002	0.038±0.007
Cr	0.000	0.003±0.001	0.000
Cu	0.058±0.000	0.084±0.016	0.093±0.032
Pb	0.302±0.108	0.508±0.037	0.642±0.013
Fe	0.913±0.149	1.233±0.028	2.943±0.018
Zn	4.211±0.946	2.943±1.035	5.741±1.242
Co	0.000	0.000	0.153±0.023

A: Upstream, B: Midstream, C: Downstream

Discussion

The results of the concentrations of trace metals in the sediments of Unwana River are presented in Table 4. The results showed that the concentrations of Cd were 0.00 ± 0.00mg/kg, 0.05 ± 0.00mg/kg and 0.04 ± 0.01mg/kg for Locations A, B and C, respectively. The concentrations pose no threat of any kind as the level was below the permissible limit of international standard. High concentration of Cd can cause osteomalacia and osteoporosis in both humans and animals. Figure 1 shows that Location C had the highest concentration of Cu while Location A had the lowest of Cu. Again, Figure 1 shows that the Cd concentration was highest in Location B and lowest in Location A. The Cr was below the detectable limit except in Location B that showed a very low concentration and would not pose any danger to the sediment quality of the river. The metals varied in the order B > C > A. These concentrations were below the legislative limit of sediment quality of Cd according to WHO [11], which is 3.00mg/kg.

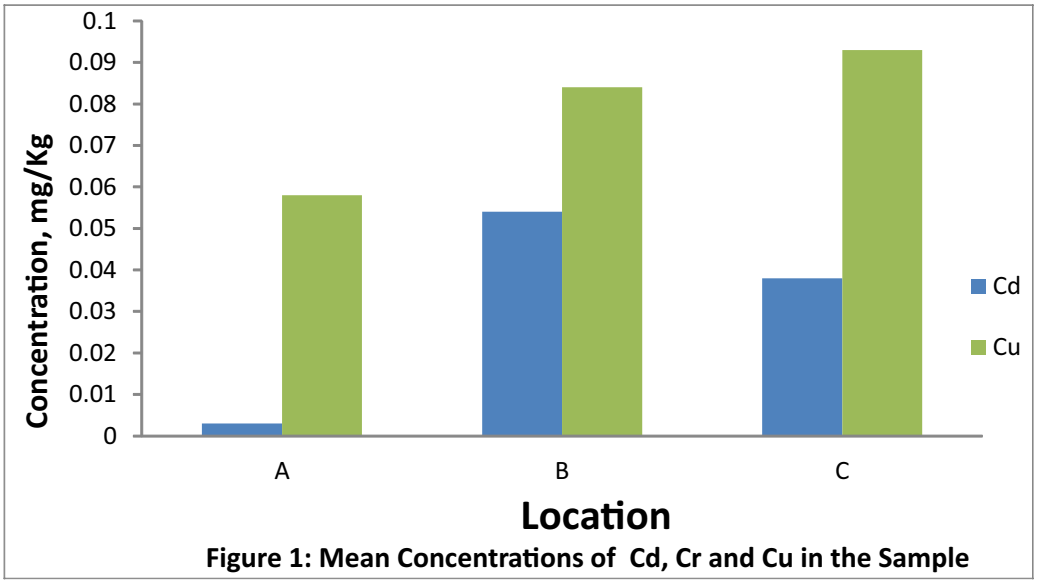

Figure 1: Mean Concentrations of Cd, Cr and Cu in the Sample

The mean concentrations of Cu were 0.058 ± 0.000mg/kg, 0.084 ± 0.016mg/kg and 0.093 ± 0.320mg/kg for locations A, B and C respectively. Figure 1 shows that the Concentration of Cu varied in the order C >B > A. These concentrations were below the permissible limit of Cr concentration in sediment according WHO [12], which is 50.000mg/kg.

The mean concentrations of Pb were 0.302 ± 0.108mg/kg, 0.508 ± 0.037mg/kg and 0.642 ± 0.013mg/kg for Locations A, B and C respectively. Therefore, they varied in the order C > B > A respectively. These mean concentrations were below the permissible limit of Pb concentration in sediment according to WHO [11], which is 300.00mg/kg. High concentration of Pb in the sample is not good as high concentration of lead causes anemia and brain disorder.

The mean concentrations of Fe were 0.943 ± 0.149mg/kg, 1.233 ± 0.028mg/kg and 2.942 ± 0.018mg/kg for Locations A, B and C respectively. Therefore, they varied in the order C > B > A as represented in Figure 2. Excessive iron in the sample could cause health challenges. The presence of iron in the sediments could be due to the dumping of metal scraps in the body of the water which settled at the bottom of the River.

The mean concentrations of Zn were 0.946mg/kg, 2.943 ± 0.035mg/kg and 5.741 ± 1.242mg/kg for Locations A, B and C respectively. Therefore, they varied in the order C > A > B as represented in Figure 2. These concentrations are below the permissible concentration of Zn in sediment according to WHO [11], which is 300.000mg/kg.

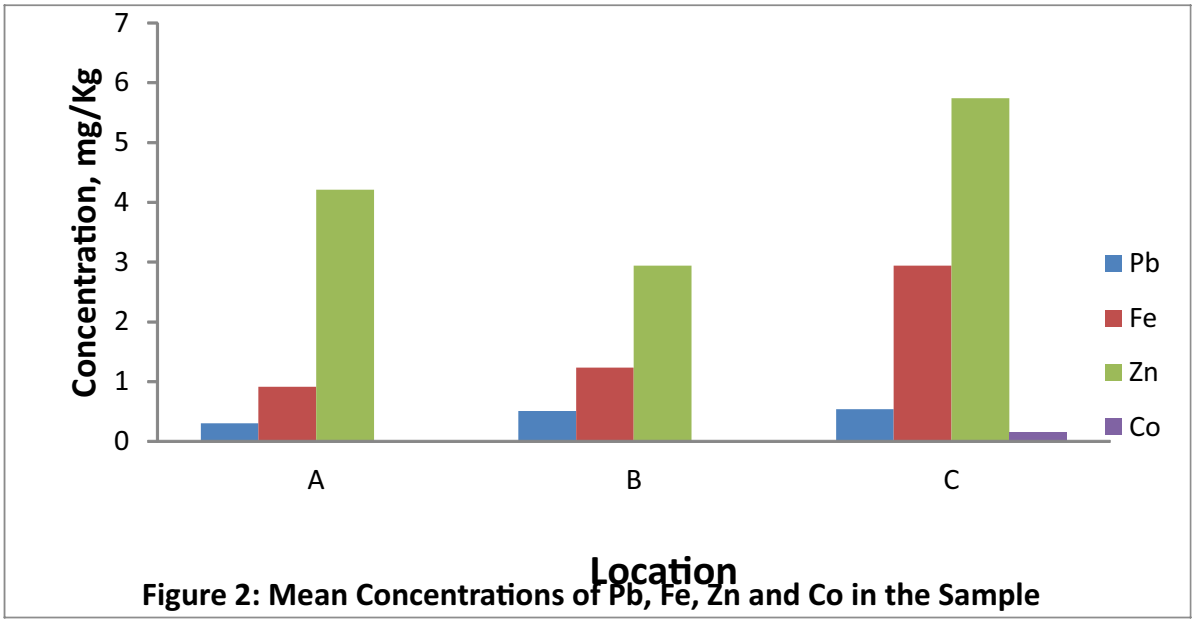

Figure 2: Mean Concentrations of Pb, Fe, Zn and Co in the Sample

They mean concentrations of Co were 0.000mg/kg, 0.000mg/kg and 0.153 ± 0.023mg/kg for locations A, B and C respectively. Therefore, they varied in the order C > A = B as represented in Figure 2.

Conclusion

Trace metals, such as cadmium, chromium, copper, lead, iron, zinc and cobalt were determined with respect to their concentrations in samples of sediment from Unwana River using Atomic Absorption Spectrophotometer (AAS). Cadmium, copper, lead, iron and zinc were detected in all the samples. However, chromium was only detected in sample B, and cobalt was only detected in sample C. Their concentrations were found to be below the permissible limits legislative limit of sediment quality.

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Determination of Nitrate in table Water Samples Sold in Owerri Urban Madu K.C., Ahanotu C.C.,Ikemezie M.N. and Maduagwu E.C.

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Abstract

Studies were carried out to determine the nitrate levels of table water sold in Owerri Urban. About sixteen different table water samples were being produced and distributed within Owerri Urban presently. World Health Organization had recommended 50mg/L as the maximum contaminant level for nitrate for a drinking water quality.Out of the sixteen table water samples, only two samples (Sample G and Sample H) with concentration of 51.64±8.47 and 52.20±6.37 have its nitrate concentration above that recommended by WHO for a drinking water while the rest were within the stipulated maximum contaminant level.

Key words: Nitrates, Nitrite, WHO, Table water, Owerri

INTRODUCTION

Water (H_2O) is a polar inorganic compound that is at room temperature a tasteless and odorless liquid, nearly colourless with a hint of blue. This simplest hydrogen chalcogenide is by far the most studied chemical compound and is described as the "universal solvent" for its ability to dissolve many substances. This allows it to be the"solvent of life"[18] It is a common substance to exist as a solid, liquid and gas on earth surface.[19]. Water is amphoteric, meaning it is both an acid and a base – it produces H^+ and OH^- ions by self-ionization. This regulates the concentrations of H^+ and OH^- ions presnt[1]

Water is a ubiquitous chemical substance that is essential for the survival of all known forms of life. It is an incredibly important aspect of our daily lives. Water is absolutely essential to the human body's survival because a person can live for about two weeks without food but under optimal condition can live for only ten days without water[2].

Accessibility and availability of fresh clean water is a key to sustainable development and an essential element in health, food production and poverty reduction. A communiqué issued after the Third World Water Forum on water in 2003, reported that an estimated 1.2billion people around the world lack access to safe water and close to 2.5billion are not provided with adequate sanitation[3]. Safe and portable water supplies in urban centers in Nigeria are still inadequate in spite of over fifty years of independence and several efforts from various governments. In Owerri, the Imo State Capital, thewater from the Government water board is able to serve only a small proportion of the urban dwellers while majority depend solely in borehole water supply. Most often, the borehole are located near sources of pollution and the quality is rather poor[5]. Rain water is seasonal and there are no organized storage facilities[6]. Ponds and streams are easy and accessible sources of guinea worm, schistosomiasis and other waterborne infections and endemic diseases[7]. The result has been increase in patronizing water vendors who collect, pack and supply different brands of water of doubtful quality

Heavy metal pollutants, nitrates, phosphates among others have been known to be present at very high concentrations in waters that are not properly treated[9]. For instance, nitrate is a natural contaminant of water, soil, plant and food[10]. It is formed when microorganisms in the environment breakdown organic materials such as plants, inorganic fertilizers, animal manure, and sewage[10]. Nitrate can get into drinking water from runoff or sewage into ground water from farms and gardens. Other sources of

nitrate in water include landfills, poorly managed animal feedlots, faulty septic systems and also poorly constructed or improperly located wells. Nitrate has been implicated in health outcomes such as cancer via the bacterial production of N-nitroso compounds[11]. The acute health hazard associated with drinking water with nitrate occurs when bacteria in the digestive system transform nitrate to nitrite. The nitrite reacts with iron in the haemoglobin of red blood cells to form methemoglobin, which lacks the oxygen carrying ability of haemoglobin. This creates the condition known as methemoglobinemia (sometimes referred to as blue baby syndrome), in which blood lacks the ability to carry sufficient oxygen to the individual body cells. Infants under one year of age have the highest risk of developing methemoglobinemia (Met Hb). This age group is the most susceptible because of a combination of factors[11], include

i) A higher gastric pH, which allows greater bacterial invasion of the stomach and hence enhanced conversion of ingested nitrate to nitrite. A greater fluid intake relative to the body weight.

ii) A greater proportion of fetalhaemoglobin (which may be more rapidly oxidized to MetHb than adult Met Hb)

iii) Lower NaOH dependent MetHb reductase activity (the enzyme that converts Met Hb to Haemoglobin)

Older persons who have a gastrointestinal system disorder resulting in increased bacteria growth maybe at greater risk than the general population. In addition, individuals who have a generally impaired enzyme system for metabolizing methemoglobin may be at greater risk[12].

Concentrations of nitrate above 100mg/L can affect pregnant women, and those adults with a rare metabolic phosphate dehydrogenase deficiency[13]. Chronic nitrate toxicity has also been implicated in spontaneous abortion, infant and fetal deaths, reduced vitality, increasing still births, slow weight gain in livestock, central nervous system, birth defects, diabetes and changes to the immune system[14]. Long term effects of lifetime exposure to nitrate above maximum contaminant level (50mg/l) include diuresis, increased starchy deposits and haemorrhaging of the spleen. Nitrate can be removed from water using reverse osmosis, distillation systems, or deionization. However, boiling and simple filtration in line filters do not remove nitrate but instead increase its concentration. This study was aimed at investigating the nitrate level in the table water samples sold in Owerri and to compare the results with WHO drinking water standards (WHO DWS).

EXPERIMENTAL

The sixteen table water used (samples A – P) which were bought from retail outlets in Owerri Urban, Imo State, Nigeria.Standard method of quantitative determination of nitrate and general water analysis as described by American Public Health Association was employed[15].

RESULTS AND DISCUSSION

TABLE 1: Nitrate level in mg/L of the sixteentable water sample

SAMPLE	BATCH NO	MANUF. DATE	EXPIRE DATE	Mean Nitrate Conc.	WHO STANDARD
A	AQ3	24/01/2017	24/01/2018	10.70±2.61	50mg/l
B	B0021	21/03/2017	21/03/2018	12.03±0.61	50mg/l
C	D0331	03/02/2017	03/02/2018	10.06±2.47	50mg/l
D	B0044	09/01/2017	09/01/2018	11.56±0.85	50mg/l
E	E0122	08/10/2016	08/10/2017	10.17±0.55	50mg/l
F	U0012	09/09/2016	09/09/2017	12.76±0.47	50mg/l
G	P0011	07/04/2017	07/04/2017	12.82±1.35	50mg/l
H	H1012	07/03/2017	07/03/2018	10.37±0.48	50mg/l
I	O0014	11/04/2017	11/04/2017	14.67±4.89	50mg/l
J	IOO12	12/01/2017	12/01/2018	11.45±3.35	50mg/l
K	G0066	05/02/2017	05/02/2017	10.06±0.64	50mg/l
L	H0222	04/04/2017	04/04/2018	11.00±0.68	50mg/l
M	K0888	07/02/2017	04/02/2018	10.09±2.88	50mg/l
N	B0101	09/05/2017	09/05/2018	12.00±0.66	50mg/l
O	K0122	22/01/2017	22/01/2018	12.01±0.68	50mg/l
P	J0101	16/11/2016	16/16/2016	13.01±0.66	50mg/l

The results of Table 1 showed that almost all the water samples analyzed for have their nitrate concentrations below the maximum contamination level of 50mg/L allowed by WHO for drinking water.Of the sixteen water samples analyzed, only Samples G and H sachet water with mean nitrate concentration of *51.64±8.47 and 52.20±6.37* exceeded the maximum contaminant level allowed by WHO for drinking water quality. The health implications on people consuming this water as described in literature are methemoglobinaemia (especially for infants), central nervous system birth defects, still births, diabetes, changes to the immune system, haemorrhaging of the spleen[12,13,14].

Samples D, J and M sachet waters with mean nitrate concentrations of 30.56±0.85, 38.45±3.35 and 35.09±2.88 respectively, although below the maximum contaminant level allowed by WHO, may have their nitrate level rise above that recommended if not properly treated.

Conclusion

The result of the study showed that fourteen out of the sixteen water samples analyzed were safe for human consumption with respect to their nitrate level. There is therefore a need to monitor all those involved in water business to comply with the guidelines for water quality. Similar studies should be carried out in all cities, nooks and crannies of our nation to determine the safety level of our table water as regards their nitrate level, since the problems associated with unacceptable levels of nitrate in drinking water is on the increase globally.

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IR Spectroscopic Analysis On Flavonoid Of The Seed Of *Bucchhlozia Coriacea* (Wonderful Cola)

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Abstract

The seed of bucchholzia coriacea of the family, capparidacceae which is widely acclaimed to be useful in ethnomedical practices was preliminary investigated using phytochemical screening, chromatography and IR spectroscopic analysis. The ethanolic extract revealed the presence of saponin, flavonoid, steroid, and alkaloid, which supported the use of this seed in ethnomedical due to their therapeutic values. The RF value of the major components from the isolated flavonoid was 0.92. This RF value suggests the isolated flavonoid to be quceretin which has the same RF value in the same solvent system (n-butanol:ethanol:ammonia; 6:2:2). The IR analysis confirmed the identified cation of the following functional groups. The O-H stretch group (3474-3268cm^{-1}), C=O group (1868-1758cm^{-1}), C-H of alkyl (2963-2449cm^{-1}) and (1462-1316cm^{-1}). C=C of aromatics (2283-2000cm^{-1} and 1658-1630cm^{-1}), which suggests that the compound is a flavonoid and likely to be quceretin or a substituted quceretin owing to its absorption pattern.

Keywords: *Bucchholzia coriacea*, Chromatography, IR spectroscopy, Flavonoid.

Introduction

Bucchholzia coriacea belongs to the family of *capparidaceae* (named after Bucchholz who collected the plants in Cameroon in the 19th century) [1]. In Nigeria, the seeds of *Bucchholzia coriacea* are used for the treatment of asthma [2], veneral diseases, stomach upset [3], and ear and eye disease and for flushing out of staphylococcus, a bacterium that affects the reproductive organs of both men and women [4]. This plant has been known to be used for the treatment of syphilis, dressing of wounds, chronic ulcers and for the treatment of snake bites [5],and is used as anti-diabetic and anti-therapeutic [6], anti-oxidant [7]. It can also serve as protection against the IR- radiation. The therapeutic properties of this plant are as a result of some natural products like flavonols and flavones [8], alkaloids, tannins and saponins etc. It also contains large amounts of caffeine and theobromine, and is used as stimulant [9,10]. Therefore, it is necessary to source drugs from natural resources as they are reported to work better than synthetic ones [11], as has been advocated by the federal government for the continuous preservation of these natural reservoirs [12]. In its industrial uses [13], *Bucchholzia coriacea* (Wonderful kola) is used in the manufacture of dyes and cola group of beverage drinks. The kola husk has been used in the manufacture of liquid detergent and organic fertilizers [14], wines and candles [15,16]. Agro-industrial by-products, kola nut husk, kola testa, etc. have proved to be valuable in replacing certain proportion of maize in monogastric nutrition [17]. It has been reported that kola nut husk meal shared similarity with cocoa pod husk but has higher crude protein and lower crude fibre contents than cocoa pod husk [18].

Materials and Methods

Fresh seeds of *Bucchholzia coriacea* were bought at Eke market in Ekwulobia in Aguata L.G.A. of Anambra State. The seeds were thoroughly washed in clean water, sliced into its pieces and were shade dried at 25^{o}C for 2 weeks (when constant weight was obtained). The dried seeds were ground to powder using manual grinder. 100 g of the ground powder was placed in a 500 mL volumetric flask and 50 mL of ethanol was poured in it, the flask was tightly covered. The set up was allowed to stand for 48 hours. The extract was decanted, filtered and filtrate was heated in a water bath to evaporate the solvent. The concentrated extract obtained was used to test for the presence of flavonoids, alkaloids,

steroids, saponins and tannins [19].

Phytochemical analysis of the extract of *bucchholzia coriacea*

Test for Alkaloids: 2 mL of the ethanolic extract was placed in a tube and 2 drops of Dragendroff's reagent was added and cream coloured precipitate was observed. 2 mL of the extract was placed in a test tube and 2 drops of Meyer's reagent was added and yellow precipitate was observed.

2 mL of the extract was placed in a test tube and few drops of the Wagner's reagent was added and observed for reddish precipitate.

Test for Flavonoids: 2 mL of the extract was placed in a test tube and few drops of NaOH solution was added and yellow colour was observed, which disappeared on addition of dilute HCl.

2 mL of the extract was placed in a test tube and 10% $FeCl_3$ solution was added and green coloured solution was observed.

2 mL of the extract was placed in a test tube and 5% lead acetate solution was added and a yellow colour was noted.

Test for Steroids: 2 Drops of acetic anhydride was added to 2 mL of the extract and 1mL of conc. H_2SO_4 was also added and shook. Reddish-violet colour at the junction of the two layers was observed.

Test for Saponins: 3 mL of the extract was placed in a test tube and 6 mL of distilled water was added. It was shaken vigorously for 30 seconds, persistent frothing was observed. 5 mL of the extract was placed in a test tube and 5 mL of Fehling's solution was added, a brick red precipitate on warming was observed.

Test for Tannins: 2 mL of the ethanolic extract was placed in a test tube and few drops of 6% ferric chloride solution were added, blue-black colour was observed.

Isolation of flavonoid from the extract

Small amount of the seed extract was placed in a test tube; it was acidified with 2M HCl and heated in a water bath for 30-40 min at 100^{o}C . Then cooled and filtered. The filtrate was extracted using 2 mL ethyl acetate. The ethyl acetate extract was concentrated to dryness in an oven (Bs model) at temperatures of 55-60^{O}C to get the crude extract. The extract was tested for the presence of flavonoids. The mixture of flavonoids obtained was separated into their individual components using thin layer chromatography.

Chromatographic separation

Thin layer chromatography was used for the separation of flavonoid. A glass of thickness 0.1cm, length 7.6 cm and width 2.6 cm was used. Slurry of silica gel and chloroform in the ratio of 1:3 was prepared. The glass was washed with distilled water to remove dirt and slurry was poured on the glass. It was air dried for 10 min. The crude sample was spotted at 1cm distance from the base of the glass using thin capillary tube. The spotted plate was placed in chromatographic tank containing, butanol:ethanol:ammonia in the ratio of 6:2:2 respectively, and was covered for proper development and separation. Only one spot was observed after the development. The Rf value of the flavonoid was determined using the formular below: Rf = Distance travelled by the sample / Distance travelled by the solvent.

The Infra-red Spectrometric determination was performed at Springboard Research Laboratory, Awka, Anambra State.

Results

Table 1: Results of the phytochemical screening of the ethanolic extract of the sample

Compound	Inference
Alkaloid	++
Tannin	-
Saponin	+++
Flavonoid	+++
Steroid	+

Note: +++ =Highly present, ++ = Moderately present,+ = present and - = Absent.

