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Research article

COMPARATIVE STUDY OF INVITRO ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *Piper guineense, Curmuma longa, Gongronemalati folium, Allium sativum, Ocimum gratissimum*

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ABSTRACT

In an attempt to explain the scientific basis for the medicinal and nutritional benefits of some spices; the Phytochemical constituents, Antioxidant and Antibacterial activity were assessed. In this study, the Antioxidant properties of ethanolic*Ocimumgratissimum***,** *Gongronemalatifolium*, *Piper guineense* (leaves), *Allium sativum* (bulb) and *Curcuma longa*extracts were evaluated using Lipid Peroxidation and Nitric Oxide Scavenging activity. The extracts were subsequently analysed for their phytochemical constituent and the ability of the extracts to inhibit the growth of some Bacteria (*Staphylococcus aureus, Streptococcus pneumonia, Escherichia coli, Proteus mirabilis, Pseudomonasaeruginosa*). The result of the study revealed that Piper giuneese (leaves), *Curcuma longa, Allium sativum*, *Ocimumgratissimum*,*Gongronemalatifoliumextracts* contain Tannin, Phenols, Alkaloid, Flavonoid and Hydroden cyanide (HCN). The total phenol content of the extracts is 0.54% for Allium sativum, 0.33% for Curcuma longa, 0.22% for *G. latifolium*, 0.27% for *O. gratissimum*, 0.16% for *P. guineense* per 5 g of each sample, overall percentage inhibition Lipid Peroxidation activity of *C. longa* was highest followed by *G. latifolium*, *O. gratissimum*, *A. sativum* and then *P. guineense*. Allium sativum and *P. guineense* showed lowest percentage inhibition of Nitric Oxide Scavenging activity. All the ethanolic extracts exhibited Antioxidant activity significantly. The inhibition of *S. aureus*, *S. pneumonia*, *E. coli*, *P. mirabilis*, *P. aeruginosa* were found to be concentration dependent; the lowest concentration- 15.5mg/ml did not inhibit any of the Bacteria. It could be therefore concluded that the consumption of these spices would exert several benefial effects by virtue of their antioxidant and antibacterial activity.

KEY WORDS: Piper giuneense,Curmuma,Gongronemalatifolium,Allium ativum,Ocimumgratisumum, Antimicrobial, Antioxidant,spices

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INTRODUCTION

Nature has been a source of medicinal treatment for thousands of years and plant-based systems continue to play an essential role in the primary health care of 80% of the world's underdeveloped and developing countries (Kalpana and Kodukkur, 2011).Plants have formed the basis of traditional medicine system that has been the way of life for thousands of years. Mostly, herbs and species contain polyphenols which are most powerful natural antioxidants and are highly valued for their antioxidant, anti-ageing antimicrobial effects. Antioxidants are widely used as ingredients in dietary supplements and are exploited to maintain health and prevent oxidative stress-mediated diseases. Antioxidant compounds like phenolic acids, polyphenols and flavoniodsinhibit the mechanism that leads to degenerative diseases (Hamid et al., 2010).

Under certain condition; oxygen can seriously affect our well being through the formation of reactive oxygen species (ROS) representing both free radical and non-free radical species which leads to the potential deleterious effects such as atherosclerosis, ischemic heart disease, ageing, inflammation, diabetes, immune suppression, neurodegenerative diseases, cancer and other diseases (Jadhav and Blutani, 2002).
Therefore, antioxidants with free radical scavenging activities may have great significance in the prevention and therapeutics of free radical mediated diseases.

Antioxidant compound in food play an important roles as a health-protecting factor. Plant sourced food antioxidants like vitamin C, vitamin E, Carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk (Prakash et al., 2007).

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavoniods scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (parkash et al., 2007).

Free radicals are created when cells use oxygen to generate energy. These bye-products are generally reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide that result from cellular redox process. At low or moderate concentrations, ROS exert beneficial effects on cellular responses and immune function but at high levels, free radicals and oxidants generate oxidative stress, a deleterious process that can damage cell structures, including lipids, proteins and DNA (Pham-Huyet al., 2008). Oxidative stress plays a major part in the development of chronic and degenerative ailments such as cancer, autoimmune disorders, rheumatoid arthritis, cataract, aging, cardiorascular and neurodegerative diseases (Willcox et al., 2004; Pham-Huy et al., 2008).

The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced insitu, or externally supplied through food and /or supplements. These antioxidants act as free radical scavengers by preventing and repairing damages caused by ROS, and therefore can enhance their immune defence and lower the risk of cancer and degenerative diseases (Pharm-Huy et al, 2008).
The most effective components seen to be flavonoids and phenolic compounds of many plant raw materials, particularly in herbs, seeds, and fruits. Their metal-chelating capabilities and radical-scavenging properties have enabled phenolic compounds to be thought of as effective free radical scavengers and inhibitors of lipid peroxidation (terao and Piskula, 1997).
Parameters used in this study to detect antioxidant activity include Nitric oxide scavenging activity, lipid peroxidation activity.

Lipid peroxidation can be defined as the oxidative deterioration of lipids containing any number of carbon-carbon double bonds.Lipid peroxidation is a major cause of food deterioration, leading to a loss of functional properties and nutritional value (yen et al., 1999). Oxidized polyunsaturated fatty acids may induce aging and carcinogenesis. The major pathway of lipid peroxidation contains a self-catalytic free radical chain reaction. However, lipid peroxidation can be catalyzed by environmental factors, such as light, oxygen, free radicals and metal ions (frankel, 1991). Synthetic antioxidants such as butylatedhydroxyanisole (BHA), butylatedhydroxytoulene (BHT), propyl gallate (PG) and terbutylhydroquinone(TBHQ) have been dominant since their introduction. They have been used as an antioxidant in foods for years. However, some physical properties of BHT and BHA such as their high rolatility

and instability at elevated temperatures, side effects and strict legislation (even recently by the country's food and drug regulating body NAFDAC) on the use of synthetic food additives. Consumers are increasingly avoiding foods prepared with preservatives of chemical origin, and natural alternatives are therefore needed to achieve sufficiently a long shelf life of foods and a high degree of safety. Therefore, the commercial development of plants as sources of antioxidant to enhance health and food preservation is of current interest. Many medicinal plants possess potent antioxidant activity and examples of them are rosemary and sage. Herbs are also found to be potent sources of natural antioxidants as well as retard lipid oxidative rancidity in foods.
The nitric oxide radical (NO) is a labile molecule, which is generated in mammalian cells. Many different biological functions have been ascribed to nitric oxide, among which vasorelaxation, inhibition of plate aggregation, cell-mediated immue responses and neuro transmission.
Apart from its role in physiological processes, NO also have toxic properties, especially after reaction with oxygen or superoxide anion radicals. The reaction products which are formed, NO_x and $ONOO^-$(peroxynitrite), are able to inflict sever cellular damage. In lung tissue, the toxicity of NO is of specialinterest, since the oxygen concentration is high and expose to both endogenous and exogenous NO occurs. Exogenous NO in the lung may arise from cigarette smoke or air pollution.Severe studies report the formation of s-nitrosothiols from the reaction of NO with sulfur- containing compounds.
Gaston et al demonstrated that the concentration of s-nitrosothiols in air way epithelial lining fluids is increases at pathological conditions and upon inhalation of exogenous NO. These findings may indicate that sulfur-containing compounds play a role in the protection against NO toxicity.