Table 2: Results of confirmator y test for flavonoid on the isolated extract

	Test	Inference
i.	Iron III chloride test	Present
ii.	Lead acetate test	Present

Table 3: The Infra-Red spectrum wave number and their functional groups

Wave number (cm^{-1})	Functional group (Description)
3474 and 3268	O-H stretch for phenols and alcohols
2963	
2764	C-H stretch for alkyl groups
2591	
2449	
2283	
2209	C-C of alkenes or aromatics
2131	
2000	
1868	
1758	C=O stretch of carbonyl
1658	C=C stretch for aromatics
754	C-H stretch for substituted aromatic compounds
876	C-H stretch for alkenes

Discussion

The results of the phytochemical analysis of *Bucchholzia coriacea* in Table 1 showed that the seed contains some secondary metabolites such as flavonoids, saponins, alkaloids and steroids. The presence of these compounds in the investigated plant, accounts for its usefulness as medicinal plant.

Flavonoids are known to have antioxidant property and protect our cells against oxidative damage and this reduces the risk of developing certain type of cancer [20]. It lowers the risk of heart diseases and equally protects the gastric mucosa from erosion. It also acts as anticonvulsant through prevention or inhibition of vitamin B_6metabolism.

The saponin content in this seed is high as well showing that if the seed is taken, it can help to eliminate toxic waste in the body fluid [21] and thereby boost the energy needed. It also serves as natural antibiotic and inhibits the growth of cancer. Alkaloids have been known from the ancient time to have strong medicinal value and at such have being used in curing some ailments when taken in smaller quantities [22].

Again, the presence of steroid in this seed shows that the seed can also be used to reduce the risk of coronary heart disease, cancer and even high blood pressure when also taken in small quantities [23].

Some other medicinal plants have been reported to contain these important natural products, for instance, *musa saplentum* pseudo-stem is used to beat down blood pressure due to its high content in secondary metabolites, yet very low in salt.

The retention factor (Rf) result obtained from TLC analysis was 0.92, which is comparable to the Rf value standard of quceretin, which from literature was reported to be 0.85≈0.9. Therefore the isolated flavonoid is likely to be quceretin.

The absorption band at 3474 cm^{-1} corresponds to the O-H of phenols and alcohols, while the bands from 2963-2449 cm^{-1} indicates the presence of alkyl groups in the compound. Furthermore, that the compound is aroma`tic is indicated by the absorption bands 2283-2000 cm^{-1}, and was also supported by the C-C absorption at 876 cm^{-1}. The stretching frequency at 1758 cm^{-1} corresponds to the carbonyl (C=O) absorption. Based on all these results, the compound is likely to be flavonoid. This is because the main functional groups present are the O-H of alcohols and phenols, C=C of aromatics, C-O of carbonyl and C-H of alkyls, which goes again to suggest that the compound is likely to be quercetin shown in Figure 1.

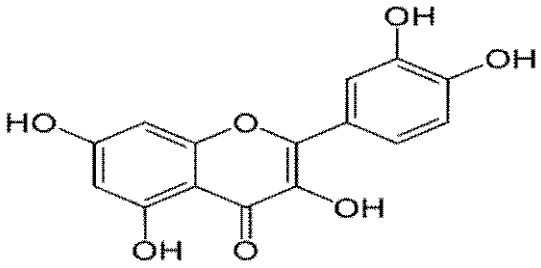

Figure 1: The structure of Quercetin

Conclusion

The findings of this work showed that *Bucchholzia coriacea* contained alkaloids, saponins, steroids, and flavonoids, which are the major constituents of medicinal plants and are known to possess therapeutic potentials. The presence of flavonoid can be said to be the more reason why the seed is highly active and useful in treatment of cancer, inflammation, headache, toothache, bacterial infections and so many more other ailments.

The chromatographic analysis suggests the isolate to be quercetin based on its Rf value.

The IR result of the isolate proposed the compound to be a flavonoid and likely to be quercetin based

on the absorption pattern. This study has therefore supported the use of *Bucchholzia coriacea* in ethnomedical practice as well as a good raw material for medicinal application based on the proposed flavonoid.

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Hydrogen Peroxide Scavenging Capacity and Antibacterial Activity of Stem Bark of *Buchholzia Coriacea* Engler

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ABSTRACT

Hydrogen peroxide scavenging capacity of stem bark of *B. coriacea* was carried out using standard technique. Antibacterial studies were carried out in methanol, ethanol, chloroform and *n*-hexane, using clinical isolates of *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Salmonella typhimurium.* Results of IC_{50} values, were 0.197mg/ml for methanol extract and 0.211mg/ml for standard ascorbic acid, showing higher hydrogen peroxide scavenging activity of stem bark of *Buchholzia coriacea* than ascorbic acid. There was a strong dose-dependent inhibition of hydrogen peroxide, which could be ascertained by the regressions coefficient R^2 values of 0.9261 and 0.9373 for the extract and ascorbic acid respectively. The methanolic extracts yielded the highest zone diameter of inhibition against the test microorganisms in comparison with the other extracts. Minimum Inhibitory Concentration (MIC) of the methanolic extract ranged from 50mg/ml to 200mg/ml . The Minimum Bactericidal Concentration (MBC) of the methanolic extracts of as ranged from 100mg/ml to 200mg/ml. Stem bark of *B. coriacea* can be used as a natural source of both antioxidant and antibacterial agents.

Keywords: Hydrogen Peroxide Scavenging Capacity, Antibacterial Activity, *Buchholzia Coriacea*

INTRODUCTION

Medicinal plants have been a valuable source of therapeutic agents, and still many of today's drugs are plant-derived natural products or their derivatives . Many intensive studies for natural therapies are ongoing (Gislene et al., 2000). According to World Health Organization as reported by Santos, Oliveira, and Tomassini, (1995), medicinal plants would be the best source to obtain a variety of drugs. Medicinal plants are considered new resources for producing agents that could act as alternatives to antibiotics in the treatment of antibiotic-resistant bacteria (Ayman and Mazen 2014).

Some of the reactive oxygen species, including hydrogen peroxide, singlet oxygen, hydroxyl and superoxide radicals, have played positive roles in energy production in vivo systems, phagocytosis, intercellular signal transfer, regulation of cell growth and the synthesis of important biological compounds (Packer et al, 2008). However, reactive oxygen species are capable of modifying DNA and membranes by attacking the lipids, proteins, and carbohydrates in cell membranes and tissues (Jung et al, 2009). There is a balance between the rates of production and removal of free radical in the organism. This is known as oxidative balance. Disruption of this balance by increase in the rate of production or decrease in the rate of removal of free radicals increases the levels of reactive oxygen species. This condition, is known as oxidative stress. The result is tissue damage due to the production of free radicals which overrides the antioxidant defense systems (Wells et al, 2009). Many natural products from medicinal plants contain large amounts of antioxidants other than vitamin C, E and carotinoids (Javanmardi *et al*., 2003) These antioxidants play important roles in delaying, intercepting, and preventing oxidative reactions (Vilioglu *et al*., 1998). These oxidative reactions are catalysed by free radicals. the process involved is called Antioxidant activity and may be as a result of the presence of phenolic components such as flavonoids (Pietta, 1998), phenolic acids and phenolic diterpenes (Shahidi *et al*., 1992). Pathological conditions such as inflammation, metabolic disorders, cells ageing, atherosclerosis and carcinogenesis are caused by the presence of Free radicals, Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) (Roback *et al*., 1988; Ames *et al*., 1993). According to Yildrim *et al*. (2001), the ROS are involved in more than one hundred diseases including, malaria, acquired immunodeficiency syndrome, diabetes, anaemia and cardiovascular diseases. Coupled with the resistance of many synthetic antibiotics and chemotherapeutic agents (Hiroshi, 2010), the problems of liver toxicity and carcinogenicity have led to restrictions being imposed on synthetic antioxidants (Grice, 1986; Wichi, 1988). There is need therefore to research into the development and utilization of more effective antioxidants and antibacterial agents of natural origin.

Buchholzia coriacea is commonly called 'Wonderful kola' and was named after R.W. *Buchholz*, who collected the plant in Cameroun in the late 19th century (Anowi, 2012). It is a perennial plant which grows as a tree and belongs to the family *capparaceae* its English name is the musk tree (Olaiya, C. O., & Omolekan, T. O. 2013). Its local names include *'Uworo'* in Yoruba, *'Owi'* in Edo, *'Uke'* in Igbo (Quattrochi-Umberto, 2007). Wonderful kola is is a forest tree with large glossy and leathery leaves; conspicuous leaves at the end of its branches (Mbata *et. al.,* 2009). It is an evergreen shrub growing up to 20 metres tall, found in Nigeria, Cameroun, Ghana, Central African Republic, Gabon and Angola (Ezekiel *et. al.,* 2009). The seeds are either cooked or eaten raw (Lemmens, 2013). The bark of the plant *Buchholzia coriacea* is smooth, blackish- brown or dark green (Erhirhie, 2015). Wonderful kola is used locally in the treatment of fever, gastrointestinal infections. Okoliet al. (2010) reported the anti-plasmodial properties of the plant, the ground seeds were therefore routinely mixed with palm oil and taken orally as treatment for malaria (Adjanohounet al., in Chinaka et *al*., 2012). The Cameroonians use the seed as remedy to relieve chest pain (Thomaset al., in Chinaka et al., 2012). It was also reported to have analgesic effects (Ezejaet al., 2011) and anthelminthic potentials (Nweze and Asuzu, 2006). In view of the use of *Buchholzia coriacea* in folk medicine to treat various infections and degenerative diseases, this research the results of this study will confirm the antimicrobial and antioxidant properties of the plant material, thereby validating its use in folk medicine.

METHODOLOGY

Stem bark of *Buchholzia coriacea* was harvested from its tree in Umulolo, in Ihitte-Ubi, Ahiazu Mbaise Local Government Area of Imo State, Nigeria, on 15th October, 2014. The plant material was authenticated at the Forestry department of Michael Okpara University of Agriculture, Umudike, Abia State.

Hydrogen Peroxide Scavenging Activity

The scavenging activity of extract towards hydrogen peroxide radicals was determined by the modified method of Ngoda, (2013). Solution of hydrogen peroxide (40Mm) was prepared in phosphate buffer pH 7.4 and its concentration was determined by measuring the absorbance at 560nm using UV spectrophotometer. 0.1mg/ml of the extract was added to hydrogen peroxide solution and absorbance measured at 560nm using UV spectrophotometer against a blank solution containing phosphate buffer without hydrogen peroxide. The experiment was repeated in triplicate. The percentage of hydrogen peroxide scavenging by the extract and standard compound was calculated using the given formula:

$$\%\, H_2O_2\ Scavenging\ activity = 1 - \frac{Absorbance\ of\ test\ sample}{Absorbance\ of\ control} \times 100$$

Calculation of IC_{50}

Various concentrations (0.625-1mg/ml) of methanolic extracts of *B. Coriacea* were taken for the study and IC_{50} values which shows 50% inhibition was calculated using regression analysis in MS excel.

Statistical analysis

All experimental measurements were carried out in triplicate and were expressed as average of three analyses ±standard deviation. Statistical analyses were performed by one sample t-test and p-values were done by one way ANOVA. The p-value<0.05 were regarded as significant.

Antibacterial Activity

Preparation of Test Organisms

The clinical isolates of *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Salmonella typhimurium* were collected from the Microbiology Department of Federal Medical Center, Owerri and identified. The identified isolates were then sub-cultured on sterile nutrient agar .The microbial culture were diluted with peptone water until the final suspension that contained about $1.0x10^8$ cfu/ml (0.5 McFarlands Standard) according to the method of Akujiobi *et al*., (2004). The McFarlands Standard was prepared by adding 0.1ml of 1% $BaCl_2$ into 9.9ml of 1% Sulfuric acid.

Antibacterial Susceptibility Testing of the *B. coriacea* Extracts

The disc technique as described by Osadebe and Ukwueze (2004) was adopted for this study to evaluate the antibacterial activity of the extracts. 0.2ml aliquot of each of each of the extracts was asceptically dropped into agar wells (of 6 millimetres in diameter) bored on already inoculated nutrient agar plates containing the test organisms. The plates were then incubated at 37^0C for 24 hours and 27^oC for 48 hours respectively. The zones of inhibition were measured with a meter rule.

Tests for Antimicrobial Susceptibility of the Dilutions and Minimum Inhibitory Concentrations (MIC) of the Plant Extracts

For the MIC (mg/ml), three drops of overnight broth cultures of the test organisms were inoculated into the dilutions (250, 200, 100, 50, 25, 12.5, 6.25 and 3.125) in each case of the test organisms (Akujobi et al., 2004). The tubes were then incubated at 37^0C for 24 hours. The lowest concentration of each of the extracts in each case of the methanolic, n-hexane and chloroform extracts that inhibited the growth of the test organisms were recorded as the MIC.

Test for the Minimum Bactericidal Concentration of the Extracts

Tubes showing no visible growth from the MIC test were sub cultured onto sterile Nutrient agar plates and incubated at 37^0C for 24 hours and at 27^oC for 48 hours. The lowest concentration of the extracts yielding no growth was recorded as the Minimum Bactericidal Concentration.

Results and Discussion

Peroxide Scavenging Capacity

Table 1: Data of hydrogen peroxide scarvenging capacity of stem bark of *B. coriacea*

Conc. (mg/ml)	% Inhibition of Sample	% Inhibition of Ascorbic Acid
0.0625	26.7	21.7
0.125	31.3	24.0
0.25	58.6	51.0
0.5	66.0	58.3
1.0	72.6	69.0

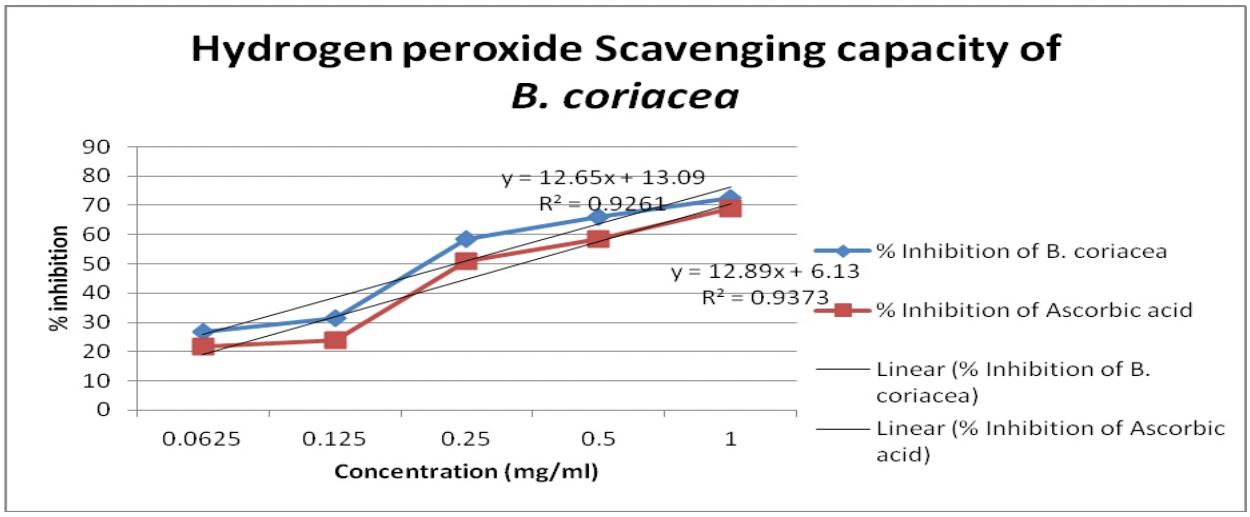

Figure 1: Line chart showing hydrogen peroxide scarvenging capacity of stem bark of *Buchholzia coriacea*

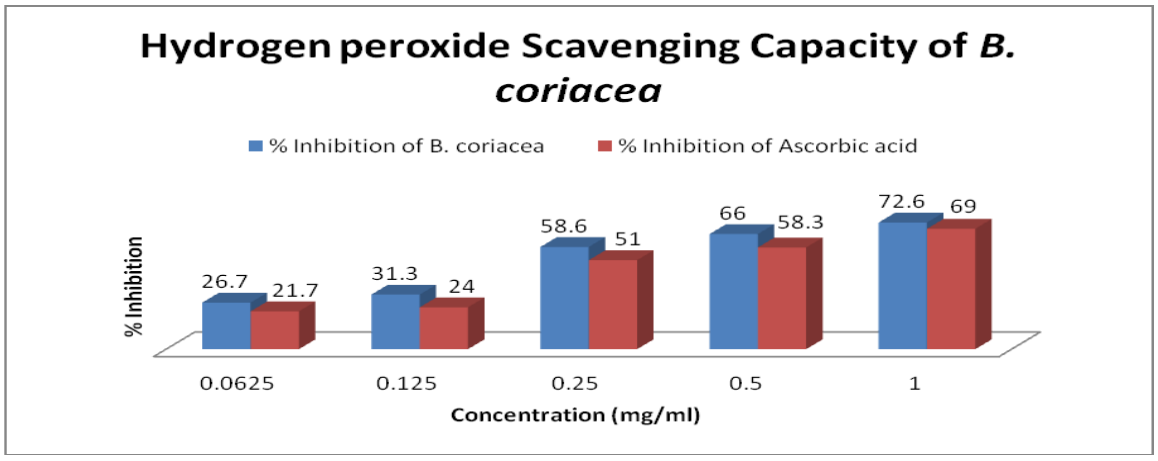

Figure 2: Bar chart showing hydrogen peroxide scarvenging capacity of stem bark of *B. coriacea*

Table 1 and figure 2 show that B. *coriacea* extract and ascorbic acid caused a strong dose-dependent inhibition of hydrogen peroxide. Figure 1 shows that the graphical equations give regressions coefficient R^2 which was found to be 0.9261 and 0.9373 for the methanolic extract and standard respectively. At a concentration of 1mg/ml, the scavenging percentages were 72.6 and 69.0 for methanol extract and standard respectively. P values were found to be significant ($p<0.05$) for both standard and extracts. The methanol extract showed good scavenging capacity compared to Ascorbic acid which was used as the standard. This was corroborated by IC_{50} values, which for the extract was found to be 0.197mg/ml compared to standard ascorbic acid 0.211mg/ml.

Antibacterial Activity

Table 2: Zone Diameter of Inhibition (ZDI) of the extracts of *Buchholzia coriacea* against the test organisms

Test organisms	Extracts			Control
	Methanol	Chloroform	n- Hexane (mm)	Chloamphenicol (mm)
Escherichia coli	19	-	10	24
Staphylococcus aureus	23	20	-	27
Streptococcus pyogenes	18	15	10	20
Pseudomonas aeruginosa	18	-	10	20
Salmonella typhimurium	20	15	12	23

Table 3: Zone Diameter of Inhibition (ZDI) of the concentrations of the extracts of *Buccholzia coriacea* against the test organisms

Test organism	Extracts	Concentration				
		100	50	25	12.5	6.25
E. coli	M	15	12	10	-	-
	C	-	-	-	-	-
	N	10	10	-	-	-
S. aureus	M	19	15	10	-	-
	C	-	-	-	-	-
	N	-	-	-	-	-
S. pyogenes	M	15	12	11	10	-
	C	10	-	-	-	-
	N	10	9	-	-	-
P. aerugino sa	M	12	10	-	-	-
	C	-	-	-	-	-
	N	-	-	-	-	-
S. typhimurium	M	15	11	-	-	-
	C	10	9	-	-	-

Table 4: Minimum Inhibitory Concentration (MIC) of the extracts of *Buchholzia coriacea*

Test organisms	Extract	Concentration							MIC (mg/ml)
		250	200	100	50	25	12.5	6.25	
E. coli	M	-	-	-	+	+	+	+	100
	C	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	N	-	-	+	+	+	+	+	200
S. aureus	M	-	-	-	-	+	+	+	50
	C	-	+	+	+	+	+	+	250
	N	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
S. pyogenes	M	-	-	+	+	+	+	+	200
	C	-	-	+	+	+	+	+	200
	N	-	+	+	+	+	+	+	250
P. aeruginosa	M	-	-	-	+	+	+	+	100
	C	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	N	+	+	+	+	+	+	+	>250
S. typimurium	M	-	-	-	+	+	+	+	100
	C	-	-	+	+	+	+	+	200
	N	+	+	+	+	+	+	+	>250

Key : N.D = Not Done; + = growth; - = no growth; > = greater than.

Table 5: Minimum Bactericidal (MBC) of the extracts of *Buchholzia coriacea*

Test organisms	Extract	Concentration 250	200	100	50	25	12.5	6.25	MIC (mg/ml)
E. coli	M		-	+	+	+	+	+	200
	C	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	N	-	+	+	+	+	+	+	250
S. aureus	M	-	-	-	+	+	+	+	100
	C	+	+	+	+	+	+	+	> 250
	N	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
S. pyogenes	M	-	-	+	+	+	+	+	200
	C	-	+	+	+	+	+	+	250
	N	-	+	+	+	+	+	+	250
P. aeruginosa	M	-	+	+	+	+	+	+	200
	C	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	N	+	+	+	+	+	+	+	>250
S. typimurium	M	-	-	-	+	+	+	+	100
	C	-	-	+	+	+	+	+	200
	N	+	+	+	+	+	+	+	>250

Key: N.D = Not Done; + = growth; - = no growth; > = greater than.

The results of the zone diameter of inhibition of the extracts of *Buchholzia coriacea* as presented in Table 2 revealed that the zone of inhibition of the methanolic extracts against the bacterial test organisms ranged from 18mm to 23mm. *Staphylococcus aureus* was the most susceptible to the methanolic extracts. The zones of inhibition of the chloroform extracts against the bacterial test organisms ranged from 15mm to 20mm, with *Escherichia coli* and *Pseudomonas aeruginosa* (both gram negative) being resistant. The *n*-hexane extracts also yielded zones of inhibition of 10mm against *E. coli, Streptococcus pyogenes* and *Pseudomonas aeruginosa;* zones of inhibition of 12mm against *Salmonella typhimurium*. The methanolic extracts yielded the highest zone diameter of inhibition against the test microorganisms in comparison with the other extracts. *Streptococcus pyogenes* and *Salmonella typhimurium* were susceptible to all the extracts of *B. coriacea* used in this study. Mbata *et al.* (2009) reported the antibacterial activity of the crude seed extracts of *B. coriacea* in some pathogenic bacteria. In their study, methanolic extracts also recorded higher antimicrobial potency when compared with the hot water extracts. Their result is in agreement with the outcome of this study. Oluseyi and Francisa (2009) also reported antimicrobial activities of *B. coriacea* against *S.aureus and E. coli.* Similar

results were also obtained in this study. Umeokoli *et al.,* (2016) reported that *n*-hexane extracts of *B. coriacea* showed antibacterial activity against *S. aureus.* The methanolic extracts also yielded better antimicrobial activities in their study. This corroborates the outcome of this study. The results of the zone diameter of inhibition of the different concentrations of the extracts (viz: 100mg/ml, 50mg/ml, 25mg/ml 12.5mg/ml and 6.25mg/ml) as presented in table 3 showed that the higher the concentrations of the extracts, the higher the antibacterial activity. However, the values of the zones of inhibition of the different concentrations were lower than those of the raw undiluted extracts. The 100mg/ml concentration yielded the highest antibacterial activity followed by the 50mg/ml, then the 25mg/ml and 12.5mg/ml. None of the 6.25 mg/ml concentration of each of the extracts showed any zone diameter of inhibition while only the 12.5mg/ml of the methanolic extract showed zone of inhibition of 10mm against *Streptococcus pyogenes.*

The Minimum Inhibitory Concentration (MIC) of the extracts of *B. coriacea* against the test organisms as stated in table 4 revealed that the Minimum Inhibitory Concentration (MIC) of the methanolic extract against the bacterial test organisms ranged from 50mg/ml to 200mg/ml against the susceptible bacteria ; that of the chloroform extracts were 200mg/ml while those of the *n*-hexane extracts ranged from 200mg/ml to >250mg/ml. The Minimum Bactericidal Concentration (MBC) of the methanolic extracts of *Buchholzia coriacea* as presented in table 5 showed that the methanolic extracts had MBC ranging from 100mg/ml to 200mg/ml; those of the chloroform extracts had MBC ranging from 200mg/ml to 250mg/ml against the susceptible bacteria while the *n*-hexane extracts had MBC ranging from 250mg/ml to >250mg/ml. These results further revealed that the methanolic extracts had higher efficacy against the test organisms. The extracts of *B. coriacea* can therefore be used in herbal remedies since their antimicrobial efficacy have been proven in this study.

CONCLUSION

The results of this work indicated that stem bark of *B. coriacea* posseses stronger antioxidant properties that ascorbic acid. Also the methanolic extract of stem bark of *B. coriacea* exhibited higher antibacterial activity than chloramphenicol. From these results it can be concluded that stem bark of *B. coriacea* can be used as a natural source of both antioxidant and antibacterial agents.

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Determination of dielectric constant, the activation parameters, and the Arrhenius parameters on the electron-transfer reaction of [N-(2-hydroxyl-ethyl) ethylenediamine-N, N', N', - triacetate cobalt (II) by copper (II) cation.

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ABSTRACT

The effects of dieletric constant and temperature in the Kinetics of the oxidation- reduction reactions (involing electron transfer) of N-(2- hydroxyl-ethyl) ethylenediamine- N,N', N' -triacetatocobalt (II) by Cu^{2+} cation were determined. The effect of diaelectric constant (D) on the rate of reactions between the ($CoHEDTAH_2O$) and Cu^{2+} ions was investigated at constant concentration of oxidant and reductant. While the temperature, acidity and ionic strength of the medium were kept constant, the dielectric constant of the medium was varied from 63.25 to 43.78, using acetone and water mixture. It was found that the rates of reaction did not show any appreciable change. The temprature dependence of rates on this reaction was investigated at 35°C, 40°C, 50°C, 55°C, and 60°C respectively. Hence, the activation parameters and the Arrhenius parameters were calculated.

INTRODUCTION

A vitamin known as coenzymes B_{12} is the only organometallic compound in nature containing metal-carbon $_{\sigma}$-bond (Crosanoe et al, 2002)[1,2]. It incorporates cobalt into a corrin ring- structure.

Vitamin B_{12} is an important Co complex. The vitamin was isolated from liver after it was found that eating large quantities of raw liver was an effective treatment for pernicious anemia. Injection of vitamin B_{12} are now used for treatment (more pleasant than eating raw liver).

Vitamin B_{12} is a coenzyme, and serves as a prosthetic group which is tightly bound to several enzymes in the body. The precise role of vitamin B_{12} is not fully understood (Lee, 1996)[3] hence this study.

Dorothy Crowfoot Hodgkin was awarded the Nobel Prize for Chemistry in 1964 for X-ray crystallographic work including solving the structure of this enzymes[3].

Vitamin B_{12} is required in humans for several transformations, such as the AdoCbl- dependent conversion of (R) – methylmalonyl co- enzyme A(CoA) into succinyl CoA[4].

And the MeCbl –dependent conversion of (S) homocysteine into methionine[4]:

$HSCH_2CH_2CH(NH_3^+)COO^-$ ---> $MeSCH_2CH_2CH(NH_3^+)COO^-$

There are a number of related reactions in which a substrate >CH-CX < is rearranged to >CX – CH<. The mechanistic details are obscured (Golding, 1990)[4].

Cobalt is biologically important in some enzymes, glutamic mutase is involved in the metabolism of amino acids and ribonucleotide reductase in the biosynthesis of DNA[3].

Traces of cobalt (1 – 1.5ppm) are added to beer to make it froth better. This has been linked with an increased rate of heart failure among heavy beer drinkers who have a dietary deficiency of protein or thiamine (Nicholls, 1973; Phipps, 1976)[5,6].

EXPERIMENT

All reagents used were of analar grade. The stock solutions of [$CoHEDTAOH_2$] were prepared

according to the method of Mansour (2003)[7-9], Copper (II) tetraoxosulphate (VI) was prepared by dissolving accurate weighed amount of the salt in a known volume of distilled water. The $_{max}$(510nm) was determined by running the electronic spectrum of the solution of [$CoHEDTAOH_2$] in the wavelength range of 340 -700nm, and plotting a graph of the absorbance against wavelength.

A stock solution of perchloric acid was made by diluting analar grade acid (70%, specific gravity 1.67) and standardizing titrimetrically. Analar grade sodium perchlorate ($NaClO_4$) was used to maintain the ionic strength.

KINETICS

The wavelength of maximum absorption, $_{max}$ of [$CoHEDTAOH_2$] was 510nm using spectrum lab 330 – 1000 spectronic 23_A spectrophotometer. The rate of the reaction of [$CoHEDTAOH_2$] with Cu^{2+} ion was studied at this $_{max}$ by observing the change in absorbance of [$CoHEDTAOH_2$] at 28°C and 0.05moldm^{-3} ($NaClO_4$) ionic strength.

The plots of log (A_t - A∞) versus time were made. From the gradient, the pseudo – first order rate constants k, were determined as given by the equation.

$$\text{Log}(A_t - A\infty) = \frac{K_1 t}{2.303} + \log(A_O - A\infty) \qquad \text{(I).}$$

Where A∞, A_t are the absorbances of the reaction mixture at time infinity, and t, respectively. The second order rate constants (k_2) were obtained from k, as $k_1/[Cu^{2+}]$.

RESULTS/ DISCUSSION

STOICHIOMETRY

The stoichiometry of the [$CoHEDTAOH_2$] with Cu^{2+} reaction was determined by spectrophotometric titration using the mole ratio method. The concentration of the $[CoHEDTAOH_2]^-$ was kept constant at 1x 10^{-4}moldm^{-3}, while that of Cu^{2+} was varied from 1.5 x 10^{-5} - 1x10^{-4}moldm^{-3} at ionic strength, 1= 0.05moldm^{-3} ($NaClO_4$) and [H^+] = 5x 10^{-3}moldm^{-3}.

The reactions were allowed to go to completion and the absorbances of the solutions were taken at 510nm. The stoichiometry was determined from the plot of absorbance versus mole ratio [Cu^{2+}] / [$CoHEDTAOH_2$][10-13].

On the basis of the stoichiometry, final absorbances at completion of reaction were plotted against mole ratio. The result indicated that one mole of [$CoHEDTAOH_2$] reacted with one mole of [Cu^{2+}]. The stoichiometric equation for the reaction is presented as equation 2.

$$[CoHEDTAOH_2] + Cu^{II} \text{ ----> } [CoHEDTAOH_2]^- + Cu^{I} \qquad (2)$$

THE EFFECT OF DIELECTRIC CONSTANT[14-17]

The effect of dielectric constant (D) on rate of reactions between the ($Co^{II}HEDTAH_2O$) and Cu^{II} ions was investigated at constant concentration of oxidant and reductant.

While the temperature, acidity and ionic strength of the medium was kept constant, the dielectric constant of the medium was varied using acetone and water mixture.

The oxidation of $(Co^{II}HEDTAH_2O)^-$ by Cu^{II} at $[H^+] = 5.0 \times 10^{-3}$ moldm^{-3}, I = 0.05 moldm^{-3}, ($NaClO_4$) and T = 29.0 ± 1 °C showed independence of rates on the dielectric constant D[13].

Decreasing the dielectric constant from 63.25, 61.09, 58.93, 56.76, 54.60, 52.44, and 43.78 (CH_3COCH_3/H_2O) as shown in Table 1 below, did not change k_{obs}.

TABLE 1: Dependence of rate on dielectric constant (D) for oxidation of $[Co^{II}HEDTAH_2O]$ by Cu^{2+} at $[Cu^{2+}] = 2.0 \times 10^{-3}$ mol dm^{-3}, $[Co^{II}HEDTAH_2O] = 1.0 \times 10^{-4}$ mol dm^{-3}, $[H^+] = 5.0 \times 10^{-3}$ mol dm^{-3}, I = 0.05 mol dm^{-3} ($NaClO_4$), λ = 510nm, T= 29 ± 1^{0}C

D	63.25	61.09	58.93	56.76	54.60	52.44	43.78
$10^3 k_{obs}$ (S^{-1})	2.44	2.63	2.63	2.07	1.92	1.91	2.99

From the table above, it is noticed that the rate of reaction is fluctuating as the dielectric constant is decreased from 63.25-43.78.

TEMPERATURE DEPENDENCE OF RATES OF REACTIONS

The dependence of temperature on the rates of the reactions for the oxidation of $[Co^{II}HEDTAH_2O]^-$ by Cu^{2+} was investigated at 35 °C, 40 °C, 50 °C, 55 °C and 60 °C respectively. The rate constants determined are reported in Table 2.

From Eyring equation and thermodynamics[18],

$$\text{Log } k_{obs}/T = \log k/h + \triangle S^{\#}/2.303R - \triangle H^{\#}/2.303RT^2 \quad (3)$$

Where k_{obs} = Temperature dependent rate constant.

k= Boltzmann's constant

h= Planks constant

$\triangle S^{\#}$ = Entropy of activation

$\triangle H^{\#}$ = Enthalpy of activation

R = universal gas constant

T = Temperature

From the plots of log k_{obs}/T versus $1/T^2$, the activation parameters were determined from the slopes and intercepts as in figure 1.

Table 2: Tempera ture dependence of rate constants for the oxidation - reduction reaction of [Co ^{II}HEDTAH$_2$O] and Cu $^{2+}$, at [Co ^{II}HEDTAH$_2$O] = 1.0 x 10 $^{-4}$ mol dm $^{-3}$, [Cu^{2+}] = 2.0 x 10^{-3} mol dm $^{-3}$, [H$^+$] = 5.0 x 10^{-3} mol dm^{-3}, I= 0.05 mol dm^{-3}, ($NaClO_4$), λ_{max} = 510nm.

Temp. (K)	10^2 k_{obs}(S^{-1})	$Logk_{obs}/T$	10^5 $1/T^2$
308	1.7		1.05
313	1.9	-4.22	1.02
323	2.1	-4.19	0.96
328	3.2	-4.01	0.93
333	3.5	-3.98	0.90

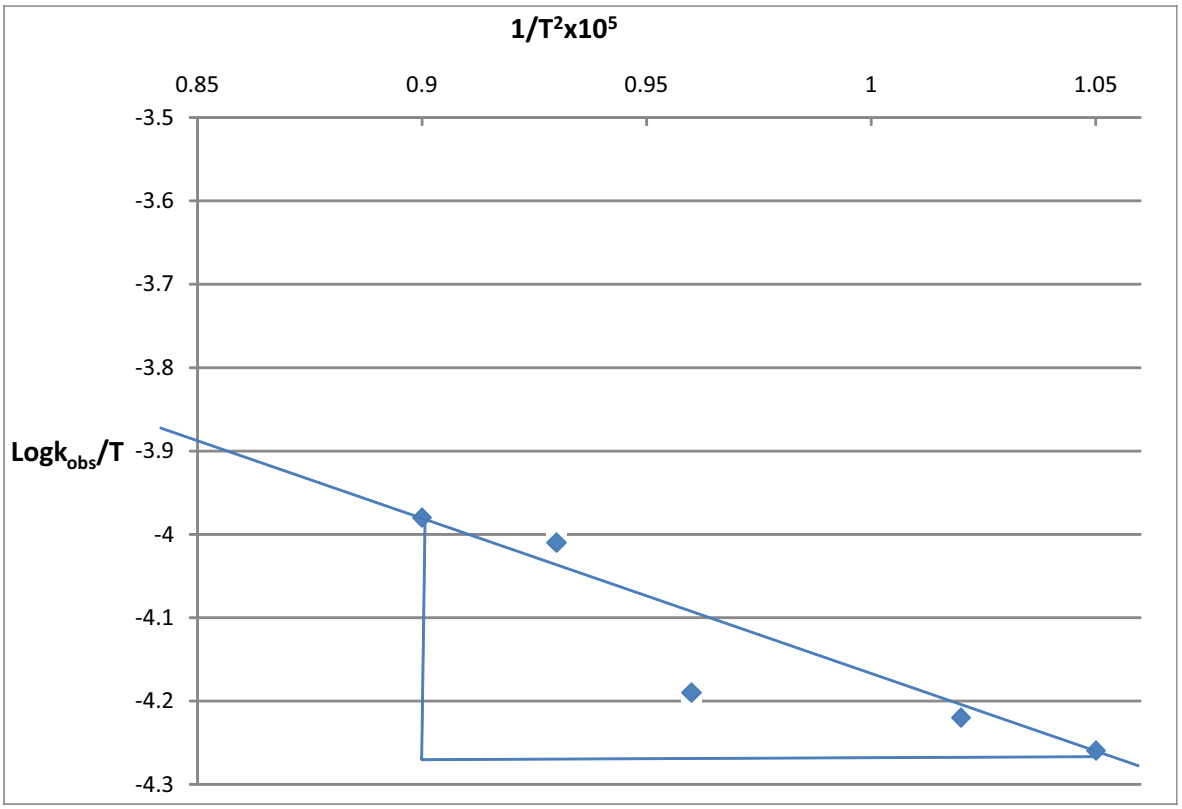

Fig 1. A graph of logk $_{obs}$ / T versus $10^5 1/T^2$

Table 3: Activation parameter for t he oxidation - reduction reaction of [Co^{II}HEDTAH$_2$O] and Cu^{2+}, at [Co ^{II}HEDTAH$_2$O] = 1.0 x 10 $^{-4}$ mol dm^{-3}, [Cu^{2+}] = 2.0 x 10^{-3} mol dm $^{-3}$, [H$^+$] = 5.0 x 10^{-3} mol dm^{-3}, I= 0.05 mol dm^{-3}, ($NaClO_4$), λ_{max} = 510nm.

Oxidant	$\triangle H^{\#}$ (kJmol^{-1})	$\triangle S^{\#}$ (JK^{-1}mol^{-1})	$\triangle G^{\#}$(kJmol^{-1})
Cu^{2+}	-3622.52	-272.63	-3713.31

For the determination of Ea (activation energy and the frequency factor A) Arrhenius equation $K = Ae^{-Ea/RT}$ is used. The constant A is called the frequency factor or pre-exponential factor; Ea is the activation energy. Collectively, the two quantities are called Arrhenius parameters[18,19]. Converting the above equation to logarithmic form, gives

$$Log_{10}K = Log_{10}A - Ea^{\#}/2.303RT \quad (4)$$

It is apparent that by determining the values of K at several temperatures, the plot of $log_{10}k$ versus 1/T will yield the activation energy from the slope of the curve and the frequency factor from the intercept. Although the frequency factor may depend slightly on temperature, unless the temperature range is very great. The determination of the activation energy is an important objective of any kinetic investigation.

Table 4: Table for graph of Logk $_{obs}$ versus 1/T(k^{-1}).

Temp.	k_{obs}	Log k_{obs}	10^3 $1/T^{(k-1)}$
308	0.017	-1.77	3.25
313	0.019	-1.72	3.19
323	0.021	-1.68	3.10
328	0.032	-1.49	3.04
333	0.035	-1.46	3.00

From the Table 4, it is seen that the rate of reaction increases as the temperature of the reaction increases.

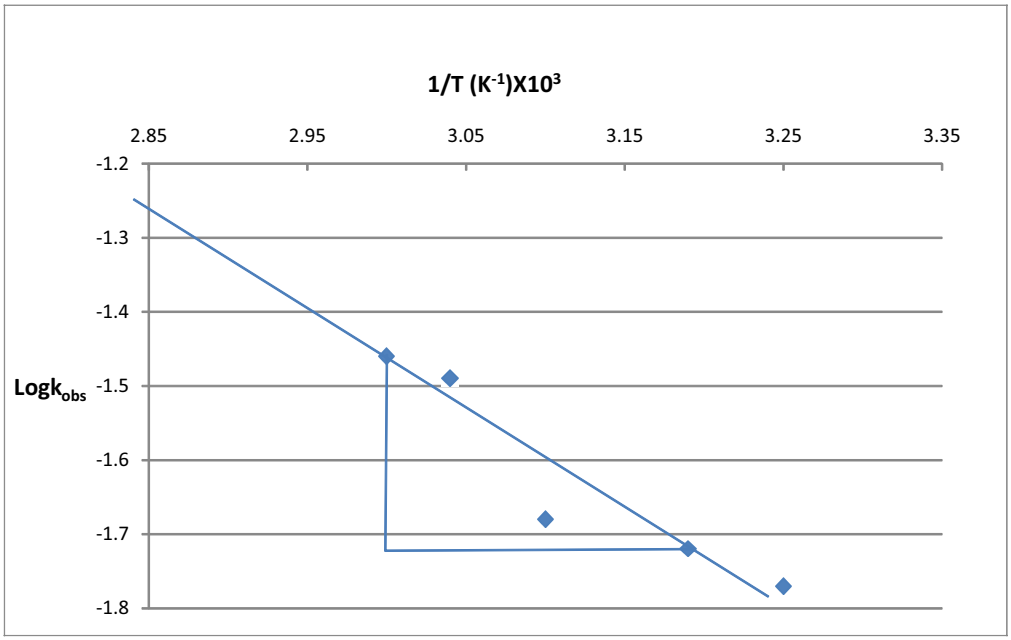

Fig 2. A graph of k_{obs} versus 1/T

From the graph, the intercept is -1.26 = 0.055 = A.

Hence, the Arrhenius parameters are as shown in table 5.

Table 5: The Arrhenius parameters for the reactions of [Co ^{II}HEDTAH$_2$O] and Cu^{2+}.

Oxidant	$Ea^{\#}$(kJ mol^{-1})	A
Cu^{2+}	-23.188	0.055

CONCLUSION

We have been able to establish the effects of dielectric constant and have calculated both the activation parameters and Arrhenius parameters in the reaction in question. Our next step is to establish mechanism of this reaction and hence compare it with some of the already known mechanisms of enzymatic reactions that are going on in some biological processes.

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Quantitative Analysis Of Two Different Species Of Cocos Nucifera Fruits.

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Abstract

A quantitative analysis of two species of coconut fruit aimed at determining the levels of alkaloid, saponin, tannin, flavoroid, iodine, iron, phosphorus, zinc, calcium, fat, protein, vitamin A and C were carried out using standard methods. The results show that the alkaloid content were 0.560% and 1.140%, saponins (0.200% and 2.800), tannins (0.450% and 3.000%), flavonoids (0.026% and 0.006%), iodine (7.400mgI/g and 6.300mgI/g), iron (3.473ppm and 4.996ppm) and calcium (5.663ppm and 7.169ppm). Other results include vitamin A (61.548 mg/kg) and 147.405 mg/kg), vitamin C (5.243 mg/kg and 7.845 mg/kg), fat (16.000% and 9.000%) and protein (4.800% and 1.120%). The study shows that the samples contain phytonutrients in varying proportions and therefore highly recommended for human consumption.

Introduction

Cocos nucifera, the coconut palm, is a monocot perennial member of the Aracaceae (palm family) cultivated in tropical areas worldwide for its fruit and fibre[1]. It has been spread widely by man but also by natural means[2]. The plant is originally from South East Asia and the Island between the Indian and pacific oceans. From that region, the fruit of coconut palm is believed to have been brought to India, East African, West Africa and to other tropical regions of the globe[3].

The coconut fruit is almost spherical to oval in shape and measures between 7 – 12 inches in width. Its rough outer husk which is the exocarp is light green but turns gray when it becomes dry. The exocarp is about 1 – 2 inches in thickness and made of tough fibres. Underneath the husk, there is woody shell enclosing the inner edible meat[4].

The main fruit comprises an outer epicarp, a mesocarp and an inner endocarp. The epicarp is the outer skin of the fruit while the mesocarp is the heavy fibrous layer, usually tanned when dry and has many industrial uses. The endocarp is the hard dark core, inside is a solid white albumen of varied thickness (depending on the age of the fruit) with an oily pulp consistency and a luquid albumen called coconut water that is thick, sweet and slightly acidic[5].

There are two main varieties of coconut tree. Tall coconut (Typica) and dwarf coconut (Nana). The Tall coconut trees which is sometimes called 'the six year coconut tree' grows tall and naturally cross pollinate. They may grow more than 1-5 feet 50 centimeters annually and grow between 65-70 feet high. Tall coconut flower and produce their first fruit at six to ten years & and their economic life is between 60-70 years. They bear fruits throughout the year at an average of 40 fruits (nuts) per year. They are slower in growth, less common, has longer leaf at the bottom and tapers slightly along the leaf up the tip[6].

Dwarf coconuts usually grow 26-32 feet (8 – 10 meters) high. They self-pollinate and start flowering at three years thus the name “the three year coconut tree” and they bear fruit seasonally with an average of about 80-100 nuts per year. They have economic life of 30-40 years and are more common than the typical variety. The leaves of this dwarf type are more uniform in shape, being as slender at the base of the leaf up to about four to five inches from the tapering tip[6].

There is another variety of coconut known as hybrid coconut.this can be formed naturally or bred internationally to produce nut size of tall coconut and volume output of dwarf coconuts. A hybrid variety combines the sought after characteristics of abundant fruit yield and fast growing nature[6].

Coconut fruits are highly nutritious and rich in fibre, vitamins C, E, B1, B3, B5 and B6; and also minerals like iron, selenium, sodium, calcium, magnesium and phosphorus[7]

Experimental

Sample Collection & Preparation

Two varieties of coconut were purchased at Oko in Aguata Local Government Area of Anambra State. The samples were broken and the epicarp and mesocarp removed, washed and ground with a mortar separately for analysis.