It is also reported that s-nitrosothiols may release NO again or exert NO-like activity. This indicates that sulphur containing compounds play a dualistic role; they scavenge NO, but may also prolong the physiological activity of NO. Because of the pivotal role of NO in physiology, it is of importance to have a fast screening method for the reaction of NO with both exogenous and endogenous compounds.

Medicinal plants possessing natural antioxidants polyphenols such as anthraquinones, flavonoids, aromatic acids, and tannins have been shown to have ROS scavenging and lipid peroxidation prevention effects (Hong et al., 1994; Houghton,2008). The commercial development of plants as sources of antioxidants to enhance health and food preservation is of current interest (Rice-Evans et al., 1997). Epidemiological studies have suggested positive associations between the consumption of phenolic-rich foods or beverages and the prevention or diseases (Scalbert and Williamson, 2000). These effects have been attributed to antioxidant components such as plant phenolics, including flavonoids and phenylpropanoids among others (rice-Evans et al., 1997).

Evidence is mounting for the role of these dietary phytochemicals, including flavonoids, ascorbic acid, α-tocopherol, and carotenoids, in the maintenance of health and protection from diseases (Torel et al., 1996 ;Cos et al., 1998). As plants produce antioxidants to control the oxidative stresscaused by sunlight and oxygen,they because a source of useful new compounds with antioxidant activity. Therefore, there is a growing interest in natural and safe antioxidant for food applications and a growing trend in consumer preference towards natural antioxidants, all of which have given impetus to the attempts, to explore actual sources of antioxidants (Gulcin, 2007).

Recently, there is an increasing interest in finding natural antioxidants from plant materials to replace synthetic ones. Natural antioxidants in plants are capable of terminating a free radical mediated oxidative reaction and would have beneficial activities in protecting the human body from such diseases (Havsteen, 2006;).

The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. Down the ages extracts of plants have evoked interest as source of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases (Tepe et al., 2004).The World Health Organization (WHO) noted that majority of the world's population depends on traditional medicine and constitutesa major source of natural organic compounds.

Antimicrobial substances are those substances that are capable of destroying or inhibiting the growth of microorganism (Houghton, 2006).Existence of microorganisms causes food spoilage and results in deterioration of the quality and quantity of processed food products. Some plant-based biologically active compounds isolated from herbs have been explored for the growth inhibition of pathogenic microbes because of their antimicrobial potential

(Abubakar et al.,2008). The medicinal value and multiple biological functionalities of several plants are defined by their phytochemical constituents (Fallah et al., 2005). Many herbal species being a promising source of bioactive compounds such as phenolics, anthocyanins, flavonoids, and carotenoids, are usually used to impart flavor and enhance the shelf-life of dishes and processed food products, recently reported work was (Nisar et al., 2010a,). Due to their high antioxidant potency, the consumption of many such plants species is recommended (Ozsoy et al., 2009).

Therefore, action must be taken to reduce this problem, such as controlling the use of antibiotics, carrying out research to better understand the genetic mechanisms of resistance, and continuing investigations aiming at the development of synthetic or natural new drugs(Omale et al., 2010).
The aim of this study is to evaluate and compare the invitro antioxidant and antibacterial activities of ethanolic extracts of some spices (*Piper guinense, Curcuma longa, Ocimumgratissimum, Gongronenalatifolium, Allium sativum)*

GONGRONEMA LATIFOLIUM (UTAZI)

Figure 1:*Gongronemalatifolium* plant.

OCIMUM GRATISSIMUM

Figure 2:*Ocimumgratissimum*

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PIPER GUIEENSE (UZIZA)

Figure 3:*Piper guineense*

ALLIUM SATIVUM (GARLIC)

Figure 4:*Allium sativum*

2.10 CURCUMA LONGA (TUMERIC)

Figure 5:*Curcumalonga*

MATERIALS AND METHODS

MATERIALS

Plant materials	Parts of plant used	Local name
Alliumsativum	Bulb	Garlic
Curcuma longa	Rhizome	Turmeric
Gongronema latifolium	Leaves	Utazi
Ocimum gratissimum	Leaves	Nchuanwu
Piperguineense	Leaves	Uziza

CHEMICALS

Ascorbic acid, sodium nitro prusside, Feric chloride, disodium phosphate, phosphoric acid, sulphalinamide, monosodium phosphate, thiobarbittiric acid (TBA), trichloroacetic acid (TCA) and α napthylethylenediaminedichlonide, ethanol, distilled water were purchased fromUhil Chemicals WWLS, Aba .

COLLECTION OF PLANT MATERIALS AND EXTRACTS PREPARATION

Plant material of *Ocimumgratissimum, Gongronemalatifolium* and *Piper guineense* (leaves) where collected from UmuahiaAlaocha, Umuahia North L.G.A, Abia State. *Curcuma longa* was collected from National Root Crop Institute, and *Allium sativum* was bought from Umuahiamain market. They were identified by Prof. F.Ekeleme of the College of Crop and Soil Sciences, MichealOkpara University of Agriculture, Umudike.

The plants were dried and the dried samples were powdered with electrical blender and made into coarse powder and stored in airtight container at room temperature.

Approximately 50g or the sample were taken and extracted using cold percolation method, using ethanol (250ml) as the organic solvent. The extractions were carried out for approximately 48hrs.
Extracts were vacuum evapourated, dried and stored in an air tight container for further analysis.

PHYTOCHEMICAL ANALYSIS

The extracts were subjected to quantitative chemical test. Ethanol and aqueous extracts revealed the presence of tannins, alkaloids, phenol, flavoniods, and hydrogen cyanide (HCN).

a) Determination for Tannin

Tannin content of the sample was determined by Folin Denis colometric method (Kirk and Sayer1998). A measured weight of the processed sample (5.0g) was mixed with distilled water in the ratio of 1:10(w/v). The mixture was shaken for 30 minutes at room temperature and filtered to obtain the extract.