Quantitative Analysis

The phytochemical parameters – alkaloids, saponins, tannins and flavonoids were analyzed using standard methods as enumerated in Harbone, 1998; the iodine, phosphate and heavy metals were determined using APHA, 1998 while vitamins A and C, proteins and fat were from Kirk and Sawyer, 1991.

Results and Discussion

Table 1: Phytochemical composition of tall and dwarf varieties of cocos nucifera.

Parameters	Samples A (%)	Sample B (%)
Alkaloid	0.560	1.140
Tannin	0.450	3.000
Saponin	0.200	2.800
Flavonoid	0.026	0.006

Sample A = Dwarf variety

Sample B = Tall variety

The alkaloid content of both species were very low (0.560% and 1.140%), however the greater amount of the tall variety shows more antimalarial, antiasthma and anticancer properties.

The tannin content of the tall specie of cocos nucifera (3.00%) was found to be higher than that of the dwarf species (0.450%). This indicates that it is less likely to be attacked by pests because tannins plays a role in plant protection from predation and also in its growth regulation.

The higher percentage of sample B in terms of the saponin level makes it easily digestible than sample A because saponins enhances nutrient absorption[11].

The low flavonoid content of the two varieties (0.026% and 0.006%) is an indication that they are not rich in flavonoid content.

Table 2: Mineral composition of the tall and dwarf varieties of coconut in ppm.

Parameters	Sample A (ppm)	Sample B (ppm)
Iodine	7.400	6.300
Calcium	5.663	7.169
Iron	3.473	4.996
Zinc	0.971	0.764
Phosphorus	1.150	1.260

The mineral composition of the two varieties of coconut reveals that it is a rich source of iodine with 7.400ppm for the dwarf specie and 6.300ppm for the tall variety. The dwarf specie having the higher value of iodine is highly recommended for pregnant woman and infants as it aids in proper bone and brain development.

The calcium, iron and phosphorus values for the tall variety (7.169ppm, 4.996ppm and 1.260ppm) were higher than that of the dwarf variety (5.663pmm, 3.473ppm and 1.150ppm) except for zinc; the dwarf variety has a zinc value of 0.971ppm while the tall variety has 0.764ppm. These metals, though varying concentration, are very useful for human growth and development.

Table 3: Proximate composition of the two varieties of cocos nucifera.

Parameter	Sample A	Sample B
Vitamin A (mg/kg)	61.548	147.4 05
Vitamin C (mg/kg)	5.243	7.845
Protein (%)	4.800	1.120
Fat (%)	16.000	9.000

The vitamin A content of Sample A (61.548mg/kg) was lower than that of Sample B (147.405mg/kg) thereby making the tall specie a very rich source of this vitamin. The same was applicable to vitamin C, the value for the tall specie was higher than that of the dwarf specie. These vitamins are necessary for good vision, growth, development and repair of body tissue.

The values of the protein and fat contents for sample A were higher than that of sample B making sample A a rich source of both protein and fat.

Conclusion

In this study, both species of cocos nucifera have comparable values in the parameters analyzed with the tall variety having much higher. Significantly, the varieties contain quality phytonutrients useful to humans.

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Comparative study of Proximate and phytochemical analysis of coconut (cocosnucifera) and turmeric (curcuma longa)

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In this study, the proximate and phytochemical compositions of extracts from two common Nigerian plants coconut and turmeric were investigated. The samples were obtained from a farm in Anambra State. The proximate and phytochemical analyses were performed using standard methods in both plants. The results of proximate mean composition of coconut and turmeric (in percentages) for moisture, fat, and crude fiber contents (%) were (44±0.12 and 22.63 ±0.9); (3.5±1.5 and7.3 ±0.3)and (20±0.43 and 43.7±1.7 respectively and percentage protein, ash and carbohydrate (15.3± 0.4 and 1.8±0.01); (10±0.03 and 16.8±1.03) and (6.87 and 7.8) respectively. The phytochemical estimation showed the presence of alkaloid, tannin, flavonoid, phenol, glycoside in both plant. The results showed high level of fat (tocopherols), ash (mineral content) and flavonoid (19.50 ±0.02 and 37.5±0.6) in tumerric. The high content of bioflavonoid in these plants proved their efficacy as food providing anti-inflammatory and antioxidant therapies. Theses can also be used as novel antibiotics when properly processed and formulated, and hence recommended as food supplement for prevention of many dangerous sicknesses like cancer and inflammation of the system.

Keywords: Phytochemical; Proximate; Analysis and Antioxidant.

Introduction

Phytochemical is a plantcomprising a wide variety of compounds that occur naturally in plants that have biological activity in humans like bioactive phytochemicals. **(**Yancui and Indika, 2016.) .According to Elke, Arendt, Emanuele and Zannini,(2013) Phytochemicals are non-nutritive components present in a plant-based diet ('phyto' is from the Greek word meaning plant) that exert protective or disease-preventing effects.Amadi, Agomuo andIbegbulem (2004) They have been associated with protection from and/or treatment of chronic diseases such as heart disease, cancer, hypertension, diabetes and other medical conditions (Surh, 2003). Examples areessential oils, phenolic compounds, flavonoids, carbohydrates, proteins, alkaloids, glycosides saponins, steroids, triterpens, sesquiterpenoides and tannin (Kensa, 2011)

Proximate analysis

Proximate analysis involve an assay for all constituents of a sample apart from the major food constituents; even including antinutrients, mineral, vita**mins** (Amadi, Agomuo and Ibegbulem,2004). Examples are moisture, fats/lipids, protein, ash (mineral content) crude fiber and carbohydrate.

Moisture is the material lost by foodstuff on heating not much higher than the temperature of boiling water; or by allowing to stand over a dehydrating agent, or by heating in a vaccum. It is generally considered to be water but actually is the total volatile matter lost or driven off at this temperature, until successive weighing show no further loss. (Morris, 1999)

Protein is calculated from nitrogen content. Proteins are made by linking in individual aminoacidstogetherin long chains. Amino acids are made up principal of carbon, hydrogen,oxygenand nitrogen. Protein is essential to all life. In animals, they help form supporting and protective structures such as cartilage, skin,nails,hair, andmuscle. They are major constituents of

enzymes, antibodies, many hormones, and body fluids such as blood, eggwhite and milk.(Norman, 1996)

Carbohydrates (from hydrates of carbon) are organic compounds with the basic structure $C_x(H_2O)_Y$. carbohydrates play a major role in biological systems and in foods. They are produced by photosynthesis in green plant and are nature's way of storing energy from sunlight.(Norman,1996).

Fats and Oil

Fats and oils are made up of fatty acids esters of glycerol. Edible fats and oil come from both plants and animal sources. And have important functional properties in foods.The extraction is done by solvent extraction method. The oil is removed from the powdered ground sample with a non-toxic fat solvent such as hexane. The solvent is percolated through the seed and after, the oil is extracted. The solvent distilled from the oil is recovered for reuse. The yield of the oil is high than in the normal pressing method.

Turmeric extract and its medicinal value

Turmeric extract contain a number of nutrients and anti-nutrients.It is a wonder plant with antiseptic and anti-inflammatory properties.These health benefits is due to the many phytochemicals, vitamins, minerals and nutrients it contains, but it is perhaps most famous for its high concentration of **curcumin,** which is an extremely potent antioxidant that is connected to a number of different health effects. (Judy,2003). The yellow flavonoids are structurally related to anthocyanins and comprise a large group of chemicals which are widely found in plant foods. They are p^H sensitive and deeper yellow in alkaline media.

Curcumin can eliminate inflammation in tissues by neutralizing free radicals and preventing oxidative stress from occurring.

Antioxidant Activity

Turmeric active ingredient, curcumin chemical makes up 2-6% of the spice, and can counteract the activity of free radicals within the body.

Coconut oil (*Cocos nucifera*)

A fruit with a hard stony covering enclosing the seed and comes from the word *drupa*meaning overripe olive. A coconut, and all drupes, have three layers: the exocarp (outer layer), the mesocarp (fleshy middle layer), and the endocarp (hard, woody layer that surrounds the seed). Coconut oil comes from the nut (fruit) of the coconut palm. The oil of the nut is used to make medicine. Some coconut oil products are referred to as "virgin" coconut oil. Unlike olive oil, there is no industry standard for the meaning of "virgin" coconut oil. (Davidson, 2001). It contains nutritive and anti-nutritive components in the extracts.

How does Coconut extract work?

Coconut extract is high in a saturated fat called medium chain triglycerides. When applied to the skin, coconut oil has a moisturizing effect. Coconut oil is sometimes applied to the skin as a moisturizer, for neonatal health, and to treat eczema and a skin condition called psoriasis. Coconut oil is also used in hair products to prevent hair damage. Research suggests that applying virgin coconut oil to the skin twice daily for 8 weeks improves symptoms about 30% more than mineral oil in children with eczema (Duke, 2001)

Malnutrition in children,pregnant and nursing mothers are high due to lack of essential nutrients from coconut and turmeric. This has resulted in a number of mortality rates in them. Also there is serious problem because of lack of major mineral for metabolism of glucose, production of cellular energy to create protein. Hence, high levelrheumatism, muscle pains, arthritis and cardiovascular disease in the country.

Poor processing as regards quality assurance and control in packaging of food, ignorance on storage facilities and procedures, poor balancing of food supplement in the country and difficulty in the extraction of the essential oil from plants are research problems that needs to be addressed.

In this research, we seek to obtain ethanolic extractoftwo abundant Nigerian plants *Cocos nucifera*and*curcuma longa*and *assess their* qualitative and quantitative phytochemicals constituents and estimate their nutritive compositions to improve health and the quality of life.

Materials and Methods

The work was carried out at J3F Research Laboratory Oko. The equipment used areDistillation apparatus, water bath , soxhlet extractor apparatus, analytical weighing balance, crucibles and lids, desiccators, Khijhdal apparatus, thermostat oven, muffle furnace.

Reagents used

Analytical n-hexane, glacial acetic acid, starch solution, bromine water,diethyl ether, carbon tetrachloride, ethanol, phenolphthalein indicator, CONC. HCL, CONC H_2SO_4, Boric acid, sodium hydroxide, water was used.

Collection and Preparation of Plant Materials

The fresh*Cocos nucifera, a*nd *curcuma longa*samples were obtained from a farm in Anambra State. The proximate and phytochemical analyses were performed using standard methods in both plants.

Extraction of Plant Material

The plant materials powdered*Cocos nucifera, a*nd *curcuma longa*were extracted using soxhlet extract and ethanol solvent.

Experimental AnalysisCarried Out

Proximate andphytochemical analysis

Table: Result of proximate analysis

Parameters*(%)Cocos nucifera (%)*	*curcuma longa(%)*
Moisture 44.0	22.63
Fat 3.5	7.3
Crude Fiber 2.0	43
Protein 15.3	1.8
Crude Ash 10.0	16.8
Carbohydrate 6.87	7.8

Table: 2 Result of phytochemical analysis

Phytochemicals *Cocos nucifera (%)*	*curcuma longa(%)*
Alkaloids 1.8	12.8
Tannins 11	14.0
Flavonoids 23	17.5
Saponins 9.9	11.96
Steroid 10.8	13.2
Phenol 6.4	16.5
Glycosides 60	26

Discussion

The analysis in Table 1.and 2 showed results of the primary and secondary metabolites. it was observed from Table 1, that results of proximate mean compositions of coconut and tumeric contain highest composition of moisture (44±0.12 and 22.63 ±0.9);. The phytochemical estimation showed the presence of alkaloid, tannin, flavonid, phenol, glycoside in both plant. The results showed high level of fat (tocopherols), ash (mineral content) and flavonoid (19.50 ±0.02 and 37.5±0.6) in tumerric.

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Oxalate-Calcium Ratios of Common Cereals Consumed in Eastern Nigeria

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Abstract

The importance of oxalate content of an individual plant in limiting total dietary calcium availability is of significance when the ratio oxalic to calcium (oxa:cal.) is greater than one. Food with oxal: cal ratio greater than two, have no utilizable calcium and contain excess oxalate while foodstuff with oxal:cal. of about one provide little calcium but do not inhibit the utilization of calcium provided by other products. The study analysed three commonly consumed cereals of oxalic acid and calcium by rigorous classical quantitative method of precipitation/titration and atomic absorption spectrophotometry respectively. The result should oxal:cal. ratios > 1 for all the cereals indicating that the cereals have no utilizable calcium, thus contain excess oxalate which not only complex the calcium contained in the food but also that derived from other food sources eaten together. This can result in deficiency of such an essential mineral as calcium in the body and even render the body prone to bone disease and possibility of developing kidney stones due calcium oxalate formation (calcium oxalate being the most common component of kidney stone).

Keywords:

Introduction

Oxalic acid (ethanedioic acid) shown in fig 1 is an organic acid that is found in many higher plants, including a large variety of commonly consumed food plants. It is a decarboxylic acid and due to the proximity of these two joined carboxyl group is one of the strongest organic acid. The anions of oxalic acid as well as its salts and esters are known as oxalates [1], this oxalates are salts or esters of oxalic acid. They are crystallines solids that are only slightly soluble in water, strongly acidic and poisonous. The importance of oxalic acid in limiting the utilization of dietary calcium was first realised in 1918 when mccluage and mendel showed that dogs retained less calcium from spinach than carrot [2], since from these observation, many studies have been made with laboratory animals and human subjects showing that dietary calcium is poorly utilized from oxalic rich food [3]. Also oxalates binds calcium present in food thereby rendering calcium unavailable for normal physiological and biochemical roles such as maintenance of strong bones and teeth, cofactor in enzymatic reactions, nerve impulse transmission and as a cloting factor in the blood [4]. The calcium oxalate which is insoluble may also precipitate around soft tissue such as kidney, causing kidney stone which are associated with blockage of renal tubules [5].

Corn or maize is one of the most popular cereals in the world. It forms the staple food of numerous people in different countries. Corn is rich in Phosphorus, magnesium, manganese, zinc, copper, iron and selenium. It also has small amounts of potassium. It has traces of vitamin A and E but more of vitamin B, B_6, niacin, riboflavin and foliate [6].

There are a number of health benefits of corn, apart from the fact that it provides necessary calories for daily metabolism of the body and maintaining low cholesterol and prevention of neutral-tube defects at birth, its high fibre content ensures that it plays a role of prevention of digestive ailments like constipation and haemorrhoids as well as colorectal cancer. The antioxidants present in corn also act as anti-cancers and prevent Alzheimer's disease [7].

Rice is a good source of phosphorus and iron, but most of the nutrient in rice are concentrated in the outer brown layers known as husk and germ. Rice also contain vitamin B is small quantities [8]. There is no other food item that provides energy to the world as provided by rice. It is not wrong to say that most of the people in the world are able to do their daily activities due to rice. Rice is about 345 calories per 100g, it is very easy to digest and hence most of these calories are absorbed by the body. Health benefits of rice include, providing fast and instant energy, good bowel movement, stabilizing blood sugar and providing essential source of vitamin B1 to human body [8].

Calcium on the other hand is an essential mineral required daily in quantity ranging from 210mg for infants to 1300mg in growing children and adolescent 1350mg [9]. Calcium is absorbed primarily in the upper part of the small intestines as it requires a pH below 6 to stay in solution [10]. The likelihood of oxalate to bind minerals as calcium and form insoluble salt is dependent upon its solubility constant (Ksp). Ksp is the product of the concentration of ions of substances in a saturated solution of the substrate [10[. For a given substance, the smaller the Ksp values the less soluble the substance will be (at a given temperature and pH). Calcium has a low solubility constant (Ksp) of 2.7×10^{-9} and is therefore likely to bind the divalent oxalate ion ($C_2O_4^{2}$).

To achieve and maintain a healthy oxalate calcium ratio, it is important to get sufficient calcium in our diet while also avoiding excessive oxalate.

Oxalate education is important not only for those looking up to promote kidney health and nutrition, but also for any hoping to become a more informed, health conscious consumer [11].

Poor kidney function is associated with diabetes, hypertension and heart disease. Good kidney function is essential for maintaining homeostasis in our body, including our pH level and electrolyte balance: our kidney also produce hormones that make red blood cells and regulate blood pressure [12]. Excess oxalate show symptoms as painful or inflamed joints, similar to arthritis, burning urine flow, intestinal cystitis aka burning bladder often associated with hyperoxalurea, burning bowel movement, vulvodymia – external female genital pain or irritation, kidney stones i.e. oxalates combines with calcium, chelating of toxic metals like mercury [13].

Scientist continues to research the connection between calcium and oxalate in food and a recent research [14], has shown that a low calcium, high oxalate food may place an individual at risk for calcium oxalate kidney stones [12].

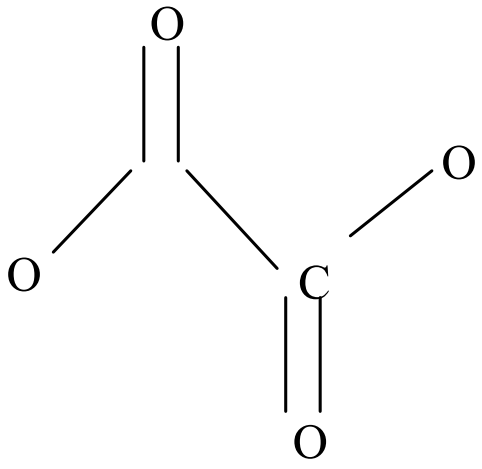


Figure 1: Oxalic acid

Experimental

The cereals were bought from a main market, Ose Market in Onitsha Anambra State, where most of the cereals from different part of Nigeria are brought for sale. They were identified at the School of Agriculture Federal College of Education (Technical) Asaba. The samples were crushed raw and from the crushed sample, a portion was taken for analysis to spring board research lab. Awka Anambra State Nigeria.

Determination of Oxalic acid

The total content of oxalate in the cereals samples was determined according to the precipitating method [15]. The extraction was done by boiling 2g of each of the samples in 40ml of water for 30minutes in a reflux condenser. 10ml of 20% $NaCO_3$ was added to each of the samples and boiled for another 30minutes. The liquid extract was filtered and washed with hot water till wash-water showed no alkaline reaction. The combined water-washed and filtrate was concentrated to a small volume and cooled.

HCl (1:1) was added drop-wise with constant stirring until the final acid concentration after neutralization was about 1% at which stage a heavy precipitate appeared, which was allowed to flocculate. Extract was carefully filtered into 250ml flask, made to mark and kept overnight. Supernatant liquid was filtered through a dry filter paper in a dry beaker. An aliquot of the filtrate in 400ml beaker was diluted with water to 200ml and reacidified with acetic acid. 10ml of a 10% calcium chloride ($CaCl_2$) solution was added to the medium and stirred very well to induce calcium oxalate precipitate to appear and left to settle overnight. The clear supernatant liquid was carefully decanted off through whatman No. 42 filter paper.

The precipitate was dissolved in HCl (1:1) solution, was made basic by adjusting the pH with ammonium hydroxide solution. The content was boiled and allowed to settle overnight. Oxalic acid was determined by titrating against 0.05N $KMnO_4$ solution.

1ml of 0.05N $KMnO_4$ = 0.00225 anhydrous oxalic acid.

$$\text{Oxalic acid (\%)} = \text{Titre} \frac{\text{Value} \times 0.00225}{2\,(\text{wt. of sample})}$$

Sample digestion for AAS analysis

2.0g of the crushed, samples (10) were weighed into a digestion flask and 200ml of acid mixture (650ml conc. HNO_3; 50ml per chloric acid; 20ml conc. H_2SO_4) added, the flask was heated until a clear digest was obtained. The digest was diluted with distilled water to the 50ml mark.

Determination of calcium content of the samples using Atomic Absorption Spectrophotometer (AAS)

The solution of the ashed samples of the food was aspirated into varian AA240 AAS according to the method of APHA [16] and was heated to atomise the element. A beam of radiation was passed and the absorbance of the samples was taken. The machine runs in triplicate and the average absorbance of the sample was calculated by referring to the appropriate calibrated curve drawn by the in-built computer interface. The concentrations were equally red out from the print-out from the computer.

Statistical analysis of the result

Data obtained from the experiment were analysed using bar chart and graphs, the statistical package for social sciences (SPSS) software for windows version 17 (SPSS inc. Chicago Illinois, USA). All the data were expressed as mean plus or minus standard deviation. The limits of significance was set at $P<0.05$. Data obtained were subjected to test of significance (ANOVA) to determine if significant difference exists between the mean of the test groups. The percentages and ratios were obtained by comparing the mean values and calculating their simple percentage and ratio from the value.

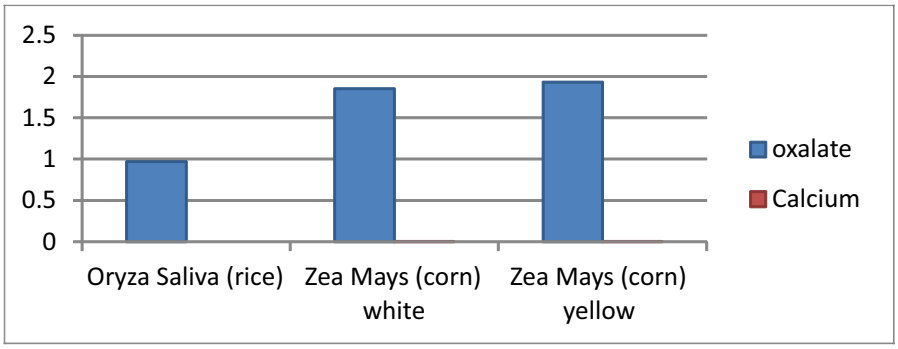
Results and Discussion

The mean oxalate and calcium content in gram percentage and mg/g respectively plus or minus standard deviation of the cereal species are reported in table 1 and shown in fig 2.

Table 1: Oxalate and calcium content of the cereal species

S/N	CEREAL SPECIES	OXALIC ACID	CALCIUM
1.	Oryza saliva (rice)	0.197 ± 0.01	^{N}D
2.	Zea mays (corn) white	1.85 ± 0.01	0.004 ± 0.00
3.	Zea mays (corn) yellows	1.93 ± 0.01	0.006 ± 0.00

Content and variation of oxalate and Calcium of some cereals



The results showed that oxalate content ranged between 0.97 ± 0.01 in rice to 1.93+ 0.01 in yellow corn. Thus rice (0.97) showed lowest oxalic content followed by white corn (1.85) and then yellow corn (1.93). The mean differences were all significant ($p>0.05$).