A standard tannic acid solution was prepared, 2ml of the standard solution and equal volume of distilled water were dispersed into a separate 50ml volumetric Flasks to serve as standard and reagent blank respectively. Then 2mls of each of the sample extracts was put in their respective labeled Flask.

The content of each Flask was mixed with 35ml distilled water and 1ml of the Folin Denis reagent was added to each. This was followed by 2.5mls of saturate Na_2CO_2 solution. Thereafter each flask war diluted to the 50ml mark wit distilled water and incubated for 90 minutes at room temperature. Their absorbance was measured at 760nm in a spectrophotometer with the reagent blank at zero. The Tannin content was calculated as show below.

% Tannin-100/w x au/as x c x vt/va
w =weight or sample
au =absorbance of test sample
as =absorbance of standard tannin solution
c =concentration of standard tannin solution
va =volume of extract analyzed.

b) Determination of Alkaloid

The Alkaline precipitation gravimetric method (harborne, 1975) was used.
A measured weight of the processed sample (5g) was dispersed in 100mls of 10% acetic acid in ethanol solution. The mixture was shaken well and allowed strand for 4 hours at room temperature being shaken every 30 minutes. At the end of this period, the mixture was filtered through what man No 42 grade of filter paper.

The filtrate (Extract) was concentrated by evaporation, to a quarter of it's original volume the extract was treated with drop wise addition of concentrated NH_3 solution to precipitate the alkaloid. The dilution was done until the $NH_{3\ was}$ in excess.

The alkaloid precipitate was removed by filteration using weighted whatman No 42 Filter paper. After washing with 1% NH_4 OH solution, the precipitate in the filter paper was dried at 60^0c and weighted after cooling in desiccators. The content was calculated as shown below.

$$\% \text{ Alkaloid} = \frac{W_2 - W_1 X}{\text{Wt or sample}} \quad \frac{100}{1}$$

Where W_1 = weight of empty filter paper

W_2 = weight of filter paper + alkaloid ppt.

c) Determination of Phenol

This was determined by the Folin-ciocateau spectrophotometer (AOAC, 1990). The total phenol was extracted in 200mg of the sample with 10ml concentrated methanol. The mixture was shaken for 30minutes at room temperature. The mixture was centrifuged at 500rpm for 15 minutes and the supernatant (extract) was used for the analysis.

1ml portion of the extract from each sample was treated with equal volume of Folin-ciocateau reagent followed by the addition of 2mls of 2% Na_2CO_3 solution. Mean while, standard phenol solution was prepared and diluted to a desired concentration.

1ml of the standard solution was also treated with the F-D reagent and Na_2 Co_3. The intensity of the resulting blue colouration was measured (absorbance) in a spectrophotometer at 560nmwavelength. Measurement was made with a reagent blank at Zero. The phenol content was calculated using the formula below.

$$\%\text{phenol} = \frac{100}{W} \times \frac{au}{as} \times c \times \frac{vt}{va}$$

Where W =weight of sample
au =absorbance of test sample
as =absorbance of standard phenol sample
c =concentration of standard phenol sample
va =volume of extract analyzed.

d) Determination of Flavoniods

Flavonoid was determined suing the method described by Harbone (1975). A measured (5g) weight of the processed sample was boiled in 100mls of 2MHCL solution under reflux for 40 minutes. It was allowed to cool before being filtered. The filterate was treated with equal volume of ethyl acetate (contained in the ethyl acetate portion) was received by filteration using weighted filter paper. The weight was obtained after dying in the oven and cooling in a desicator. The weight was expressed as a percentage of the weight analyzed. It was calculated as shown below:

$$\%\ \text{Flavonoid} = \frac{W_2 - W_1}{\text{Wt of sample}} \times \frac{100}{1}$$

W_2 =weight of filter paper x flavonoid precipitate

W_1 =weight of filter paper alone

e) Determination of Hydrogen Cyanide (HCN)

This was determined by alkaline pikratecolourimeter method by Balogopalin et al (1988)
1.02g of the sample was dispersed in 50ml of distilled water in a 25.0ml conical flack. An alkaline pikrate paper was hung over the sample mixture and the blank in their respective flasks.

The set up were incubated overnight and each pikrate paper was eluted (ordipped) into a 60ml of distilled water. A standard cyanide solution was prepared and diluted to a required concentration. The absorbance of the eluted sample solution and the standard were measured
spectophotometrically at 540nm wavelength with the reagent blank at zero.
The cyanide content was determined by the formular shown below.

$$\text{HCN mg/kg} = 1000/w \times au/as \times c \times D$$

Where W =weight of sample analyzed
au = absorbance of standard HCN solution
c= concentration of the standard in mg (d)
D=dilution factor where applicable.

DETERMINATION OF ANTIOXIDANT ACTIVITY

a) AntilipidPeroxidation Activity

Animal material: goat liver was collected from local market in Umuahia. Goat liver perfused with normal saline through hepatic portal vein was harvested and its lobes were briefly dried between filter papers to remove excess blood and thin cut with a heavy-duty blade. The small pieces were then transferred in a sterile vessel containing phosphate buffer (pH 7.4) solution. After draining the buffer solution as completely as possible the live was

immediately grinded to make a tissue homogenate(1gml) using freshly prepared phosphate buffer (pH 7.4)(Dinakaan et al., 2011).
The Ethanolic extracts of the samples were used in various concentration (3000,2000,1500, 1000 and 500 µg/m1) individually. 3ml of liver homogenate was added with 100µl of 15mm ferric chloride solution and was shaken for 30min. from collected mixture, 100µl of different concentration of both plant extracts individually in different test tables.

The same procedure was followed for control and blank. Water was used as a control and ascorbic acid (100µg/ml) as standard. All the test tables were incubated for 4hrs at 37^0c. After incubation, Trichloracetic acid (TCA) was added to all tubes containing the mixture in 1:1 ratio and centrifuged for 30min. the supernated liquid was collected and thiobarbituric acid (TBA) was added in 1:1 ratio and heated for 1hr in water bath, cooled and absorbance was measured at 530nm. By using the following formula then measured at 530nm. By using the following formula the percentage of anti-lipid peroxidation activity was calculated. (Dinakaan et al., 2011).

$$\% \text{ antilipid peroxidation} = \frac{(\text{control-sample})}{(\text{control})} \times 100$$

b) Nitric Oxide Scavenging Activity

The nitric oxide scavenging activity was conducted based on the greiss assay method 2.0ml of 10mm sodium nitro prusside and 5.0ml of phosphate buffer were mixed with 0.5ml of different concentrations of the plant extracts and incubated at 250c for 150min. the sample was run as above but the blank was replaced with the same amount of water. After the incubation period, 2ml of the above incubated solution was added to 2ml of greiss reagent and incubated at room temperature for a period of 30mins.
The absorbance was measured immediately at 546nm and percentage of scavenging activity was measured with reference to ascorbic acid as standard.
Greiss reagent (1% sulphanilamide 0.1% naphthylethylethylenediamine dichloride and 3% phosphoric acid) (Jaiswal et al., 2010).