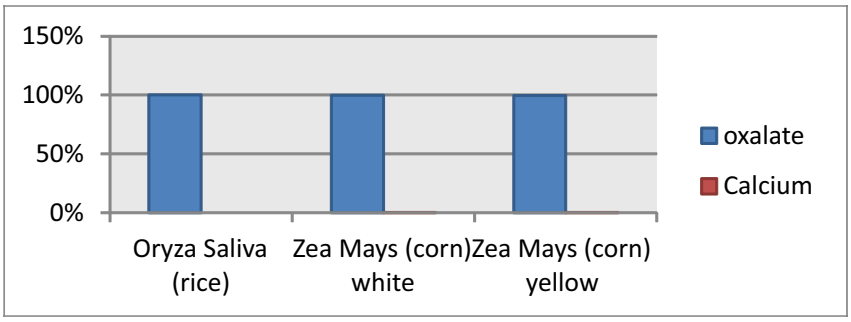
The calcium content ranged from zero (0) in rice to 0.006 ± 0.00 in yellow corn. The calcium content of the cereals appeared very low compared to the levels of oxalic acid and the mean calcium content for all the cereals where all insignificant.

Table 2, showed the percentages and ratios of the oxalate:calcium. The results showed that these staple cereals contain high oxalic acid than calcium, simply indicating oxal:cal. ratio greater than one. The implication is that since these staple have oxal:cal. greater than one, there is no utilizable calcium but excess oxalate which not only complex the calcium contained in the food but also calcium present in other food eaten with them thereby making the body deficient in calcium and susceptible to oxalaurea and kidney stones.

Table 2: Percentages of the oxalate and calcium

S/N	SPECIES	OXALATE	CALCIUM
1.	Orypa saliva	(100%) 100	0(0%)
2.	Zea mays (corn) white	(99.79%) 99.79	0.21 (0.21%)
3.	Zea mays (corn (yellow	(99.69%) 99.69	0.31 (0.31%)

Fig 2: Content and Variation of Oxalate Calcium of some cereals in percent



CONCLUSION

The cereals are good sources of oxalic acid, an antinutrient (phyto chemical) and are poor sources of calcium an essential macro minerals. The high content of their oxalic acid as compared with their calcium content resulted in oxal:cal ratio greater than one. This indicates poor utilization of calcium due to calcium oxalate formation. Thus suggesting complementing and or supplementing these foods with calcium and possibility of oxalaurea and possible kidney stones when these staples are constantly eaten with inadequate calcium supplements.

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Comparative Evaluation Of The Quality Of Water From Two Springs (Ogbarala And Iyintaokuku) In Ohumola And Amorji Igbere In Bende Lga Of Abia State Okoro, O.A., Ukpabi C., Ndulaka J.C. And Agwu K.A.

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ABSTRACT

Water samples collected from Ogbaralaand Iyintaokuku early in the morning were subjected to some physicochemical tests to find out their suitability for domestic uses. Their temperatures checked with thermometer in situ, gave values higher than WHO's standard: (Ogbarala=29°c,Iyintaokuku= 28°c) as against 25°c.While their pH wasfoundlower thanWHO's standard (6.5-8.5). The pH of the two springs gave 5.0 respectively with the pH test paper and average values of 5.17 for Ogbarala and 4.67 for lyintaokuku with pH meter. Other physical parameters checked (color, odor, taste and total dissolved solids) for the two springs were within maximum permissible limit for drinking water.The springs were odorless, colorless and tasteless.Their total dissolved solids were 84mg/l for Ogbarala and 74mg/l for lyintaokuku. Their conductivities, acidities, total alkalinites, total hardness, chloride, Caand Mg were within WHO's standards for drinking water these results were analyzed statistically by Pearson product moment correlation coefficient at 95% confident level and found to be correlated. Their metallic concentrations were determined:Mg (1.63mg/l and 5.65mg/l) Na andK were analyzed using model 6420 flame photometer and sudden was found to be 1.0mg/l each for both springs and 1.1mg/l K for Ogbarala and Iyintaokuku respectively which were below WHO's maximum acceptable limit hence they are not harmful to users. Ca, Mg and the heavy metals were analyzed using Unicam solar 969 Atomic Absorption spectrophotometer and their values found to be within WHO's permissible limit except Cd and Zn which were slightly higher (0.0139mg/l) and much higher (2.1653) for Iyintaokuku respectively. The ANOVA statistical test conducted on the metal concentrations of these two springs at 95% confidence level showed that there is no significant difference in the metal concentrations of the two springs analyzed.

Keywords:*Two springs, Physicochemicalproperties, water samples, chemical analysis, metalconcentrations.*

\INTRODUCTION

Water is needed for the survival of every living thing on earth. It is generally obtained from two principal sources namely; surface water (lakes, rivers, streams, etc) and ground water (bore-holes water and well water)[1]. More than one billion people in the world do not have suitable drinking water and two to three billions lack access to basic sanitation services. Due to the activities of human beings and animals, water is adversely affected and many unwanted and harmful substances enter into water and cause its pollution[2]. About three to five million people die annually from water-related diseases[1]. Water plays an important role in the bodily intake of trace elements by human. Although some trace elements are essential to man, at high levels essential and non essential elements can cause morphological abnormalities, reduce growth, increased mortality and mutagenic effects[3].

Everyday lack of access to clean water and sanitation kills thousands ofpeople, leaving others with reduced quality of life[4]. The major water pollutants are organic materials, dissolved gases, inorganic dissolved solutes, suspended solids, elemental components of fertilizer, pathogenic organisms and heat[2].

Ogbarala and Iyintaokuku are two springs used largely by two communities (Ohumola and Amaoji) for drinking and cooking.

The aim of this work is to find out if these springs are clean and safe for domestic uses as these communities believe. To do this, their physicochemical and metal concentrations were investigated.

MATERIALS AND METHODS

Two samples (2) each of Ogabarala and Iyintaokuku springs were collected in the morning time using plastic containers (table water bottles). Their temperatures were measured in situ at the point of collection by dipping thermometer inside each of the two springs and checking their temperatures. Their pH was measured thrice, firstly in situ at their points of collection using pH test papers,and later twice in the laboratory using model PHS-3C Precision pH meter. The results of pH with the pH test meter are the ones presented on table 1.0.

Conductivities of the water samples were measured using digital conductivity meter (TRONICS EQ-660A) in accordance with standard methods for the examination of water and waste water by the American Public Health Association (APHA)[5].

The following physicochemical analyses were conducted: colour, odour, taste, acidity, total alkalinity, total hardness, total dissolved solids and chlorides were done using standard methods as described by[6] and [7]. Sodium and Potassium contained in the water samples were analyzed using model 6420 flame photometer. While Calcium and magnesium and heavy metals were analyzed using Unicam Solar 969 Atomic Absorption Spectrophotometer.

RESULTS AND DISCUSSION

Table 1.0 represents the physicochemical parameters of the two springs (Ogbarala and Iyintaokuku) analyzed. The pH values of the two springs were low: Ogbarala (5.17) and Iyintaokuku (4.67) respectively as against WHO'sstandard of 6.5-8.5. The low pH has characterized most studied ground water resources in Nigeria[8]. The values of pH of ground water reported by researchers include a range of 4.8 to 5.1 in Onitsha and mean value of 5.56 in Benin, a range of 4.3 to 7.8 in Lagos, mean of 6.3 in Akure, a range of 5.9 to 7.0 inNsukka and a range of 5.8 to 7.4 in Gboko[8].

The low pH of these springs could be as a result of their geochemistry. The conductivity values of these springs were quite low: 0.021yscm^{-1} for Ogbarala and 0.041yscm^{-1} for Iyintaokuku, so they are within WHO's acceptable limit. These low values agree with thelow concentrations of the metallic ions got: calcium (mg/l) 3.8901 and 7.7482; Magnesium (mg/l): 1.6330 and 5.6280; sodium (mg/l): 1.0 for each spring; potassium (mg/l): 1.1 for each spring sample tested. Also, the same low values of metals concentrations are applicable to the heavy metals tested. It is said that magnesium deficiency can increase calcium imbalance, worsenblood vessel calcification and potentially lead to type 2 diabetes[9].

The acidity values of these springs were 16mg/l$CaCO_3$ for Ogbarala and 9.2mg/l $CaCO_3$ for Iyintaokuku respectively. The total alkalinity values of these springs are 30.00 and 31.50mg/l for Ogbarala and Iyintaokuku respectively which are quite below WHO'sstandard of 50mg/l for drinking water.

Total hardness for these springs were got to be 10mg/l for Ogbarala and 16mg/l for Iyintaokuku which shows that these springs are soft water. Their values are quite below WHO'smaximum permissible limit of 150-500mg/l $CaCO_3$. Soft water has been said to have been associated with rickets in children and cardiovascular diseases while hard water was said to be associated with rheumatic pains and goiter[8].

The values of total dissolved solids and chloride concentration were quite low, below WHO's maximum permissible limit. Their values for total dissolved solids were 84mg/l for Ogbarala and 74mg/l for Iyintaokuku as against WHO's permissible limit of 1000mg/l and their chloride concentration values were 50.48mg/l for Ogbarala and 36.30mg/l for Iyintaokukurespectively as against WHO's permissible limit of 250mg/l.

Table 1.0: Physicochemical parameters of two springs in Ohumola and Amaoji communities

Parameter	Ogbarala	Iyintaokuku	*WHO
Temperature	29°c	28°c	25°c
pH	5.17	4.67	6.5-8.5
Conductivity ($yscm^{-1}$)	0.021	0.041	1400
Acidity (mg/l $CaCO_3$)	16	9.2	
Total alkalinity (mg/l)	30	31.50	50
Total hardness (mg/l)	10	16	500
Total dissolved solid (mg/l)	84	74	1000
Chloride (mg/l)	50.48	36.30	250

*Source: Egereonu*et al.,* 2012; EPA, 2011.

All but two of the heavy metals analyzed were within acceptable limit set by WHO. Cadmium and zinc concentrations in Iyintaokuku were slightly above and much above WHO's permissible limit respectively. Their values for Iyintaokuku were 0.0139mg/l and 2.1653mg/l. Their values for Ogbaralawere within acceptable limit of WHO (< 0.0001mg/l and 0.0014mg/l).

The zinc concentration of Iyintaokuku is alarmingly higher than WHO's standard. Its zinc concentration was 2.1653mg/l as against WHO's standard limit of 0.10mg/l. This may be as a result of human activities near this spring, such as dumping of old rusted zinc roofing sheets and cadmium batteries near this spring. Such activities should be discouraged for the safety of users of this spring. Zinc imparts undesirable astringent taste. This high level of zinc could also suggest that there may be appreciable quantity of zinc ore there. The high level of zinc explains why the cadmium content of this spring is slightly higher (0.0139mg/l) than the WHO's standard limit of 0.01mg/l, and much higher than that of Ogbarala (<0.0014mg/l). Some zinc ores concentrates from sulfide zinc ores (ZnS) contain up to 1.4% cadmium[10]. Cadmium is naturally found in very low concentration in rocks, coal and petroleum and could enter ground and surface water and become widespread[8]. Cadmium is toxic, its presence in water should not be encouraged at all.

With the exception of the peculiar case of cadmium and zinc concentrations in Iyintaokuku the values obtained in this work are in agreement with the previous work done on ground water[11], and spring water[12,1] respectively.

Table 2.0: Metal concentrations of the two springs in Ohumola and Amaoji communities

Parameter	Ogbarala (mg/l)	Iyintaokuku (mg/l)	WHO standard (mg/l)
Pb	<0.0001	<0.0001	0.05
Cd	<0.0014	0.0139	0.01
Fe	0.0118	0.1266	0.30
Cu	<0.0001	<0.0001	1.00
Zn	<0.0001	2.1653	0.10
Cr	<0.0001	<0.0001	0.05
Ca	3.8901	7.7482	50
Mg	1.6330	5.6280	30
Na	1.0000	1.0000	250
K	1.1000	1.1000	<20

*Source: Egereonu*et al.,* 2012.

CONCLUSION

Generally, from the values of the parameters analyzed in this work, it can be concluded that one of the two springs (Ogbarala) is of good quality and safe for drinking. It may also be necessary to analyze water from these springs at different times of the year to know which time is the safest time for their use domestically. And, for Iyintaokuku, having high concentrations of cadmium and zinc, human activities should be controlled near this spring to avoid further pollution.

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Antiulcer and Anticonvulsant Properties Of 4-Hydroxyl-3-Methoxybenzylidene-4,(4-Chlorophenyl)-Semicarbazone And Its Ni(Ii), Co(Ii) And Cu(Ii) Complexes .

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ABSTRACT

The urgent need to revolutionize the treatment of ulcer and epilepsy has led to the synthesis of 4-hydrox-3-methoxybenzylidene-4,(4-chlorophenyl)semicarbazone(ligand)andits nickel(II),cobalt(II) and cupper(II). All synthesized compounds were characterized using elemental analysis and infrared spectroscopy.The conductivity, solubility and melting point values revealed that all reported compounds were non-conducting and are environmentally stable. At low (30mg/Kg) and high (60mg/Kg) doses the ligand exhibited highest percentage ulcer inhibition of 91.63 and93.75% respectively. At a low dose of 30mg/kg body weight, the ligand was active against organophosphorous pesticide induced convulsion on albino rats. It was observed that on increasing the dosage administered, tendency of ulceration represented as ulcer index and area of ulcerated lesions decreased while volume and pH ofmucos increased for each animal group. Comparing with standard antiulcer drugs; cimetidine (50mg/kg) and omeperazole (20mg/kg), it was found that L, CuL_2 and NiL_2 showed higher ulcer inhibition abilities than cimetidine and omeperazole at the doses studied. Meanwhile, the standard drugs cimetidine and omeperazole possessed better ulcer inhibition properties than CoL_2 both at low and high doses. However, the ligand and its metal complexes can be considered as potential antiulcer agents

Keywords: phenylsemicarbazone, antiulcer activity, anticonvulsant activity

INTRODUCTION

Among new chemical pharmacophores with broad spectrum of activities and less neurotoxicity; semicarbazones, thiosemicarbazones and their metal complexes have become of considerable attention to medicinal chemists because of their potentially beneficial pharmacological properties and wide variation in their mode of bonding and stereochemistry (Njar*et al*,1995; Mohamed *et al.,* 2012; Agarwal *et al*., (2005(b)). Peptic ulcers are induced by an imbalance between the aggressive factors such as acid or pepsin and the gastrointestinal mucosal resistance to the aggressive factors while gastric ulcer is mainly induced by weakening of the defensive factors (Murakamu*et al*., 1990). Thus, effective antiulcer drugs are either antisecretory agents which suppress the aggressive factors or cytoprotective agents which strengthen the defensive mechanisms of the gastrointestinal mucosa (Paiva*et al*.,1998; Turdor*et al*.,2007). In attempt to provide a lasting solution to the incidence of convulsion and ulcer which are among the top chronic diseases in sub-Sahara African region, our strategy involved the synthesis, characterization of new aryl semicarbazone and the comparative study of the anticonvulsant and antiulcer properties of its nickel, cobalt and cupper complexes on albino rats.

EXPERIMENTALS

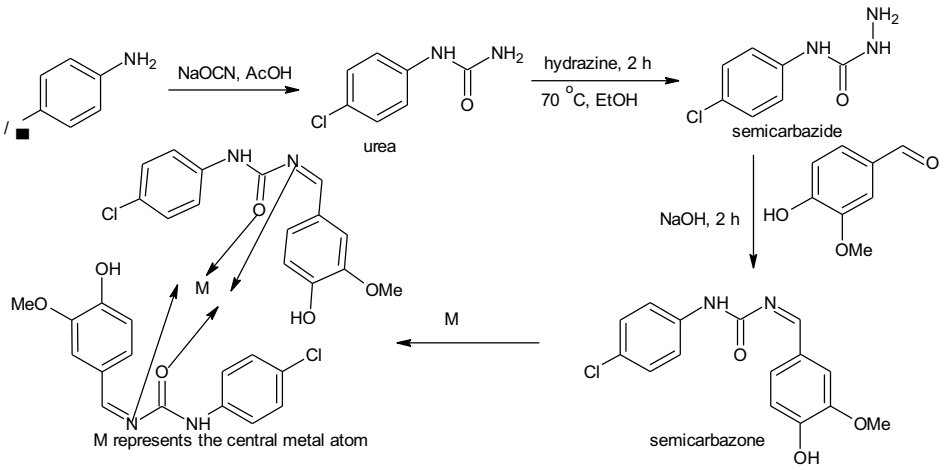
All starting chemicals and solvents were of reagent grade and were purchased from Aldrich chemical Laboratories through Bristol scientific Lagos and were used without further purification. Melting points were determined on a Yamato capillary melting point apparatus model MP-21, The carbon and hydrogen contents were determined based on ASTM-D378 standard, The nitrogen content of each sample was determined using ASTM-D3179 standard, percentage metal was determined using Agilent FS240AA Atomic Absorption Spectrophotometer according to the method of APHA 1995. Difference in percentage inhibition between the test and the control groups was studied using one way ANOVA.

Synthesis of 4-hydroxyl-3-methoxybenzy-lidene-4(4-chlorophenyl)semicarbazone (L)

4-Chloroanaline (0.1M,1.28g) was dissolved in 10ml of glacial acetic acid and diluted to 100ml with distilled water. Equimolar quantity (0.1M,0.33g) of sodium cyanate in 50ml of warm water was added to the previous solution with stirring. The reaction mixture was allowed to stand for 30min, and then filtered, washed, dried and recrystallized from boiling water. To a solution of 4-chlorophenyl urea (0.1M,0.34g) in 20ml ethanol, an equimolar (0.1M) quantity of hydrazine hydrate was added. The reaction mixture was made alkaline by adding 4g of NaOH, and refluxed at 70°C for 2h and cooled in ice. The product was filtered under suction and recrystallized from ethanol.A solution of 4-chlorophenylsemicarbazide (0.1M,2.22g) and equimolar quantity of 4-hydroxyl-3-methoxybenzaldehyde (0.1M,1.52g) in 100ml of ethanol was refluxed at 70°C for 2 h in the presence of glacial acetic acid (1ml). The product obtained after cooling was filtered and recrystallized from 95% ethanol to give pure 4-hydroxyl-3-methoxybenzy-lidene-4(4-chlorophenyl) semicarbazone (L) ligand.

Synthesis of Cobalt, Nickel and Copper Complexes

Fifty millilitreethanolic solution of metal ions [$CoCl_2.6H_2O$, $NiCl_2.6H_2O$,$CuSO_4.7H_2O$] were each added to respective 50ml ethanol solutions of (0.5M,8.9g) 4-hydroxyl-3-methoxy benzylidene-4(4-cholro phenyl)semicabarzone in 1:2 (metal:ligand) ratio. The mixture was heated under refluxed at 70°C for 1h and cooled in ice, the product was washed with distilled water and recrystallized from 95% ethanol.



Scheme 1: Synthesis of the Ligand and metal complexes

Antiulcer activities of the Ligand and Its Cobalt, Nickel and Copper Complexes

The rats were acclimatized for 48h, weighed between 60-100g. They were fasted for 24h before the experiment, but were allowed access to drinking water till two hours to the experiment. Gastric ulcer was induced according to the method described by Robert *et al,* (1979). Group1 rats were ulcer control that received 5ml of water per kg body weight orally by orogastric intubations where groups 2,3,4 and5 rats received oral low doses (30mg/kg) of the ligand, cobalt (II), nickel (II), and copper (II) complexes respectively, while groups 6,7,8 and 9 rats received a high dose (60mg/kg) of the ligand, cobalt (II), nickel (II), and copper (II) complex respectively. The surface area (mm^2) covered by each lesion was measured (Murakamu*et al*., 1990), and the sum of erosion areas per rat intestine was estimated.Thesum of erosion areasper rat intestine was estimated as follows:

Area of ulcerated lesion =

Area of ulcerated lesion = $\pi R^2$where R is radius of ulcerated lesion

Tendency of ulceration was estimated using ulcer index as follows:

$$(UI) = \frac{total\ area\ covered\ by\ ulcers}{total\ corpus\ mucosal\ surface}\ x\ \frac{100}{1}$$

The ulcer index (UI) for each rat was calculated as the mean ulcer score.

Percentage inhibition (%I) was determinedaccording to (Njar*et al*., 1995), as

$$\%I = \frac{UI\ in\ controlgroup - UI\ in\ testgroup}{UI\ in\ controlgroup}\ x\ \frac{100}{1}$$

Anticonvulsant Activities of the Ligand,Cu(II), Ni(II) and Co(II) complexes

The rats were grouped into eight and were accommodated two rats per cage. Groups 1,2,3&4 received oral low doses (30mg/kg) of HL, NiL_2, CoL_2, & CuL_2 respectively, while the rat groups 5, 6, 7 & 8 received oral high doses of (60mg/kg) of L, NiL_2, CoL_2, & CuL_2 respectively. After 100 min of this pretreatment, the rats were administered orally with equal dose of 0.5cm^3 of 5%v/v organophosphate pesticide with the use of canuler. The rats were monitored for 8 h and observations recorded. Behavioural seizures were rated according to Racine's scale; Racine, (1972).

RESULTS ANDDISCUSSION

TABLE1: PHYSICAL PROPERTIES OF THE LIGAND AND THE COMPLEXES

Compounds	Yield (%)	Colour	Melting point(^{0}C)	Conductivity(Us/cm)
Ligand	62	Pale yellow	110	.
NiL_2	60	Pale yellow	121	32
CoL_2	66	Pale yellow	128	48
CuL_2	74	Dirty green	113	38

Low molar conductivity values of the complexes suggested they are non-electrolytes while the ligand showed no signal on conductivity. Both the ligand and complexes on the basis of their conductivity values could be regarded as neutral molecules. The high melting points of the ligand and complexes suggest that the synthesized compounds are air and moisture stable [Table1]. Hence, they can be stored for months without any significant changes in their composition.

TABLE 2: Percentage Elemental Analysis

			Percentage Element Found (% Calculated)					
	Formula	**Molar mass**	**%C**	**%H**	**%N**	**%O**	**%Cl**	**%M**
L	$C_{15}H_{14}O_3N_3Cl$	319.5	56.36 (56.34)	4.40 (4.38)	13.11 (13.15)	15.00 (15.02)	11.11 (11.13)	– (–)
NiL_2	$C_{30}H_{28}O_6N_6Cl_2Ni$	698.7	50.76 (51.52)	3.89 (4.00)	11.80 (12.02)	13.69 (13.74)	9.82 (10.16)	8.60 (8.54)
CoL_2	$C_{30}H_{28}O_6N_6Cl_2Co$	698.9	51.53 (51.51)	3.88 (4.00)	11.84 (12.02)	13.72 (13.74)	10.09 (10.16)	8.62 (8.57)
CuL_2	$C_{30}H_{28}O_6N_6Cl_2Cu$	703	51.53 (51.21)	4.02 (3.98)	12.01 (11.95)	13.60 (13.66)	9.98 (10.10)	9.13 (9.10)

The complexes synthesized are monometallic, ollowing the results of percentage elemental analysis which are in good agreement with the assigned formulations. This is because the theoretically calculated percentage values are in close agreement with experimental values obtained from elemental analysis (Table 2).

Table 3: Infrared absorption frequencies (cm^{-1}) of the Ligand and Complexes.

Compounds	V(O-H)cm^{-1}	V(C=O)cm^{-1}	V(C=N)cm^{-1}	V(C-N)cm^{-1}	V(C-O)cm^{-1}	V(M-N)	V(M-O)
Ligand	3280	1707	1628	1237	1028	-	-
NiL_2	3336	___	___	1222	1028	510	522
CoL_2	3291	___	___	1155	1021	488	506
CuL_2	3336	___	___	1159	1054	410	424

The significant bands of the Ligand and their assignments are given in Table 3. In all complexes the band at 1028cm^{-1} remained almost at the same position as in the ligand confirming that C-O did not participate in chelation. The band at 1725-1705cm^{-1} for v(C=O) is absent in all complexes; which indicates an interaction with the metal since the attachment of metal could lead to displacement of the major functional group. However, the band at 1237cm^{-1} for v(C-N) was lower in complexes suggesting repulsion, elongation, lengthening and weakening of C-N bond within the complexes. Conversely, the band observed at 3280cm^{-1} for v(O-H) was higher in complexes than in the ligand an indicative of the contraction and strengthening of O-H bond resulting from metal-ligand interaction. The bands at 522cm^{-1} and 510cm^{-1} are for v(Ni-O) and v(Ni-N) while bands for v(Co-O) and v(Co-N) also appeared at 506cm^{-1} and 488cm^{-1}, bands assignable to v(Cu-O) and v(Cu-N) were observed at 424cm^{-1} and 410cm^{-1} respectively. The appearance of these bands further supports bonding of the ligand to the metal through oxygen and nitrogen atoms.

Table 4: Antiulcer Properties of the Ligand and the Complexes

Compounds Tested	Weight of Mucos (g)	pH of Mucos	Area of the intestine studied	Total Area of Ulcerated lesion (mm^2)	Ulcer Idex (%)	Percentage Inhibition (%)
Control (5ml of water)	.....	2.06±0.02	900mm^2	84.98±0.05	9.44	-
Cimetidine	0.61±0.05	3.00±0.01	1400	168±0.05	NR	88
Omeprazole	0.46±0.025	5.60±0.05	847.62	178±0.96	NR	79
Ligand						
30mg/kg	0.810±0.004	3.40±0.06	900	7.07±0.02	0.79	91.63
60mg/kg	0.850±0.007	3.70±0.04	900	5.28±0.02	0.57	93.75
CuL_2						
30mg/kg	0.68±0.01	3.00±0.14	900	9.40±0.02	1.04	88.98
60mg/kg	0.70±0.007	3.10±0.11	900	8.65±0.05	0.961	89.82
NiL_2						
30mg/kg	0.52±0.001	2.82±0.02	900	12.90±0.04	1.43	84.85
60mg/kg	0.55±0.04	2.88±0.02	900	12.55±0.02	1.39	89.26
CoL_2						
30mg/kg	0.46±0.001	2.71±0.04	900	30.10±0.02	3.34	64.62
60mg/kg	0.52±0.05	2.80±0.05	900	28.85±0.03	3.21	66.26

Rats pretreated with a low dose of (30mg/Kg) and a high dose of (60mg/Kg) of L, NiL_2, CoL_2, and CuL_2 before being given absolute ethanol had significantly reduced areas of gastric ulcer formation compared to rats pretreated with $5cm^3$ of water (Table 4). At low and high doses, the ligand exhibited highest percentage ulcer inhibition seconded by CuL_2 followed closely by NiL_2, with least inhibition observed in CoL_2. On increasing the dosage administered, tendency ofulceration represented as ulcer index decreased for each animal group. The result of this study revealed that the ligand is a more effective antiulcer drug than the complexes, yet the ligand and its complexes can be considered as potential antiulcer drugs.The antiulcer activities of the compounds can be explained on the basis of reducing the acidity of the stomach juice as it can be implied from the pH values. These results confirmed the proton pump inhibition activities of the compounds via increasing pH, suppressionof the aggressive factors and strengtheningof defensive mechanisms of the gastrointestinal mucosa.Comparing with standard antiulcer drugs; cimetidine (50mg/kg) and omeperazole (20mg/kg), it was found that L, CuL_2 and NiL_2 higher ulcer inhibition abilities than cimetidine and omeperazole at the doses studied. Meanwhile, the standard drugs cimetidine and omeperazole possessed better ulcer inhibition properties than CoL_2both at low and doses (Table 4).

Table 4: Anticonvulsant Activities of the ligand and the complexes

Compounds	Dosages Administered	
	30mg/kg	60mg/kg
HL	+	+
NiL_2	-	+
CoL_2	-	-
CuL_2	-	+

The ligand has been observed to exhibit anticonvulsant activities at low (30mg/Kg) and high (60mg/Kg) doses, the anticonvulsant activities of CuL_2 and NiL_2 were only observed at high dose of 60mg/kg body weight. At these dosage limits, no anticonvulsant activity was observed for CoL_2.

CONCLUSION

Arylsemicarbazone and their metal complexes have been synthesized and isolated as air stable microcrystalline solids in a good percentage yield. The synthesized compounds are non-electrolytes. The ligand and complexes functioned via proton pump inhibition mechanism. The ligand is a more effective antiulcer and anticonvulsant drug than the metal complexes. However, the ligand and its metal complexes can be considered as potential antiulcer agents.

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Comparative Analysis of *Dacryodes Edulis* Seed, (Bush Pear) Oil and *Cucumeropsis Mannii* Seed Oil (White Seed Melon)

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ABSTRACT

The research project studies the analysis of bush pear oil and white seed melon oil. The analysis on bush pear oil and white seed melon was carried out by solvent extraction using n-hexane. Percentage yield content was 5% and 8.04% respectively. The oils extracted were analyzed for Acid value, Saponification Value, peroxide value iodine value, Refractive index and pH value. The values obtained were for bush pear was 22.44 mg/KOH/g, 64.52 mg/KOH/g, 37 mg/KOH/g and 71.44 mg/iodine/g, 1.311 brix and 5.4 respectively while 26.93mg/KOH/g, 56.1/KOH/g, 7.4gm/KOH/g, 52.03gm/iodine/g, 1.301 brix, and 5.2 were obtained for white seed melon oil. The extracted oil were analyzed for the chemical properties (Acid values, saponification value, peroxide value, iodine value) the values obtained are and the percentage yield is 8.04%. The comparism between the oil from bush pear and white seed melon is that both of them are non drying oils and they are good in cosmetics industries. The physicochemical properties of these oils show that have both industries and cooking potentials.

Key words: Bush Pear, Solvent Extraction, N-Hexane, Saponification Value, Acid Value, Physicochemical Properties, Percentage Yield.

INTRODUCTION

Dacryode Edulis [Bush Pear] has a generic name *"Dacryode*" derived from the Greek word "*Dakuron*" meaning (tear) referring to resin droplet on the bark surface of its members while "*Edulis*"means edible emphasizing the importance of nutrient fruits in the plant cultivationTwo varieties are recognized var-paricarpa and var-edulis whose conical fruits are smaller with the pulp. Var-edulis exhibit verticulate or sub-verticulate branching while the branching is slender and opposite or biyfurcate in var- parvicarpa (Okafor, 2003). *Dacryode Edulis* is an indigenous fruit on the gulf of genius and central African countries but due to the popularity of the nutritious fruit for consumption, the plant is widely cultivated (Verheji, 2002) Lam gave four synonyms viz to the *Dacryode Edulis; Carnanimeduke Hook.F. Carnammas Phuengl, pachylobus Edulis Hook F and pachylobussa Phuengl*. However, this synonyms has long been considered as the most unambiguous synonyms (Boutelje, 2008). The common name are in English, they includes; African pear, Bush butter, Bush fruit tree, Eben tree, native pear, (Kapsue and Tchiegang, 2006) and in French the oil of fruit of *Dacryode Edulis* is a rich source of amino acids and triglycerides the fatty acid composition of fruit pulp oil of two cultivars of bush pear (cultivar 1 and cultivar 2) grown in Nigeria were determined. The oil was found in the pulp which is made up of 48% of oil and a plantation can produce 7.8 tons of oil per hectare. It is also rich in vitamins and a reach source of amino acids triglycerides (John, 2001).

In the major world, one major source of protein and vegetable oil is from oil-fruits (Williams, 2004) oil constitute a well defined class of naturally occurring substance. It is greasy, being soluble in the organic solvents but insoluble in polar solvents such as water. Oil is a liquid at room temperature. Commercially, oil as well as fats is sourced from certain plant groups mostly seeds and nuts and some parts of animal within which they occur in relatively large quantity in an easily available form (McGraw, 2007).

The existence of oil in certain plants has been known for century of years. Oil can be grouped into edible and non-edible oil depending on the amount of unsaponifiable matters and impurities contained therein. Edible oil extracted from African pear, bread fruits, cashew nuts, peanuts etc are example of vegetable oil which are naturally occurring esters of higher fatty acids and glycerol and are predominantly triglycerides with traces of mono and triglycerides, sterols and oxidants, vitamins, saturated and unsaturated free fatty acids and other minor constituents. They are widely distributed in nature and were first consumed as food. Latter, oil were discovered to be used as renewable raw materials for variety of non food production for instance, soaps, creams, disinfectants, paints, enamels, ink etc.

Cucumeropsis Mannii [White Seed Melon] is a member of the cucurbitaceae family. Vernacular names for this crops includes egusi-itoo in Yoruba, agusi in hausa and white seed melon in English, [Egunjobi,2004]. This crop is often referred to as the real egusi given its long history in West Africa, dating back 4,000years. This crop is primarily harvested for its long seeds called egusi-itoo. [Kortse., 2012]. The seed thrives in harsh climate and high yields are attainable in barren landscape. The seed increases soil quality through ground cover and suppression of weeds. (National Research Council,2006). Egusi-itoo is primarily grown for the oily seeds it produces, the seeds are commonly ground up and used to thicken soups and stews or as an ingredient in dumplings (Schaefer, 2010). Another consumption of egusi-itoo is in patty form after oil has been extracted from the seed, it is then eaten as a protein substitute. Most commonly it is dehulled and consumed as a snack. Some are soaked, fermented, boiled and wrapped in leaves to form a favourite food seasoning.

They are consumed as ball snack and ogiri (fermented condiment) melon seed contain a fairly high amount of unsaturated fatty acid, linoleic acid suggesting a possible hyprocholesteric effect. The oil extracted from the seed is used for edible purpose while the residual cake is fried and consumed as a snack in some rural parts of southern Nigeria. The inhabitants mill the seeds and shape into balls to substitute meat in their diet (Nwokolo and Sam, 2007). White seed melon oil is one of the most important rate versatile oil, due to its use in various spheres of life. The kernel of the white seed melon contains semi drying oils which can be used for making soaps, cooking and illumination. The flesh of the white seed melon is not commonly consumed raw because of its bitter taste.

This study was aimed at extracting the oil from bush pear and white seed melon oil, and characterizing it for industrial and commercial purposes.

METHODOLOGY

Sample Collection

Bush pear seeds and white seed melon were bought at eke Oko market in Oko community in Orumba North Local Government Area of Anambra State and were taken to chemistry Laboratory for further analysis.

Sample Preparation

The bush pear seeds were dehulled and cut into pieces using a sharp knife, and then grinded with electric blender, the white seed melon was also grinded with the electric blender.

Oil Extraction

The prepared samples were soaked in different bottles with n-hexane for 24 hours. The oil which was mixed with n-hexane was separated by simple distillation method with the use of soxhlet extract. A good amount of oil was extracted from the samples which were used to characterize the samples.

Physicochemical Properties

Determination of Acid Value

1g of the oil for each of the samples were weighed accurately into a conical flask and dissolved with 10ml of chloroform ($CHCl_3$), 2 drops of phenolphthalein was added and then the solution was titrated with 0.1M KOH, pink colour appeared and persisted for some time, the average titre reading was taken.

$$\text{Acid Value} = \frac{56.1 \times M \times V}{W}$$

M= molarities of KOH; V= volume of KOH used in titration; W=mass of the oil sample

Determination of Saponification Value

1g of oil for each of the sample were weighed into a conical flask and dissolved with 25ml of alcoholic KOH (ethanolic potassium hydroxide), some boiling chips were added and the solutions were boiled gently for 1hour on a water bath under reflux condenser. 2 drops of phenolphthalein indicator was added and the excess alkali (KOH) was titrated with 0.5M HCl until end point was reached. A blank titration was also carried out following the same procedure without the sample. The average titre value for the sample and the average titre value for the

blank were recorded. The saponification value was obtained using the formula below.

$$\frac{(b-a) \times 28.05}{W}$$

a = reading of the first titration with the sample; b = reading of the second titration without the sample (blank); 28.05 is the number of milligrams of KOH in 1ml of 0.5M KOH.

W= weight of sample

Determination of Peroxide Value

1.00g of oil for each of the sample was weighed in a conical flask and was dissolved in 30ml of solvent mixture containing 12ml of chloroform, and 18ml of glacial acetic acid for each of the samples. 0.5ml of saturated aqueous solution of KI (Potassium Iodide) was added, this was allowed to stand for 1hour for one minute. 30ml of H_2O was added and the solution was titrated with 0.002M $Na_2S_2O_3$ solution until the yellow colour was discharged. 1M of starch was added which gave the solution a pink colour. Titration was continued by adding the average volume of potassium iodide until the pink colour disappeared. The same procedure was applied to the blank without the sample.

$$\text{Peroxide Value} = \frac{1000(a-b)\,M}{W}$$

Where a=volume of $Na_2S_2O_3$; b =volume of $Na_2S_2O_3$ used in the blank; M = molarity of sodium thiosulphate; W =weight of the sample

Determination of Iodine Value

1g of the oil each of the samples was weighed into a conical flask and 20ml of carbon tetrachloride (CCl_4) was added to dissolve the oil. 25ml of wijs solution (8.5g of iodine +7.5g of iodinemonochloride in glacial acetic acid was added into the flask containing the sample. The flask was swirled severally to ensure an intimate mixture. The whole mixtures were kept in the dark room for 1hour at room temperature. At the end of the period, 10ml of 10%KI was added in the mixtures followed by 100ml of distilled water. The resultant solution was titrated with 0.1M $Na_2S_2O_3$ solution added gradually with constant and vigorous shaking until the yellow colour disappeared. 2ml of starch solution was added and titration continued until the colour disappeared. The procedure was repeated for the blank titration without the sample.

$$\text{Iodine Value} = \frac{(b-a)\,M \times 1.269}{W}$$

S =Titration titre of the sample; B = Titration titre of the blank; M =Molarity of the standard thiosulphate solution; W = Weight of the sample

Determination of Refractive Index

Few drops of the oil were placed on the refractometer and the readings were taken and recorded.

RESULTS

The experimental results of the analysis carried out in this project are stated in the table below.

Table 1: Percentage Yield of the Oil of Bush Pear

Parameter	Values
Weight of Bush Pear	170g
Weight of the oil extracted from the sample	8.5g
Percentage yield	5%

Table 2: Percentage Yield of the Oil of white seed melon

Parameter	Values
Weight of white seed melon	336g
Weight of the oil extracted from the sample	27g
Percentage Yield	8.04%

Table 3: Physicochemical Analysis of the Oil Extracted from Bush Pear

Parameter	Values
Acid value (mg/KOH/g)	22.44
Saponification value (mg/KOH/g)	64.52
Iodine value (mg/iodine/g)	71.44
Peroxide value (mg/KOH/g)	37
Refractive index (brix)	1.311
pH value	5.4
Unsaturation level	Moderate

Table 4: Physicochemical Analysis of the Oil Extracted from White Seed Melon

Parameter	Values
Acid value (mg/KOH/g)	26.93
Saponification value (mg/KOH/g)	56.1
Iodine value (mg/iodine/g)	52.03
Peroxide value (mg/KOH/g)	7.4
Refractive index (brix)	1.301
pHvalue	5.2
Unsaturation level	Moderate

Discussion

After the analysis, the bush pear oil was determined to be oil at room temperature and it is unsaturated oil. The iodine value which is 71.44mg/KOH/g which makes it a non drying oil, which means that it will be good in production of creams and saponification value which is 64.52g/Iodine/g makes it suitable in production of soap. The white seed melon oil was determined to be unsaturated oil which means that it will be suitable for cooking and the iodine value which is 52.03mg/iodine/g which makes it a non drying oil suitable for production of creams. Therefore bush pear oil and white seed melon oil is good quality oil and can find application in cosmetics industries.

CONCLUSION

From this research work carried out, a reasonable amount of oil was extracted from the bush pear; it is more advisable to consume this type of vegetable oil that is low in saturation than the oil from animal product. The physicochemical characteristic composition of the bush pear oil and white seed melon oil shows that they have some industrial potentials. White seed melon oil should be used for cream production while Bush pear oil should also be used in cosmetics industries because of its physicochemical properties. Further research should be based on other components of the oil, the fruit skin and the unsaponifiable components in the fruit seed as well as non lipid components of the oil.

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Phytochemical And Proximate Analysis Of Paw Paw (*Carica Papaya*) Seeds

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ABSTRACT

Phytochemical and proximate analysis of pawpaw (Carica papaya) seeds were carried out in this study. The pawpaw seeds samples were purchased from Eke market in Ekwulobia. The sample was ground into fine powder using a dry grinder. The ground samples were weighed and tested using standard method. The phytochemical screening revealed the presence of Alkaloid, flavonoid, saponins, tannins and glycoside and absence of steroid and terponoid while proximate analysis reveals high carbohydrate (27%), moisture (20%), fibre (18.5%), fat (11%) and ash (10%). The results of both phytochemicals and proximate analysis of pawpaw seeds suggest the seeds of pawpaw have medicinal activities and was rich in some required body nutrient. They can be use in the treatment of disease such as cancer, liver cirrhosis and inflammatory.

KEYWORD: Phytochemicals, Proximate, Medical Activities, Carbohydrate, Cancer, Inflammatory.

INTRODUCTION

Botanically, fruits are parts of flowering plants derived from the fertilization of specific tissues such as one or more ovarie of flowers, they are non – staple foods which make up about 39% of the food intake of persons living in developing countries of Africa. In non – technical usage, such as food preparation, fruits normally represent the fleshy, seed bearing structure of certain plants, which are edible in the raw state. Fruits are important sources of sugars, vitamins A, C and B group, low protein and lipid respectively. Also, they have been shown to contain high amounts of minerals, moisture, low ash and crude fibre. In addition, fruits contain little or no fat or sodium and being a plant food, no cholesterol, thus nutritionally healthy when consumed with other foods. Fruits can be nutritionally supplement diets in developing countries beyond nutrients seeds, such as antioxidants (e.g polyphenols), soluble fiber (e.g pectins and beta - glucan), vitamins (e.g. vitamin A, B and D groups), organic acids (e.g tartaric acids) which act as functional food materials or nutraceuticals provide specific health benefits such as prevention of diseases and growth of gut pathogens, enhancement of body immunity, protection against heart diseases, cancer, osteoporosis, hypertension etc.

Phytochemicals are naturally occurring chemicals produced by plants. The prefix “phyto” is from a Greek word meaning plant. Some phytochemicals give plants their pretty colors, like the blues in blueberries and the red in raspberries and other phytochemicals give plants their distinctive aromas. These phytochemicals help the plants by alleviating insects and other creatures to pollinate the plants or spread the seeds. Phytochemicals are biologically active and can affect your health when you eat the plants that contain the compounds. Research studies suggest it's possible that various phytochemicals may help protect from cancer or possible slow down the growth of cancer, reduce inflammation and help regulate hormones. Phytochemicals are often extracted from plants, processed and sold as dietary supplements. They're general considered to be safe, but there's not much regulation regarding their dosages or even effectiveness, so it's important to speak with your health care provider before taking these supplements, especially, if you have any health condition. When consumed in the diet, there is an increasing body of evidence to indicate that phytochemicals may reduce the risk of age – related chronic disease such as coronary disease, heart disease, diabetes, high blood pressure and certain type of cancer. Although their absence from the diet might not cause deficiency symptoms, such as those found with vitamins and minerals, they are thought to be important for health and well being throughout life especially in adulthood and in the elderly.

Proximates are used in the analysis of biological materials as a decomposition of human-consumable good into its major constituents. They are a good approximation of the contents or packaged comestible goods and serves as a cost-effective and easy verification of nutritional panels. This means that testing can be used to verify lots but cannot be used to validate food processor or food processing facility; instead a nutritional assay must be conducted on the product to quality said producers. Nutritional panels in the United States are regulated by the FDA and must undergo rigorous testing to ensure the exact and precise content of nutrient. This should prevent food processor from making unfounded claim to the public.

In industry, the standard proximates are: Ash, moisture, proteins, fat and carbohydrate. Analytically, four of the five constituents are obtained via chemical reactions and experiments. The fifth constituent carbohydrates are calculated based in the determination of the four others. Proximate should nearly always account for 100% display the resolution of the chemical test, as small variation in the way each test is performed accumulates or overlap the compositional make up. There an additional ingredient that may fall under the category of one of the fire constituents' carbohydrates, for example include but are not limited to:

- Dietary fibres, sugar, sugar alcohol where ash includes but is not limited to.
- Dietary minerals (sodium, potassium, iron, calcium)
- Vitamins (B-carotein, retinol, vitamin B3, vitamin B2, B vitamins).

Although proximate do not give the entire nutritional assay, they are an inexpensive way to track deviation in the quality of foods.

Paw-paw *(Carica papaya)* belongs to the family caricacease grown in Nigeria. At the early stage of fruit development, glucose is the main sugar but to sucrose content increases during ripening and can reach up to 80% of the total sugars. The edible portion of the ripe *papaya* fruit contain both macro and micro mineral and these are Na, K, Ca, mg, p, Fe, ca and Mn. *Carica papaya* contains carotenoid, vitamin, thiamine, riboflavin, niacin, vitamin B-b and vitamin K B (Barri *et al.,* 2009).

MATERIALS AND METHOD

Sample Collection

Paw paw (*Carica papaya*) seeds were purchased from Eke market Ekwulobia, Anambra State Nigeria.

Extraction of Seeds Materials

Powdered pawpaw seeds (200g) was weighed and soaked into ethanol for 24hours. The soaked samples were then filtered into volumetric flasks and the residue was discarded. The filtrate was placed in a flat container and was allowed to dry as ambient temperature. The resulting dry extract was stored at 4°C until when needed, when the extract was mixed with 1ml or distilled water.