DETERMINATION OF ANTI-BACTERIAL ACTIVITY

Test microorganism

For this study,*Streptococcus pneumonia, Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa* were used as test organism.

Preparation of Different Concentrations of Extracts

Sterile 1ml dropper pipettes were used to deliver 0.1ml of each concentration onto punched sterilized circular (3mm diameter) filter papers (whatmann number 1). The extract impregnated disc were dried at 40^0c, packed into sterile bottles, labeled and stored at 8^0c prior to sue. Thus, the final concentrations of extract per disc in descending order was 250mg, 125mg, 61.5ml, 31.25mg and 15.5mg (Egwari, 1999).

Screening for Antibacterial Activity of Extracts

The disc diffusion method was used in this study. The test organisms (1:100 dilution of an 18h broth cultures) were inoculated onto Mueller Hinton agar plates with sterile cotton swabs (sterlin) soaked in the inoculate. Discs of different extract concentrations were placed firmly on the surface of the inoculated agar plates and incubated at 37^0c for 18h under aerobic conditions. Zones of inhibition were measure and recorded in millimeters (Egwari, 1999).

Minimum Inhibitory Concentration

The MIC values were intepreted as the highest dilation (lowest concentration of the sample, which showed clear zone. All tests were performed in duplicates.

RESULTS

PHYTOCHEMICAL INVESTIGATION RESULT

The phytochemical screening carried out on ethanolic extract of the plant samples revealed thepresence of phenols, tannins, alkaloids, flavoniods and Hydrogen cyanide.

Table 2: Quantitative analysis of phytochemicals in the plant extracts

Sample	**Tannin**	**Phenol**	**Alkaloid**	**Flavonoid**	**HCN**
	(%)				(mg/kg)
Allium sativum	0.21	0.54	0.12	0.73	2.62
Curcuma longa	1.84	0.33	0.62	0.61	4.76
Gongronemalatifolicum	0.26	0.22	1.64	0.29	3.95
Ocimumgratissium	0.13	0.32	0.27	0.31	4.21
Piperguineense	0.30	0.16	0.14	0.22	5.93

ANTIOXIDANT ACTIVITY RESULT

The antioxidant activity of the extracts was accessed by Nitric Oxide Scavenging Activity and Lipid Peroxidation Activity.

Nitric Oxide Scavenging Activity

The percentage inhibition of Nitric Oxide Scavenging Activity of*Allium sativum*, *Piper guineense*, *Gongronemalatifolum*, *Curcuma longa*, *Ocimumgratissimum*is shown in Table.3 and Fig. 6. In table 3, the highest concentration (3.0 mg/ml) of all plant samples showed the greatest percentage inhibition activity.

TABLE 3:%Inhibition of Nitric Oxide Scavenging Activity of the 5 Samples

Concentration (mg/ml)	*Piper guineense*	*Gongronemalatifolum*	*Curcuma longa*	*Ocimumgratissimum*	*Allium sativum*
3.0	79.69±2.206	83.60±1.110	81.25±2.206	83.60±1.110	80.47±3.309
2.0	64.85±1.110	73.44±2.206	67.19±2.206	70.32±2.213	75.00±2.206
1.5	49.22±1.103	66.41±1.103	62.50±2.206	66.41±1.103	66.41±1.103

1.0	33.60±1.110	63.28±1.103	49.22±1.103	63.28±1.103	59.38±0.000
0.5	13.28±1.103	47.66±1.103	30.47±1.103	44.53±1.103	48.22±2.517
Standard Control	53.13±0.000	53.13±0.000	53.13±0.000	53.13±0.000	53.13±0.000

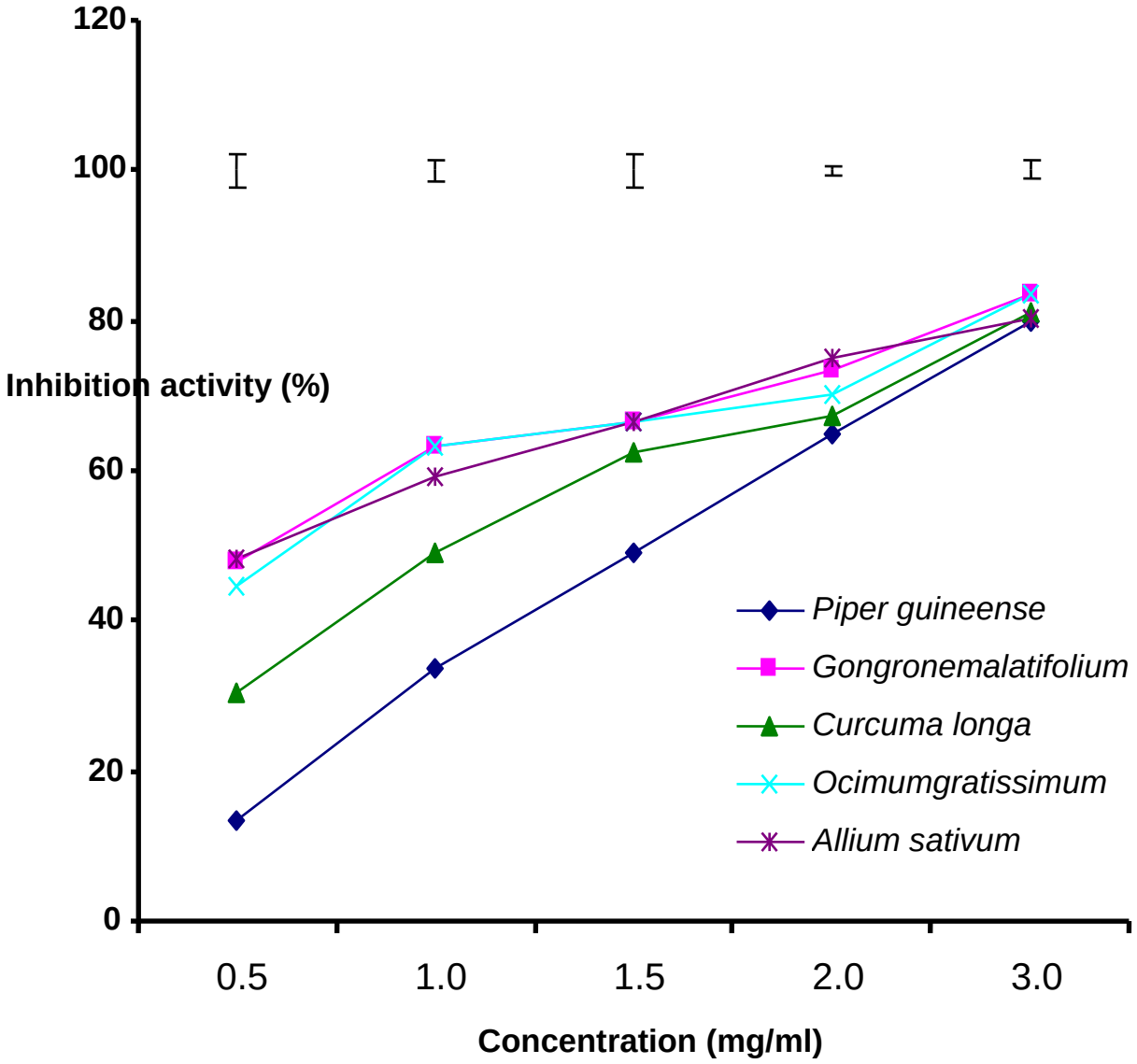

Figure 6: Effect of ethanolic extracts on Nitric oxide scavenging activity.

Lipid PeroxidationActivity

The Percentage Inhibition of Lipid PeroxidationActivity of *Allium sativum*, *Piper guineense*, *Gongronemalatifolum*, *Curcuma longa*, *Ocimumgratissimum*is shown in Table 4 and Fig. 7 . Percentage Inhibition of Lipid Peroxidation activity of *Allium sativum*is lowest compared with the other samples.

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Table 4:%Inhibition of Lipid Peroxidation Activity of the 5 Samples

Concentration (mg/ml)	*Piper guineense*	*Gongronemalatifolium*	*Curcuma longa*	*Ocimumgratissimum*	*Allium sativum*
3.0	31.21±1.287	56.36±0.000	78.03±0.431	56.06±0.424	47.27±0.863
2.0	26.67±0.856	51.21±0.424	64.54±0.424	51.22±0.431	32.73±0.865
1.5	20.91±431	46.37±0.481	63.34±0.431	50.00±0.424	30.30±0.856
1.0	9.69±0.856	34.55±0.856	62.12±0.431	46.37±0.431	6.37±0.431
0.5	7.28±0.856	23.64±0.836	60.91±0.431	39.09±0.424	2.73±0.431
Standard Control	36.97±0.000	36.97±0.000	36.97±0.000	36.97±0.000	36.97±0.000

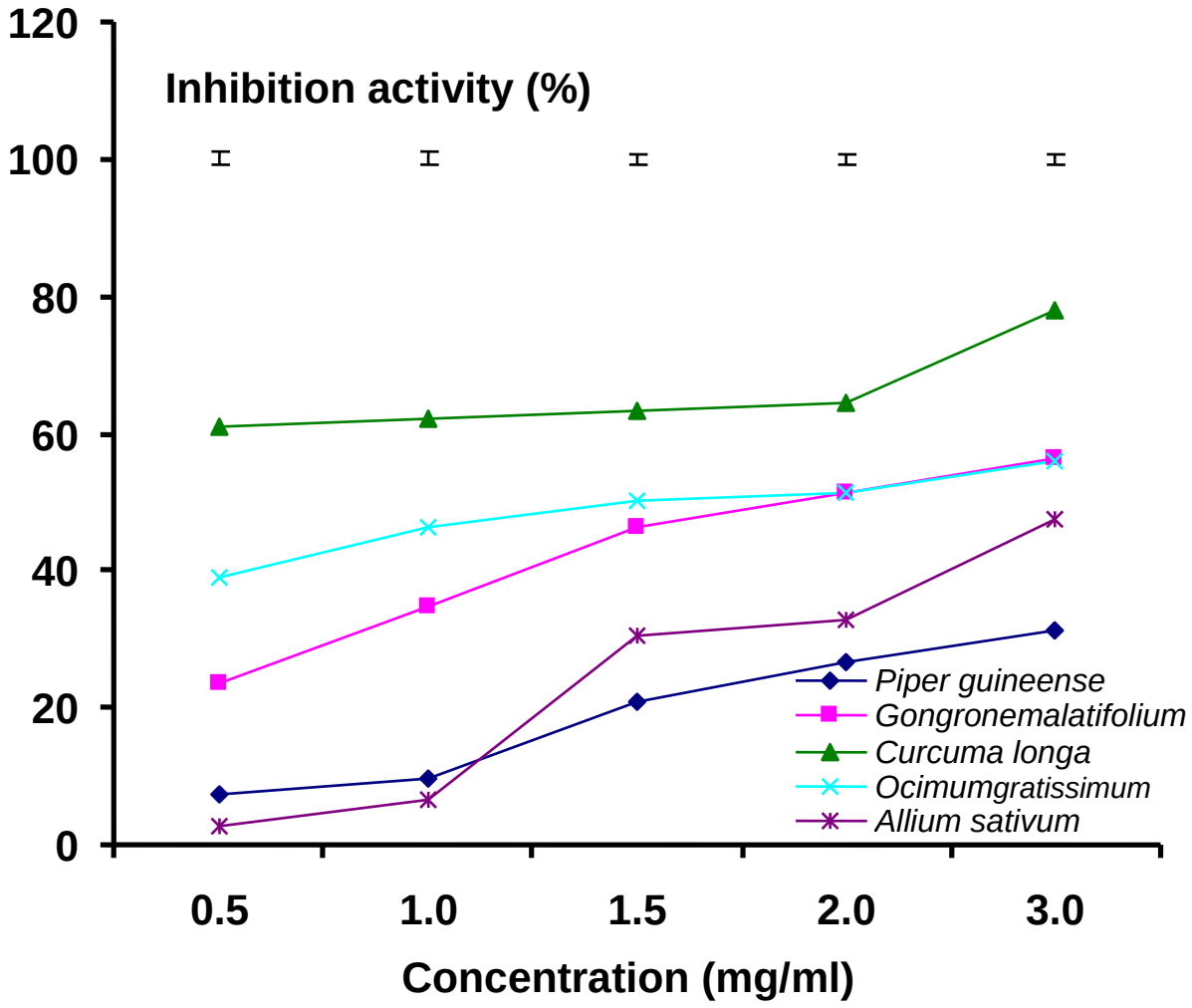

Figure 7: Effect of ethanolic extracts on lipid Peroxidation activity

ANTIBACTERIAL ACTIVITY RESULT

The result of antibacterial activity of the various plant extracts on the test organisms is shown below.