Phytochemical Screening of Pawpaw Seeds

The seeds were analysed for alkaloids, flavonoids, saponins, Tannins, Terpenoids, Cardial glycoside and steroid.

Alkaloid

1ml of 1% HCl was added to 3ml of the extract in a test tube. The mixture was heated and stirred continuously for 20 minutes in a water bath. After which the mixture was cooled and filtered.

1ml of filtrate was added to 0.5ml of mayer's reagent (potassium mercuric iodide) formation of a yellow coloured precipitate indicates the presence of alkaloids.

Flavonoids

2ml of the extract was added to 1ml of distilled water. The solution was shaken. 1ml of 10% NaOH solution was added to the mixture, formation of green precipitate show the presence of flavonoids.

Saponins

3ml of the extract was mixed with 5ml of distilled water and shaken vigorously for 30seconds. Non – persisted frothing was observed.

Glycosides

1g of the sample was placed separately into two beakers. To one of the beaker was added 5ml of dilute tetraoxoculphate (vi) acid H_2SO_4 while H_2O was added to the other. The two beakers were located for 3 minutes and the content filtered into labeled test tubes. The filtrate were made alkaline with NaOH and heated with fehling's solution for 3 minutes and a precipitate was observed in the test tube with water while that with acid no precipitate was observed. This indicates the absence of glycoside.

Terpenoids

2g of dry extract was dissolved in acetic anhydride, heated to boiling and cooling then, 1ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of a pink colour indicates the presence of terpenoids.

Steroids

2g of dry extract was dissolved in acetic anhydride heated to boiling, cooling and then concentrated sulphuric acid was added along the sides test tube. Formation of green colour indicates the presence of steroids.

Proximate Analysis

The analyses (Moisture, ash, carbohydrate, crude fibre, crude fat and crude protein) were carried out using official method of analysis by Association of Analysis Chemist (AOAC, 2002).

RESULTS

The phytochemicals screening of paw paw seeds were presented in the table below.

Table 1: Result of phytochemical analysis of paw paw seeds

S/N	Parameter	Intensity
1	Alkaloid	+++
2	Flavonoid	+++
3	Saponin	+++
4	Terpenoids	-
5	Tannins	+
6	Steroids	-
7	Glycosides	++

+++ = **Highly present; ++ = Moderately present;+ = Fairly present;- = Negative**

The proximate analysis of paw paw seeds were presented in the table below

Table 2: Result of Analysis of Pawpaw seeds

Parameter	Value (%)
Moisture	20%
Ash	10%
Fat	11%
Fibre	18.5%
Protein	14%
Carbohydrate	27%

DISCUSSION

From the results obtained, Table 1 reveals the presence of alkaloid, flavanoid, tannins, saponins and glycoside with the exception of terpenoid and steroid which were above.

Table 2 showed that moisture content was 20%, carbohydrate 27%, ash content 10%, protein 14%, fat 11%, and fibre content 18.5%. The presence of phytochemicals in *Carica papaya* seeds extract suggest possible medicinal and healing properties (Okwu, 2005). It has been reported in the flavonoids are free radical scavenging that presents oxidative cell damage and have strong anticancer activities (Delmas *et al.,* 2000). Herbs that have tannins as a component are astringent in nature and are used for microbial infection (Sofowara, 1998). Tannins are also known to be useful in the prevention of cancer (Okwu, 2005). Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents for the analgesic properties. Cardiac glycosides are important class of natural occurring drugs whose action helps in treatment of congestive heart failure (Harbone, 2003).

Glycosides have anti–inflammatory activities, acts as cardiac depressant and also appear to kill or inhibit cancer cell (Okwu, 2005). Saponins are believed to react with cholesterol rich membrane of cancer cells, thereby limiting their growth and viability (Roa *et al.,* 1995). Saponins have the property of precipitating and coagulating red blood cells (Yadar and Agarwala, 2011) some of the characteristics of saponins including formation of foams in aqeous solution, hemolytic activity, cholesterol binding properties and bitterness (Okwu, 2004). Saponins in medical plants are responsible for most biological effect related to cell growth and division in humans and have inhibitory effect on inflammation.

CONCLUSION

This work showed that *carica papya* contains a lot of potentially active secondary metabolite such as alkaloids, flavonoids, saponins, glycoside and tannins and carbohydrate moisture and fibre with low content of protein, ash and fat. The presence of these active ingredients shows that *carica papaya* has high potential medicinal values and nutrition for good source of therapeutic agents.

The result of the work has confirmed *carica papaya* seeds has important nutritional properties and be useful in the reatment of diseases. Thus, it is thereby recommended that awareness should be created to the general public in the nutritious and medicinal value of the seeds. Federal government and general public should embark on preservation of the plant from going into extinction due to values. Also, more work should be done on other parts of the plant; leaf, shoot etc.

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Assessment Of The Heavy Metal Concentrations Of Fishes From Some Concrete Ponds In Onitsha Urban In Anambra State Nigeria

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Abstract

The research focused on the level of heavy metal contamination of fish in private concrete ponds in Onitsha. The heavy metals: mercury, lead, chromium and cadmium concentrations in fishes in six concrete ponds located at different zones in Onitsha were determined using Atomic Absorption spectrophotometer. The result showed variable concentrations of these heavy metals in the fishes. The concentration of mercury was highest (0.500mg/kg) in pond Z_V and pond Z_{VI} (0.423mg/kg). Pond Z_{VI} had highest concentration of lead (0.223mg/kg). Pond Z_{II} had the highest concentration of chromium and Z_I had the highest concentration of cadmium (0.190mg/kg). The detected values though variable but were narrowly within the permissible limit/guideline as proposed by WHO 1994 guideline, WHO/FAO/EPA 1996 permissible values and FEPA 2003 Standard of heavy metals in fish.

Keywords: Concrete, pond, dementia, mercury, Clairs, batrachus

Introduction

Fish is a cheap food, but of excellent nutritional value, having high quality amino acid (Protein) which is easily digestible and good for children. Fish contain a wide range of vitamins notably Vitamins 'A' and D., prominent minerals present include iodine, magnesium, phosphorus, selenium[1] etc. Regular intake of fish meal promote human body defense mechanism for protection against invasion of pathogens because of the presence of antimicrobial peptide and equally reduce the risk of heart diseases and lower the risk of development of dementia and Alzheimer disease. Review had proved that breast fed babies whose mothers eat fish regularly have better eye sight perhaps due to the omega-3-fatty acid, content of the consumed fish[2].

The contamination of the underground water or boreholes by heavy metals has become a very worrying event. Those farming fish in pond, channel water from boreholes to the ponds. These boreholes are being contaminated by the heavy metals from different activities done in the environment. The heavy metals contamination may have devastating impacts on the ecological balance of natural water bodies causing loss of aquatic diversity. Many of these metals tend to remain in ecosystem and continue moving from one compartment to the other within the food chain. Fish is often at the peak of the aquatic food chain and absorb these heavy metals through their epithelial or mucosal surface of their skin, gills and gastrointestinal tract. Metals are accumulated in the fish even at concentration higher than that present in water or sediment [3,4]. As fish consumption dominate in the menu of many people especially Onitsha indigenes whose favourite meal is nsala soup - a delicious cuisine with preponderance of fresh fish serve with pounded yam. There is need to assess the concentration of these heavy metals in fish to ensure that man is not being exposed to these metals through fish consumption[5]. There are many species of fish, with varied nutritional content. This study focused on the walking catfish (Caris batrachus), a species of freshwater, air breathing catfish native of Southeast Asia, but also introduced outside its native range where it is considered an invasive species[6]. Walking catfish normally lives in slow moving and often stagnant waters, in ponds, swamps, streams, rivers even in flooded rice paddies. C. batrachus had the ability to use its pectoral fins to keep it upright as it makes a wiggling motion with snake like movements. Its walking skills allow the fish to move to other sources of water, to find food or suitable environment[7].

Catfish farming is increasingly becoming an attractive form of agriculture for many Nigerians especially young people using concrete ponds or plastic tanks. Using concrete pond seems to be more natural to culturing a relatively larger number of fishes as pond design can be tailored from the on set to match intended fish density. The market price of African catfish is usually ganged per kg^{-1} weight of fish and varies across several geographical locations in Nigeria. The walking catfish was selected for this study because it is commonest specie that survives in pond, always available and sold for human consumption[8].

Heavy Metals:

The term heavy metal refers to any metallic chemical element that has a relatively high density at least five times greater than that of water. The include arsenic, beryllium, cadmium, chromium, lead, manganese, mercury, nickel, selenium etc[9]. The multiple industrial, domestic, agricultural, medical and technological, applications of heavy metals have led to their wide distribution in the environment, raising concerns over their potential effects on human health and the environment. Report had shown that these heavy metals are essential nutrients needed by the body for biochemical and physiological functions. Inadequate supply of these micronutrient results to deficiency diseases and syndromes. Excessive concentration of these trace metals also lead to adverse contributions or toxicity[10]. The concerns about the high level of trace metals in foods has prompted several statutory bodies such as World Health Organization (WHO), Food and Agricultural Organization (FAO) of the United Nations State, Codex Alimentaius etc to establish maximum allowable/permissible concentrations for some of the metals in consumable substance[11].

Materials and Method

Sample Collection

Random sampling method was used to select six standard concrete built ponds from Onitsha urban. The ponds were designated Z_1 - Z_6 according to the location/zone of collection. Six grown Clarias batrachus (between three to six months old in the pond) were collected from each of the six selected fish ponds using cast net. The fishes were brought alive to the laboratory and placed on a dissection board. The weight and body length of the fishes were noted. The gills, tentacles, fins, livers and bones were removed from each of the wet-fish using clean stainless knives. The fishes were put in a clean, covered plastic container and stored in the freezer pending heavy metal determination[12].

Preparation of Sample/Reagent

All glassware used for the analysis were watched with detergent and soaked in 10%(v/v) $HN'O_3$ overnight followed by rinsing with distilled water. All reagents used were of analytical reagent grade from BDH Chemical U.K. The Standard solution of the heavy metals to be determined (mercury, cadmium, lead, chromium) were prepared by dilution of their stock solutions to the desired concentration for the corresponding metals to be analyzed, and were used to calibrate the AAS and various Absorbance of each metal. Each fish sample was homogenized thoroughly using Panasonic (Model MX 1015P2) blender with stainless steel cutters, each of the homogenized fish sample were further digested by measuring about ±0.001g of the sample into a 200ml beaker and 10ml of conc. HNO_3 was added, the beaker was covered with a watch glass and after most of the sample had dissolved by standing overnight, heated on a hot plate with boiling until vigorous reaction subsided and a clear solution obtained. The solution was allowed to cool, transferred into a 50ml volumetric flask and diluted to the mark with distilled water[12].

The prepared samples were analyzed of the heavy metals (Hg, Cd, Pb, Cr) using AA240 Atomic Absorption Spectrophotometer. The results of the heavy metals determination were calculated in mg/kg.

Result

The results of the AAS determination of heavy metals were presented in table 1.

Table 1: Heavy Metal concentration of fish from six Concrete ponds in Onitsha Urban.

Ponds	Heavy Metals Concentration (mg/kg)			
	Cd	Cr	Pb	Hg
Z_I	0.190	0.070	0.030	0.001
Z_{II}	0.000	0.101	0.041	0.037
Z_{III}	0.047	0.000	0.203	0.007
Z_{IV}	0.033	0.001	0.013	0.307
Z_V	0.012	0.032	0.001	0.500
Z_{VI}	0.073	0.020	0.223	0.423
Mean ($\sum$)	0.059	0.037	0.252	0.213
STD($\pm$)	10.064	0.028	0.384	0.225

Discussion

The results of the heavy metal concentrations of fishes from six different concrete ponds in different zones in Onitsha were presented in the table 1. The metals analyzed include Cadmium, Lead, Chromium and Mercury.

The order of concentration of heavy metals defected in the ponds are follows:

Z_I = Cd > Cr > Pb > Hg

Z_{II} = Cr > Pb > Hg - Cd not detected

Z_{III} = Pb > Cd > Hg - Cr not detected

Z_{IV} = Pb > Cd > Hg > Cr

Z_V = Hg > Pb > Cd > Cr

Z_{VI} = Hg > Pb > Cd > Cr

Pond Z_I had the highest concentration of Cadmium (0.190mg/kg) followed by pond Z_{VI} (0.073mg/kg). Cadmium was not detected in pond Z_{II}. The European community (EC)[13] considered acceptable concentration value for cadmium in fish is 0.05mg/kg and the Joint Food Agriculture Organization and World Health Organization (FAO/WHO) 1996 recommended the provisional tolerable weekly intake (PTWI) of Cd is 0.007mg/kg per body weight. The concentration of Cadmium, in these ponds were within the EC permissible Value except for pond Z_I which had a high value probable due to the heavy metal content of the bore hole from which water is channeled and activities of human in that location. But the mean and standard deviation values were (x = 0.06 and I 0.060). Cadmium is very toxic metal, high exposure/accumulation in liver, bones blood, kidney and muscle and could cause diseases like lung disease, (lung cancer) chronic rhinitis, kidney stone, hypercalcuria and hypercalciuria etc [14].

The concentration of lead was between 0.223 – 0.001mg/kg with pond Z_{IV} having the highest concentration and Z_V the lowest concentration. According to the EC (2001) and FAO/WHO/EPA(1996) guideline, the permitted lead level for fish are 0.4 and 0.5mg/kg respectively and Codex standard value is 0.03mg/kg. Apart from Codex standard, the concentration of lead in these pond fall below the required level, this is an advantage because high lead exposure could cause some

diseases like anemia, damage to the kidney, brain and even nervous system, miscarriage, hypertension, imunuetoxicity, etc [15,16].

The chromium content in the fishes was at the range of 0.070 – 0.000 mg/kg. Pond Z_1 – possessed the highest chromium content. Chromium is not detected in the pond Z_{III}. The maximum chromium level permitted for fishes is 0.3mg/kg according to Bulgarian, Food Codex Standing and 0.1mg/kg [15] also for Brazil Heavy metal content standard in fish. The mean chromium content for the selected pond is 0.037 and the standard deviation ± 0.028. The chromium content of all the ponds were below the dangerous level as recommended by the monitoring organization [15].

The highest mercury level in fish was 0.500mg/kg from pond Z_v and pond Z_I had the lowest mercury content. The maximum mercury permitted level for fishes is 0.5mg/kg according to Turkish food codex, Bulgarian food and EC regulation 1886/2006 [13,15,16,] established safety metal quantity limit from eating fishes a weekly maximum dosage commodity known as Provisional Table Weekly Intake (PTWI) perkg of body weight is 5mg total mercury kg^{-1} body weight (bw). The mercury content of fishes from these ponds fall within the referenced permissible limit. All forms of mercury are toxic and their effects include gastrointestinal toxicity, neurotoxicity, nephrotoxic etc [9,17].

Conclusion

Fish is one of the primary sources of heavy metal contamination in man [4]. The study selected four common heavy metals which are among the 176 priority pollutants tested by USEPA 1994. The results of the analysis have shown the presence of heavy metal in the fishes from the selected ponds though on a variable range, but their mean and deviation values were still within the permissible range. Among all the heavy metals studied the mean deviation values indicated that lead and mercury were prominent in Onitsha environment.

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Comparative Qualitative And Quantitative Analyses Of Phytochemicals Found In The Juice And Peels Of Citrus Sinensis (Sweet Orange)

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ABSTRACT

This study provides an explicit perception of *Citrus sinensis*portion (juice and peels) as to which one has more number of phytochemicals-chemicals that may not have nutritional property but can work towards prevention of diseases. The following parameters were determined: tannins, saponins, alkaloids, flavanoids, cardiac glycosides, phytate, oxalate, phenols, steroids and terpenoids. The result showed that the extracts of peels of *Citrus sinensis*had the highest concentration of tannins (31.20%), flavanoids (14.58%), cardiac glycosides (10.83%), oxalate (192, 600mg/100g), phenols (104.42mg/100g), and steroid (7.68mg/100mg). The peels also had moderate concentration of phytate (16.24%), terpenoid (22.16mg/100g_ and also had low concentration of sapponins (3.11%) and alkaloids (2.98%). While the juice extract of *Citrus sinensis* had the higher concentration of cardiac glycoside (4.22%) and terpenoid (22.16mg/100g). A moderate content of tannins (18.64%), alkanoids (5.48%), flavanoids (8.26%) and steroid (1.05mg/100g) was found in the juice extract. The juice extract also had a low concentration of saponin (3.32%), phytate (6.03%),oxalate (44,625mg/100g),phenols (9.95mg/100g).

Key words: Qualitative and quantitative analyses,Phytochemicals, *Citrus sinensis,* juice, peels.

INTRODUCTION

Sweet orange (*Citrus sinensisL.)* is the world's most commonly cultivated fruit tree[1]. It belongs to the family of Rutaceae which consists of mandarins, limes, lemons, grapefruits, sour and sweet oranges[2]. It is one of the most important crops in the world. It is widely cultivated in tropical and subtropical climates for its sweet fruit, peeled or cut, and eaten whole, or processed to extract orange juice[2]. Orange juice of sweet orange is the most important fruit juice consumed worldwide, its market share is continuously increasing and orange production has reached 68.4million tons worldwide[3], with Brazil as the top producer. Orange juice is a rich source of Vitamin C which is considered as the most important water soluble antioxidant. The major role of Vitamin C is the prevention of scurvy which causes the disease that leads to the formation of spots on the skin, spongy gums and bleeding from mucus membranes[4].

The consumption of Citrus fruits is also believed to confer some protection against diseases such as cardiovascular disease and cancer[2]. The peels obtained from citrus fruitsconstitute between 50 and 65% of the total weight of the fruits. When not processed further, this by-product becomes a very disturbing waste capable of causing serious environmental pollution[2]. The term phytochemicals is often used to describe a diverse range of biologically active compounds found in plants. Phytochemicals provide plants with color, flavor and natural protection against pests[2]. They are not essentially required for the sustenance of life but confer extra health benefits against pathogens[2].

MATERIALS AND METHODS

Reagents: 1%HCl, 5% lead ethanoate, 2% HCl, Wagner's reagent, Meyer's reagent, 10% Ferric chloride, 10% lead ethanoate, 2% NH_4OH, 1.0MNaOH, conc. HCl, 50%H_2SO_4, Fehling's solution (A and B), concentrated H_2SO_4, chloroform, sodium nitroprusside solution, 10% ethanoic acid, Ethanol, Conc. NH_4OH, 20% ethanoic acid, 0.1MNaOH, phenolphtalenein, methanol, 5% NaOH, 20(v/v) H_2SO_4 solution, 5% $CaCl_2$ solution,pentanol, 0.3% ammonium, thiocyanate solution, distilled water, sodium carbonate, picric acid, potassium cyanide etc.

Apparatus: Retort stand and clamp, 50ml burettes, conical flasks, boiling tubes, water bath, electronic weighing balance, Whatman No.1 filter papers, 100ml volumetric flasks, 1ml pipettes, watch glasses, beakers, glass stirring rods.

Collection of sample: fresh sweet oranges (*Citrus sinensis)*were purchased from Obirigbo market in Ekwusigo local government area of Anambra state in the month of November,

2017.

Preparation of sample: the fresh sweet oranges were washed twice with distilled water. They were peeled with sterile knife. Thejuice was removed from the fibre by pressing hard on the cut orange. The juice was filtered with the muslin cloth and the residues were discarded while the supernatant was stored in a sterile container for further use. Then the peels were cut into small pieces at the temperature of 40^0c. The dried samples were allowed to cool and pulverized using electric grinder (blender) to obtain the powdered form which was stored in an air tight container for further use.

Qualitative screening: qualitative screening was done using standard methods[5,6] to check the presence of the different phytochemicals in the *Citrus sinensis* juice and peels before qualitative analyses were done. The results of qualitative phytochemical screening of the juice and peels of *Citrus sinensis*(sweet orange) are as stated intable 1.

QUALITATIVE SCREENING:

Determination of tannin by titration

The Folling-Dennis titration method was used as described by Pearson.

100cm^3 of the petroleum ether was added to 20g of the sample in a conical flask and covered for 24hours. Then the sample was filtered and allowed to stand for 15minutes for the petroleum ether to evaporate. It was then re-extracted by soaking in 100$cm^3$of 10% ethanoic acid in ethanol for four(4) hours. The sample was thereafter filtered. To the filtrate was added 25cm^3 of ammonium hydroxide to precipitate the alkaloids. These alkaloids were heated with electric hot plate to remove some of the ammoinium hydroxide still in solution. The remaining volume was measured to be 33cm^3. 20cm^3 of ethanol was added to 5cm^3 of this remaining volume (33cm^3) and titrated with 0.1M NaOH using phenolphthalein as indicator until the solution turned pink. Tannin content was then calculated in percentage.

:. % of tannic acid content= $\dfrac{C_1 x100}{\textit{weight of sample analyzed}}$

Determination of saponin: 5g of the sample was put in 20% ethanoic acid and allowed to stand in a water bath at 50^0c for 24hours. This was filtered and concentrated using a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract till the precipitate was complete. This allowed to settle and the precipitate collected filtration and weighed. The saponin content was calculated in percentage.

% saponin content = $\dfrac{\textit{(weight of filter paper + residue) – weight of filter paper x 100}}{\textit{Weight of sample analyzed}}$

Alkaloid determination: five (5) grams of the sample was weight into a 250ml beaker and 7000cm^3 of 20% ethanoic acid in ethanol was added, it was covered and allowed to stand for four (4) hours at 25^{0}c it was then filtered with filter paper no. 42. The filtrate was concentrated (using a water-bath) to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitate was complete the precipitate was then collected and washed with 1% ammonia solution. Then the solution was filtered with pre-weighed filter paper. The alkaloid (residue) on the filter paper was dried in the oven at 80^{0}c. Finally,the alkaloid content was calculated and expressed as a percentage of the weight of the sample analyzed.