4.3.1Zone of Inhibition of*Staphylococcus aureus*by Plant Extracts

Ethanolic extract of all plant samples showed significant diameter zone of inhibition of *S.aureus.* The zone of inhibition of *S.aureus*by plant extract was concentation dependent.

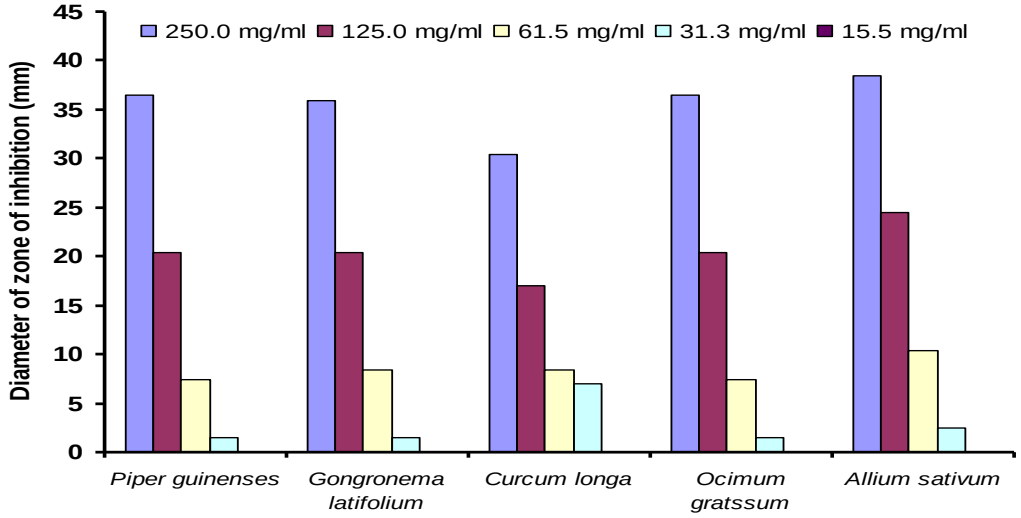

Figure 8: Zone of inhibition of *Staphylococcusaureus*

4.3.2 Zone of Inhibition of S*treptococcus pneumonia* by Plant Extracts

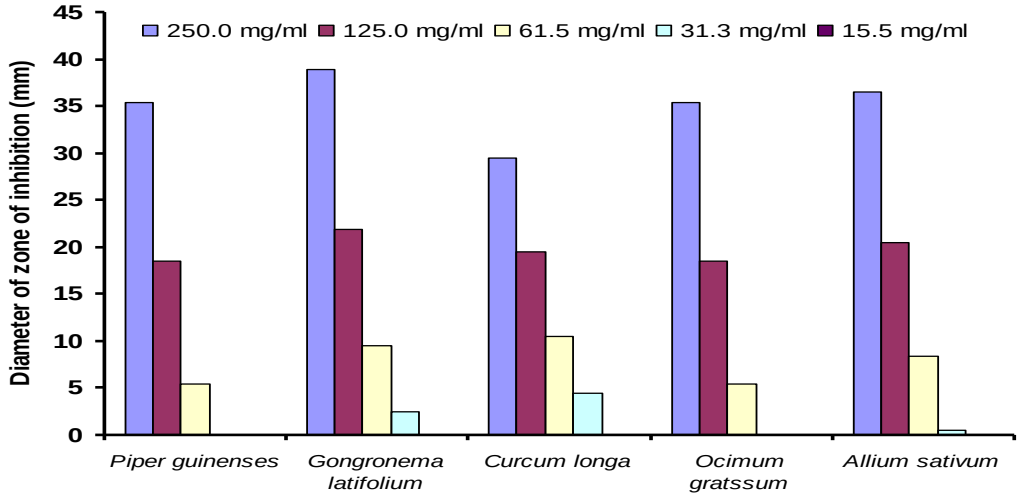

Figure 9: Zone of inhibition for *Streptococcus pneumonia*

Zone of Inhibition of Escherichia coli by Plant Extracts

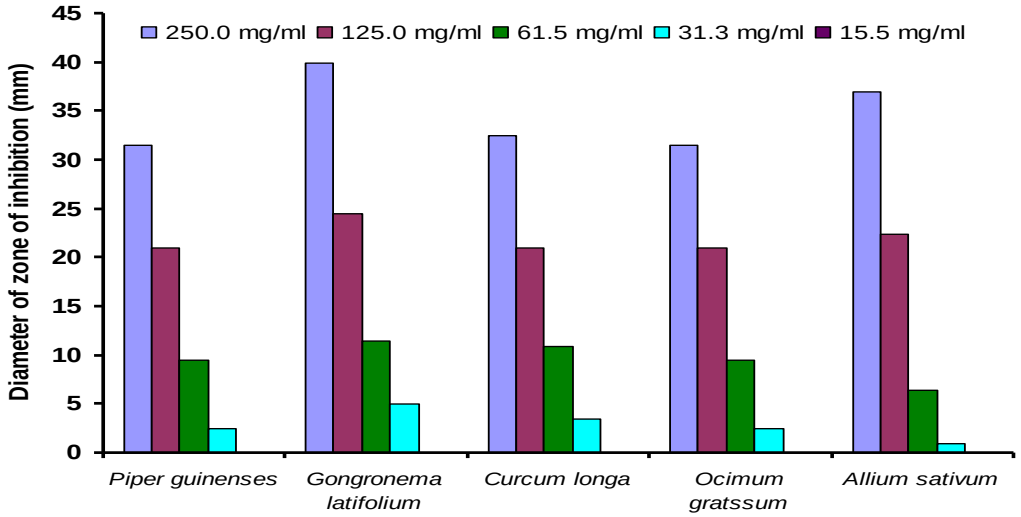

Figure 10: Zone of inhibition for *Escherichia coli*

Zone of Inhibition of *Proteus mirabilis* and *Pseudomonas aeruginosa* by Plant Extracts

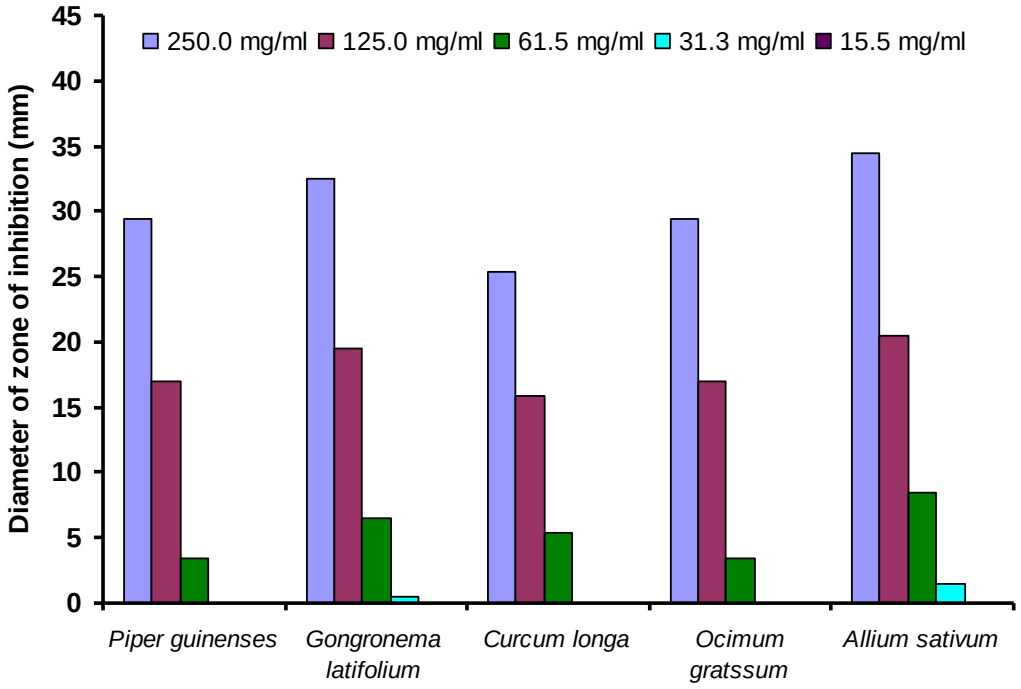

Figure 11: Zone of inhibition for *Proteus mirabilis*

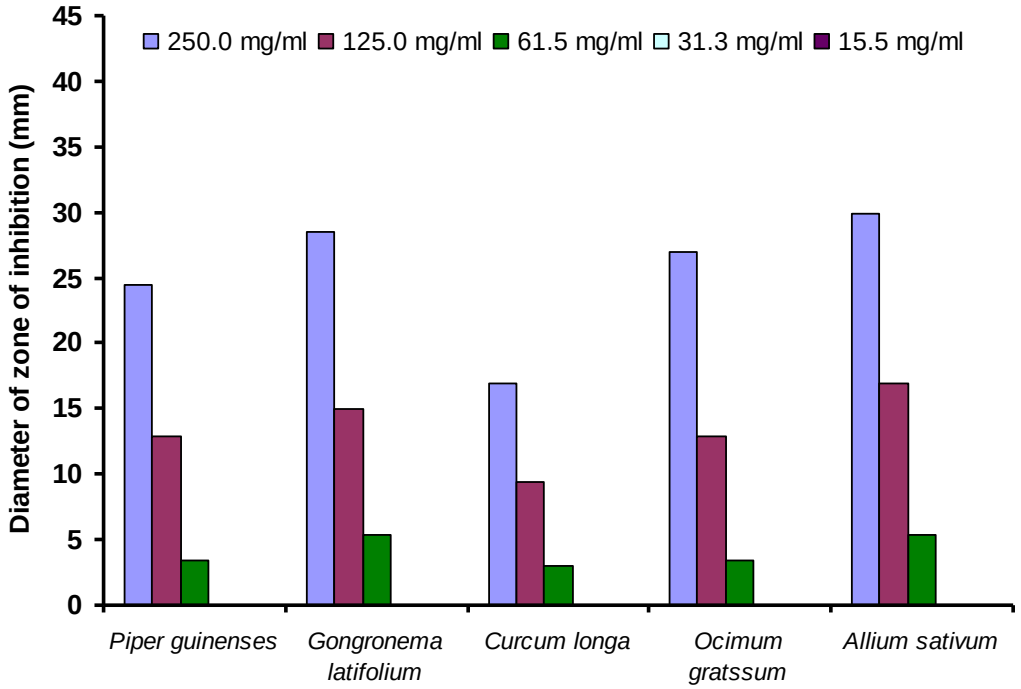

Figure 12: Zone of inhibition for *Pseudomonas aeruginosa*

DISCUSSION

Many traditional plants remedies are known in folk medicine and used for treatment and some have been validated by scientific studies to actually exert biological action against diseases and various health complications (Atangwho, 2012). This study therefore provided bases to the folkloric use of different plants as a remedy for cardiovascular, neurological, ocular andpulmonary diseases etc caused by oxidative stress. It also justifies the folklore medicinal uses about the therapeutic values of these plants as curative agent and therefore, the purification and characterization of the phytochemicals that can be isolated from these plants will be useful as a chemotherapeutic agent. All the plants exhibited potent antioxidant activity in this study. The presence of the polyphenols in all the plants is likely to be responsible for the free radical scavenging effects observed. These plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. The degree of reduction in absorbance measurement is indicative of the radical scavenging power of the extract (Mittal et al., 2012). The quantitative phytochemical composition of ethanolic extracts of the samples is shown in Table 2. The result showed that *A. sativum* had the highest flavonoid content followed by *C. longa.Gongronemalatifolium* contained the highest % Alkaloid. *Allium sativum* and *C. longa* contain more phenol than the other samples while *Piper guineense* had the lowest % phenol content. Also *C. longa* had the highest tannin content while *O. gratissimum* had the lowest.

Phytochemicals are secondary metabolites of plants known to exhibit diverse pharmacological and biochemical effects on living organisms (Trease and Evans, 1989).
These secondary metabolites have been associated with antimicrobial activities and numerous physiological activities in mammalian cells in various studies (Sofowora 1993; Abo et al., 1999; Nweze et al., 2004). This supports its use for the treatment of dysentery (Kerharo and bouquet, 1950). Many plants containing alkaloids and Flavonoids possess anti-inflammatory and antioxidant activity which highlights the importance of the plants used in this study. (Middleton et al., 2000, Sharma et al.,2009).

The effect of different concentrations of extracts of *Piper guineense*, *Curcuma longa*, *Gongronemalatifolium*, *Ocimumgratissimum*, *Allium sativum*on the Nitric oxide scavenging activityis shown in Fig. 6. The effect of extracts

of *P. guineense, C. longa, G. latifolium, O. gratissimum*and *A. sativum* at different concentrations on the Nitric oxide scavenging activity were significantly different. At the lower concentration, *P.guineense*has the lowest %inhibition activity compared with other samples. Its %inhibition activity progressively increased as concentration increases; also % inhibition effect of *P. guineense* and *C. longa* from 0.5-1.5mg is significantly different, similarly the inhibitory effect of *C. longa* and *Piper* from 0.5-1.5 mg differ from that of *O. gratissimum, G. latifolium,* and *A. sativum.* With the exception of *O. gratissimum*, the % inhibition of Nitric oxide scavenging activity increases more or less linearly as concentration increases.