Calculation:

$$\% \text{ weight of alkaloid} = \frac{(\text{weight of filter paper + residue}) - \text{weight of filter paper} \times 100}{\text{Weight of sample analyzed}}$$

Flavonoids determination: 10g of sample was repeatedly extracted with 100cm^3 of 80% aqueous methanol at room temperature. The whole solution is filtered through filter paper. Then the filtrate was transferred into a water-bath and the solution evaporated to dryness. This was dried in the oven at 180^{0}c to constant weight, finally the percentage flavonoid in the sample was calculated thus:

$$\% \text{ flavonoids} = \frac{(\text{weight of beaker + residue}) - \text{weight of beaker} \times 100}{\text{Weight of sample analyzed}}$$

Cardiac glycosides determination: to 1cm^3 of extract was added 1cm^3 of 2% solution of 3,5-dinitrosalicyclic acid in methanol and 1cm^3 of 5% aqueous sodium hydroxide. It was boiled for two(2) minutes until brick-red precipitate was observed. This was filtered, the filter paper with the absorbed residue was dried in an oven at 50^{o}c to constant weight. The weight of the filter paper with the residue was taken, and the % cardiac glycoside content of the sample was calculated thus:

$$\%\text{cardiac glycoside} = \frac{(\text{Weight of filter paper + residue}) - \text{weight of filter paper} \times 100}{\text{Weight of sample analyzed}}$$

Phytate determination: 0.2g of each of the differently processed samples were weighed into different 250ml conical flasks. Each sampleswas soaked in 100cm^3 of 2% concentrated hydrochloric acid for three (3) hours. The samples were then filtered 50cm^3 of each filtrate was placed in 250ml beaker and 100cm^3 of distilled water was added to each sample. This was followed by the addition of 100cm^3 distilled water. 100cm^3 of 0.3% ammonium thiocyanate solution was added as indicator to each sample and titrated with standard iron (iii) chloride solution (0.0012M$FeCl_3$) the percentage phytic acid was calculated using the formular:

$$\text{Phytic acid (\%)} = \frac{\text{Titre value} \times 0.00195 \times 1.19 \times 100}{\text{Weight of sample}}$$

Oxalate determination by titration method: oxalate content of *Citrus sinensis*(sweet orange) titrimetric method.

Digestion: 2g of the sample was suspended in 190cm^3 of distilled water in a 250ml volumetric flask. 10cm^3 of 5MHCl was added. This was digested at 100^{0}c for 1hour, cooled and made up to 250ml mark.

Oxalate precipitation: duplicate portions of 125cm^3 of the filtrate were measured into beakers and four drops of methyl red indicator added to each. Then ammonia solution was added in drops until the solution changes from salmon pink color to faint yellow color (pH 4-4.5). Each portion was then heated to 90^{o}c after which 10cm^3 of 5% $CaCl_2$ solution was added with constant stirring. It was cooled and left overnight at 25^{0}c. The solution was thereaftercentrifuged at 2500rpm for 5minutes. The supernatant was decanted and the precipitate completely dissolved in 10cm^3 of 20% (v/v) H_2SO_4 solution.

Permanganate titration: the total filtrate from the digestion of 2g of sample was made up to 300cm^3. Aliquots of 125cm^3 of each filtrate was heated until near boiling and then titrated against 0.05M standardized $KMnO_4$ solution to a faint pink color which persists for 30seconds. The calcium oxalate content was calculated using the formula:

Oxalate content (mg/100g) = $\frac{Tx(Vme)(Df)x10^5}{ME \times Mf}$

Where Df = $\frac{Vt}{A}$

T = titre volume of $KMNO_4$

Vme = volume-mass equivalent (i.e1ml of 0.05M $KMnO_4$ solution0.00225g anhydrous oxalic acid)

Df = the dilution factor ($^{Vt}/_{A}$ =2.4)

VT = total volume of titrant (300cm^3)

A = the aliquot used (125cm^3)

Me = the molar equivalent of $KMnO_4$ in oxalate ($KMnO_4$ redox reaction) and

Mfr =the mass of sample used.

Phenol determination: the quantity of phenols in the plant samples was determined spectrophotometrically. 2g of each sample (juice and peels) was boiled with 50cm^3 of ethanol for 50minutes. 5cm^3 of the boiled sample was then pipetted into 50ml volumetric flask and 10cm^3 of distilled water added. This was followed by the addition of 2cm^3 of ammonia solution and 5cm^3 of concentrated pentanol. The solution was made up to mark with distilled water and left for 30minutes for color development. Its absorbance was thereafter measured at 505nm wavelength.

Preparation of standard curve: 5cm^3 of the alkaline picrate and 5cm^3 of potassium cyanide solution were mixed in a test tube, and heated for 5 minutes in boiling water bath. The following volumes were pipetted into six test tubes and each volume made up to 10cm^3 with distilled water to give 5, 10, 20, 30, 40 and 50mg respectively. These were covered with rubber stopper and kept in a cool place. The absorbance was measured at 625nm.

Hydrogen Cyanide Determination:

Procedure:

- **Alkaline picrate solution:** 25g of sodium carbon and 5g of picric acid was dissolved in some quantity of distilled water in a liter volumetric flask and finally made up to mark with distilled water. Hydrogen cyanide standard was prepared by dissolving 0.241g of potassium cyanide in some quality of water in a liter volumetric flask and finally making it up to mark with distilled water this gives a solution containing 100mg hydrogen cyanide/millitres.
- 1g if the sample was dissolved in 25cm^{3}of distilled water. 3-4 drops of chloroform, was added to it and stirred. This solution was placed in 500ml conical flask the Whitman No.1 filter paper was cut into strips 10-20cm long and 0.5cm wide and saturated with the alkaline picrate solution prepared. The saturated filter paper was hanged with a cork inside the conical flask and incubated at 20^{o}C for 20-24 hour. The sodium picrate present in the filter paper is reduced to reddish compound in proportion to the amount of hydrocyanic acid evolved. This color was eluted by placing the paper in a clean test tube containing 10cm^{3}of distilled water and compared with standard at625nm.

RESULT AND DISCUSSION

The result of the qualitative analyses of the phytochemical constituents of *citrus sinensis* juice and peel extract are as summarized in table 1.0 below. While tables 2.0 and 3.0 showed the qualitative results of their phytochemical constituents.

Table 1.0 qualitative results of phytochemicals in the juice and peels of *Citrus sinensis*

Peels of *Citrus sinensis*

Parameter	**Juice**	**Peel**
Tannins	++	+++
Saponins	+	+
Alkaloids	++	+
Flavanoids	++	+++
Cardiac Glycosides	+++	+++
Phytate	+	++
Oxalate	+	+++
Phenols	+	+++
Steroids	++	+++
Terpenoids	+++	++

Key: +++=High ++=Moderate +=low

Table 2.0 Qualitative result of phytochemicals in juice of *Citrus sinensis*

PARAMETER	MILLIGRAM/100G	PERCENTAGE (%)
Tannins		18.64
Saponins		3.32
Alkaloids		5.48
Flavanoids		8.26
Cardiac Glycosides		4.22
Phytate		6.03
Oxalate	41,625	
Phenol	9.95	
Terpenoids	22.16	
Steroids	1.05	

Table 3.0 Quantitative Result of phytochemicals in *Citrus sinensis*peels

PARAMETER	MILLIGRAM/100G	PERCENTAGE (%)
Tannins		31.20
Saponins		3.11
Alkaloids		2.98
Flavanoids		14.58
Cardiac Glycosides		10.83
Phytate		16.24
Oxalate	192,600	
Phenol	104.42	
Terpenoids	21.60	
Steroids	7.68	

The preliminary phytochemical investigation revealed the presence of various constituents of the peels and juice of *Citrus sinensis*as shown in table 1.0. Theyshowed the presence of tannins, saponins, alkaloids, flavanoids, cardiac glycosides, phytate,oxalate, phenols, steroids and terpenoids in various degrees. This is in agreement with other works done on juice and peels of *Citrus sinensis*[4,7]. The variations of the concentrations of these phytochemicals found in the juice and peels of *Citrus sinensis* are in agreement with other works done on *Citrus sinensis*[9]. Other researchers reported the presence of these phytochemcials at different concentrations in the juice and peels of *Citrus sinensis*[8]. phytochemicals are known to display their health protective effects in diverse ways. They can act as antioxidants and protect cells against free radical damage or in reducing the risk of cancer by inhibiting tumor production or antibacterial activity and hormonal stimulation[9,10].*Citrus sinensis* juice and peels are rich in nutrients and contain many phytochemicals with strong potential for use in drug production or as food supplements[9,10].

The qualitative phytochemical analysis result are as reported intable 2.0 and 3.0. Theseresults showed that the juice and peels of *Citrus sinensis*contained all the phytochemicals identified in the preliminary qualitative tests (table 1.0) at various degrees. For instance, saponin content was low in both the juice (3.32%) and peel (3.11%) of *Citrus sinensis* while cardiac glycoside was low in juice (4.22%) and moderate (10.80%) in the peels. Others followed the same trend in juice and peels of the *Citrus sinensis*studied. Some of these values are not in agreement with other works done on these parameters. For instance, some researchers [11] reported that the total phenolic contents in *Citrus sinsensis*peels extract ranges from 204.90 to 517.00mg/100g, and total flavanoid content of 97.48 to 177.86mg/100g. Whereas our work showed the phenolic content to be 9.95and 104.42mg/100g in *Citrus sinensis*juice and peels respectively. These observed differences may be due to methods usedin sample preparation. Again, this our work showed *Citrus sinensis*juice and peels to contain 4.22 and 10.83% cardiac glycosides whereas a work done in 2011[12] found them absent.*Citrus sinensis* juice and peels can help the body to be healthy. The utilization of the peels of this fruitwill enhance conversion of waste to wealth. It will also help to manage solid waste and have cleaner environment.

CONCLUSION

From the result got in this work it is clear that *Citrus sinensis*juice and peels contain appreciable amount of phytochemicals at different concentration. These juice and peels of *Citrus sinensis*are potential good sources of nutrients for production of food supplement and as such, their use in this wise should be encouraged. The peels of *Citrus sinensis*also contain important phytochemicals needed to fight various kinds of infections in humans. Thus, effort should be directed towards harnessing their potential in drug formulation and development.

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Investigation On The Mechanical Properties Of Coal Briquette Blends Of Different Biomasses; Eze Nkechinyere Olivia[1], Onuegbu Theresa Uzoma [2], Afiukwa Joseph Nwode [3]

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ABSTRACT

This study investigated the effect of different biomass load and type on the mechanical properties of coal briquette blends. Agricultural wastes of mango seed shell (Mangifera indica), empty palm fruit bunch (Elaeis guineensis) and sugarcane bagasse (Saccharum officinarum) were blended with coal at different ratios and briquetted in a screw press under the compaction pressure of 13.4kPa and force of 190N. Parameters such as bulk density, durability, compressive strength, porosity index were considered to evaluate the strength of the briquettes. The results showed that incorporation of biomass into coal improved some of the useful properties of the resulting briquette blends such as porosity index, durability and compressive strength. The mechanical properties investigated were significantly influenced by the biomass load and type. The study also showed that mango seed shell biomass was most suitable for the production of coal briquette blends.

Keywords: *Coal briquette blends, mechanical properties, agro-waste, waste utilization, biofuel energy.*

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INTRODUCTION

Disposal of biomass wastes produced from different agro-industrial activities is usually an environmental problem. A key factor for such condition is the utilization of these residues for the production of energetic solid bio-fuel by increasing their properties through briquetting.[1] Briquetting is one of the evolving technologies suitable for waste material conversion to solid bio-fuels for energy purposes which has been investigated by several researchers. Briquetting processes helps to solve the problem of accumulated waste available in the environment. Indirectly, briquetting is a way to utilize the total energy available in the environment to generate electricity / thermal heat through the route of briquetting technology[2]. In producing coal briquette blends, the main point is to convert coal waste and agro-residues to agglomerated structure that can be handled and fed to a combustion chamber. This is done by densification of the coal and agro-residues using a press. The densification process creates strong and durable bonding in densified products such as pellets and briquettes. The mechanical properties of the produced briquettes are of primary importance because they are indication of the strength of the briquettes. Mechanical properties indicate the maximum force/stress that the densified products can withstand and the amount of fines produced during handling, transportation and storage. It determines the range of usefulness of a material and establishes the service life that can be expected[3]. The mechanical properties are determined by testing the compressive strength, durability, bulk density, hardness, etc. Compressive strength of a briquette is its resistance to breaking under compression, and it is an indication of the handling characteristics of a briquette. Durability is resistance to abrasion. Durability values represent the relative strength of the particle-particle bonding in the briquettes. Bulk density of a briquette is the energy/volume ratio. High density briquettes have high burning time, and stay long in the briquette stove. Mechanical properties of coal briquette blends are influenced by factors such as type and particle size of materials, binder type and quantity used in briquetting, briquetting pressure, biomass to coal ratio and mass percent of water (moisture content)[2,4].

This study investigated the effect of different biomass materials to coal ratio on the mechanical properties of coal briquette blends.

MATERIALS AND METHODS

The empty palm fruit bunch, mango seed shell and sugarcane bagasse were sourced from farmlands at Mgbabor Echara Community, where they are produced in large quantities and from different dumpsites at Abakaliki, Ebonyi State. The sub-bituminous coal fine was obtained from the Nigerian Coal Corporation, Enugu, Enugu State. The selected biomasses were milled and sieved using standard sieve to obtain particles of size 3mm in diameter. The coal fines were sieved through a standard sieve of 1mm size to achieve the desired surface area of the raw materials shown in plate 1-4.

Plate 1: Empty palm fruit bunches **Plate 2: Mango seed shell** **Plate 3: Sugarcane Bagasse** **Plate 4: Coal fines**

Briquette Formulation: The briquettes were produced using a screw press. Briquettes of varied biomass loads were produced by blending the biomasses at various ratios with coal fines in the ratios; 100:0; 80:20; 60: 40; 40: 60; 20:80 and 0:100. $Ca(OH)_2$ (5%) based on the mass of the coal was used as the desulphurizing agent and 10% cassava starch based on the entire mass of the mixture was used as the binder. The cassava starch, coal, biomass, and $Ca(OH)_2$ were mixed thoroughly, filled in the briquette press mould and briquetted at a pressure of 13.4kPa .The briquettes were removed from the moulds after a 5 min and sundried for two weeks [5,6].

Plate 5: Formulated Briquettes

Table 1: Briquette sample blend ratio and codes

Briquette sample	Coal	Coal- Mango Seed Shell					Coal - Sugarcane Bagasse					Coal - Empty Palm Fruit Bunch				
Ratio	100:0	80:20	60:40	40:60	20:80	0 : 100	80:20	60:40	40 :60	20:80	0 : 100	80:20	60:40	40:60	20:80	0:100
Code	C	CM_1	CM_2	CM_3	CM_4	CM_5	CS_1	CS_2	CS_3	CS_4	CS_5	CE_1	CE_2	CE_3	CE_4	CE_5

Determination of the mechanical properties of the briquettes

The mass of each briquette composition was measured using an electronic weighing balance Model MB 2610, England. The average mass of each briquette composition was recorded.

Bulk density: The bulk density of the briquettes was calculated as follows[2]:

$$D_{bulk}(g/cm^3) = \frac{\text{mass of the sample}}{\text{volume of the sample,}} \quad [1]$$

where volume of the briquettes $= \pi R^2 h - \pi r^2 h$

R=radius of the outer circle, r = radius of the inner circle, h=height of the briquette.

The density recorded was determined after 14 days of drying the briquettes.

Compressive strength: This was determined using a compressive strength testing machine, Model 2914, USA. The machine measures the compressive force of the briquette samples. The compressive strength of the briquettes was calculated using the equation:

$$\text{Compressive strength } (N/mm^2) = \frac{\text{compressive force (N)}}{\text{cross sectional area of the sample } (mm^2)} \quad [2]$$

Porosity index: This was determined by weighing each briquette sample (w_1) and immersing in a 500 cm^3 of water, for 2 min. They were removed from the water, left for 10 min to allow excess water to drip off and reweighed (w_2). The quantity of water absorbed by the briquette was also noted. Porosity index was calculated as the ratio of the mass of water absorbed by the samples to the mass of the sample immersed in water[2].

$$\text{Porosity index} = \frac{\text{mass of water absorbed by the sample}}{\text{mass of the sample immersed in water}} \quad [3]$$

Hardness: The hardness of the briquette samples was determined using Eseway hardness Testing machine Type DVRB-P USA, and the values were read directly from the machine. **Durability:** This was determined by weighing each briquette W_1 and keeping them in a metallic box with a cover. The box was shaken for 3 min and after which the remaining briquette was weighed W_2. Durability was calculated as[2]:

$$\text{Durability} = \frac{\text{weight of the briquette after shaking the box } (W_2)}{\text{weight of the briquette before shaking the box } (W_1)} \quad [4]$$

The durability of each briquette was rated based on the durability rating proposed by[7]

RESULTS AND DISCUSSION

Table 2: Mechanical Properties of the Briquette Samples

Briquette samples	Mass (g)	Bulk density (g/cm^3)	Compressive strength (N/mm^2)	Porosity Index	Hardness	Durability
C	333.4 ± 1.35	1.986 ± 0.06	1.21 ± 0.03	0.49 ± 0.05	658 ± 1.00	0.64 ± 0.01
CM_1	288.6 ± 0.26	1.719 ± 0.07	1.35 ± 0.04	0.68 ± 0.04	603 ± 0.58	0.70 ± 0.03
CM_2	235.1 ± 0.36	1.400 ± 0.02	3.01 ± 0.03	0.77 ± 0.00	523 ± 0.00	0.77 ± 0.02
CM_3	186.9 ± 0.36	1.128 ± 0.01	4.19 ± 0.07	1.31 ± 0.05	415 ± 0.00	0.85 ± 0.05
CM_4	167.9 ± 0.40	1.113 ± 0.00	3.82 ± 0.21	1.76 ± 0.02	404 ± 0.00	0.82 ± 0.02
CM_5	144.2 ± 0.66	0.892 ± 0.00	2.41 ± 0.00	2.17 ± 0.11	398 ± 0.00	0.80 ± 0.04
CS_1	240.8 ± 0.17	1.434 ± 0.01	1.29 ± 0.07	0.98 ± 0.02	503 ± 0.00	0.72 ± 0.01
CS_2	225.7 ± 0.26	1.344 ± 0.06	2.85 ± 0.33	1.24 ± 0.05	432 ± 0.00	0.78 ± 0.03
CS_3	186.9 ± 0.36	1.113 ± 0.01	4.05 ± 0.11	1.59 ± 0.15	338 ± 0.00	0.85 ± 0.02
CS_4	167.9 ± 0.40	1.000 ± 0.02	3.85 ± 0.06	2.17 ± 0.04	317 ± 0.58	0.83 ± 0.03
CS_5	144.2 ± 0.66	0.859 ± 0.04	2.11 ± 0.06	2.85 ± 0.10	309 ± 1.00	0.80 ± 0.04
CE_1	249.3 ± 0.40	1.485 ± 0.01	1.69 ± 0.29	0.92 ± 0.07	523 ± 0.00	0.66 ± 0.02
CE_2	245.5 ± 0.26	1.462 ± 0.04	3.77 ± 0.05	1.26 ± 0.02	463 ± 1.15	0.70 ± 0.02
CE_3	199.5 ± 0.36	1.188 ± 0.01	3.06 ± 0.12	1.52 ± 0.11	400 ± 0.00	0.80 ± 0.01
CE_4	185.7 ± 0.44	1.106 ± 0.00	2.71 ± 0.26	1.85 ± 0.08	332 ± 0.00	0.76 ± 0.03
CE_5	144.6 ± 0.40	0.861 ± 0.00	2.05 ± 0.02	2.49 ± 0.07	305 ± 0.00	0.72 ± 0.02

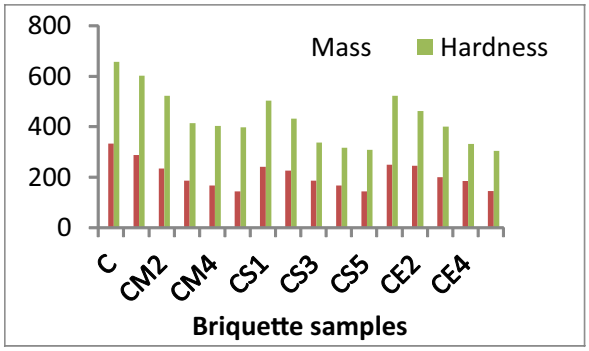

Figure 1: Mass and hardness of the briquettes porosity index

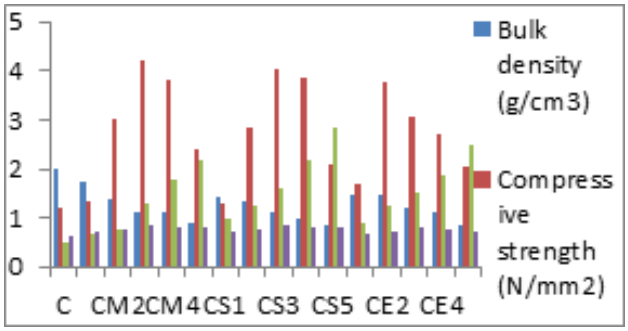

Figure 2: Bulk density, compressive strength, and durability of the briquettes.

The mass of the briquettes decreased as the biomass load increased. This was attributed to the mineral load origin of coal, which resulted to heavier molecular weight than the biomass which is of plant origin[4]. Hardness also decreased as the biomass load increased. There is a significant effect of the ratio and biomass type on the hardness and mass of the briquettes.

The bulk density of the briquettes decreased as the biomass load increased, and is as a result of biomass having low bulk density compared with coal. High density briquettes have high burning time and are desirable in terms of transportation, storage, and handling. They are therefore desirable in various kinds of thermal applications. The bulk density values obtained are comparable to those obtained by[8, 9] .The compressive strength results showed that biomass increased the compressive strength of the coal briquette to a certain point, beyond which it started to drop. The increase in compressive strength as biomass increases is attributed to biomass nature (soft, fibrous, non friable), and its ability to reduce the brittleness of the coal briquette. This finding agreed with that reported by [10]. A briquette with high compressive strength value can easily be handled, stored, and transported from one place to another without generating dust or fines. The porosity index of the coal briquette blends increased with increased biomass load and is higher than that of the coal briquette. This is attributed to the fibrous nature of the biomass which creates pores in the briquette blends, and density of the briquettes. The CS briquettes with the highest porosity index values had the lowest density values, then the CE and CM briquettes. An increase in porosity index with increase in biomass load due to decrease in density of the briquette was also reported by [7, 8, 11, 12, 13, 14]. The durability of the coal briquette blends increased with increase in biomass load up to a certain extent, and then declines. The trend observed in the durability might be attributed to the chemical composition (e.g. presence of lignin, hemicelluloses, cellulose, extractives) and particle nature of the biomasses (fibrous and non friable), since the same compaction pressure and quantity of binder was used. The coal briquette has low durability because it is brittle and easily disintegrates during shaking of the metal box.

CONCLUSION

The mechanical properties of coal briquette blends depended largely on the biomass type and the ratio of biomass to coal. Coal briquette blends from mango seed shell proved more satisfactory than those of sugarcane bagasse and empty palm fruit bunch because they have moderate porosity, higher bulk density, more durable and hard than the other briquettes. The mechanical properties of the briquettes were significantly influenced by the biomass load and biomass type. Coal briquette blends have better mechanical properties (such as porosity index, durability and compressive strength) than the single coal briquette and biomass briquettes.

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