At higher concentrations, 20-30mg there was no significant difference between % inhibition activities between the samples. At 2.0 mg there was no significant difference between %inhibition activity of *A. sativum and G. latifolium, P. gunineense and C. longa, O. gratissimum and C. longa*, but there was a significant difference in the % inhibition activity between *P. guineense and A. sativum; C. longa and G. latifolium.* At the maximum concentration Nitric oxide inhibition was similar in all the samples.

As ethanol extract of these plant showed the dose dependent antioxidant activity comparable to one another, antioxidant agent might be developed from this plant for the treatment of some human disorders associated with free radicals. Phenolic compounds containing free hydrogen are largely responsible for antioxidant activity (Evans et al., 1996; Evans et al., 1997), thus the phenolic compounds of the plant extractcan be referred to be responsible for the antioxidant activity (Ejele et al., 2011).

The effect of different concentrations of extracts *P. guineense, C. longa, A. sativum, O. gratissimum*, and *G. latifolium* on Lipid peroxidation activity is shown in Fig. 7. The effect of the ethanolic extracts of the samples differed significantly at various concentrations. *Curcum longa* was the most effective in inhibiting lipid peroxidation at all concentrations. Percentage inhibition activity by *G. latifolium and O. gratissimum* differed significantly at concentration of 0.5 – 1.5 mg/ml but were similar at 2.0 and 3.0 mg/ml. On the average *C. longa* has the highest % inhibition lipid peroxidation activity, followed by *O. gratissimum, G. latifolium and, A. satium,* and then lowest was *P. guineense.* At 0.5 and 1.0 mg *P.guineense*inhibitedlipid peroxidation activity more than *A. sativum*, but from 1.5 mg to the highest concentration *A. sativum*inhibitedlipid peroxidation activity than *P. guineense*.

These plant extracts inhibited lipid Peroxidation activity greatly due to the fact that they posses reasonable percentage polyphenols which include tannins and flavoniods (Ejele et al.,2011).

The diameter of zone of inhibition of *staphylococcus aureus* by ethanolic extract of *P. guineense,G. latifolium, C. longa, O. gratissimum,* and *A. sativum* at different concentrations is shown in Fig. 8. With *P. guineense, S. aureus* was inhibited highest at 250 mg/ml and lowest at 31.3 mg/ml. This was the case with extracts from the other plants. At lowest concentration of 15.5 mg/ml of all the samples *S. aureus* was not inhibited. Diameter zone of inhibition *S. aureus* was the same with extracts *P. guineense* and *G. latifolium* at 125 mg/ml. Amongst the samples *C. longa* has the lowest diameter of zone of inhibition at 250 mg/ml and *A. sativum*having the highest zone of inhibition at 250 mg/ml.
Literature has shown that terpenoids and phenolic compounds show most of the antibacterial activities and *S. aureus*are specifically more susceptible to phenolic compounds (Cowman, 1999).

The diameter of zone of inhibition of *Streptococcus pneumonia* by ethanolic extract of *P. guineense,G. latifolium, C. longa, O. gratissimum,* and *A. sativum* at different concentrations is shown in Fig. 9. At the highest concentration of 250 mg/ml all the extracts inhibited *S. pneumonia* with diameter of zone of inhibition between 35 mm-40 mm except *C. longa* which was about 30 mm. At 125 mg/ml *S. pneumonia* was inhibited by the all the extracts with diameter of zone of inhibition between 19 mm to about 22 mm. At 61.5 mg/ml the diameter zone of inhibition of *S. pneumonia* was between 5 mm and 10 mm and at 31.3 mg/ml, *P. guineense* and *O. gratissimum* did not inhibit *S pneumonia.* Also at 15.5 mg/ml *S. pneumonia* was not inhibited by all extracts. *Gongronemalatifolium* had the highest diameter zone of inhibition of *S. pneumonia* at 250 mg/ml and *A. sativum*had lowest diameter of zone of inhibition at 31.3mg/ml

The diameter of zone of inhibition of *Escherichia coli* by ethanolic extract of *P. guineense,G. latifolium, C. longa, O. gratissimum,* and *A. sativum* at different concentrations is shown in Fig. 10. At 250 mg/ml diameter zone of inhibition of the extracts was between 31 mm and 40 mm. At 125 mg/ml the diameter zone of inhibition was between about 21 mm and 24 mm while at 61.5 mg/ml it was between 9 mm and 11 mm and at 31.3 mg/ml it was between 1 mm and 5 mm. At 15.5 mg/ml all extracts did not inhibit *E.coli, O. gratissimum* and *P. guineense* inhibited *E. coli* at 31.3 mg with the same diameter of zone of inhibition. *O gratissimum* and *C. longa* have similar diameter of zone of inhibition at all the concentrations.It is suspected and speculated that phenols and flavoniods aid antibacterial activity of the extracts of the plant samples (Adebolu and Salau, 2005).

The diameter of zone of inhibition of *Proteus mirabilis* and *Pseudomoansaeruginosa Escherichia coli* by ethanolic extract of *P. guineense,G. latifolium, C. longa, O. gratissimum,* and *A. sativum* at different concentrations is shown in Figs. 11 and 12. At the lowest concentrations of 15.5 mg/ml *P.mirabilis* was not inhibited and at 31.3 mg/ml *P. guineense, C. longa* and *O. gratissimum*did not inhibit *P. mirabilis*. At 250 mg/ml *A. sativum*had the highest zone of inhibition (Fig. 11). At 15.5 mg/ml and 31.3 mg/ml *P. aeruginosa* was not inhibited. *Pseudomoansaeruginosa*was inhibited by *A. sativum* at 250 mg/ml and lowest by *C. longa* at 61.5 mg/ml.

CONCLUSION

From the results generally, it can be concluded that the ethanolic extract of these plants:*Piper guineense*, *Curcuma longa*, *Gongronemalatifolium*, *Ocimumgratissimum* and *Allium sativum*posses antioxidant and antimicrobial activity. Some plants, like *Curcuma longa* even at very low concentration hadsignificant antioxidant activity compared with the others had minimal effects at low concentration of their extracts. The same goes for their antimicrobial activity.They have the ability to mop-up free radicals and inhibit the growth of microorganisms especially when used generally at high concentration since they contain bioactive compounds such as tannins, saponins, and flavoniods. These results suggest that extracts from these plants may be used to treat diseases.

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