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Phytochemical and Antimicrobial Activity of Piperine Extract and Essential Oil of *Piper nigrum* Leaves

[1]OJO Olabimpe Iyabode, [1]Ezenwa Valentine Chukwuma, [2]Ezeike A.C and [3]Ojo Oluwaseun Adedayo

[1]Department of Chemical Sciences Bamidele Olumilua University of Education, Science and Technology, Ikere-Ekiti, Ekiti State., [2]Department of Science Laboratory Technology, Federal Polytechnic Oko, Anambra state Nigeria. [3]Department of Medical Microbiology, Federal University of Technology Akure.

E-mail: ojoolabimpeiyabode@gmail.com. Tel: 08066793330,

Abstract: *Phytochemical and antimicrobial activities of the Piperine extract and essential oil extract from Piper nigrum leaves were evaluated. The phytochemicals were determined using Harborn standard qualitative and quantitative analysis methods. The antimicrobial assay was carried out using agar well diffusion method and the test organisms were Staphylococcus aureus, Salmonella spp, E. coli and Aspergillus spp. The study revealed that the flavonoid, tannin, saponin, glycosides, phenol, and alkaloids content of Piperine extract of Piper nigrum are as follows 1.1 % ± 0.1, 71.58 mg/l ±0.1, 1.4 % ± 0.4, 4.7 % ± 0.03, 7.59 mg ± 0.1 and 1.5 % ± 0.3 respectively. The antimicrobial assay of Piperine extract showed zones of inhibition for Staphylococcus aureus , Salmonella spp, E. coli and Aspergillus spp are as follows: 18.0 mm, 19.0 mm, 21.0 mm and 18.0 mm respectively. While zone of inhibition for the essential oil for Staphylococcus aureus, Salmonella spp, E. coli and Aspergillus spp are as follows: 33.0 mm, 40.0 mm, 47.0 mm and 8.0 mm respectively. The result obtained from this study shows that essential oil has more zone of inhibition than Piperine extract leaves and this indicates that essential oil can be more effective in the treatment of disease caused by all these organisms.*

Key words: *Piper nigrum*, antimicrobial Activity, Piperine and essential oil.

1 Introduction

Phytochemicals are chemical compounds produced by plants as a result of primary or secondary metabolism. They generally have some biological activity in the host plant and are commonly found in the leaves, roots, bark, fruits, vegetables, nuts, legumes, and grains. (Bjeldanes and Shibamoto, 2010). The therapeutic and medicinal benefits of some plant extracts can be attributed to the presence of phytochemical constituents in them.

2. Literature Review

Traditional medicine is as old as the history of mankind, and studies have shown that about 80% of the world population presently depends on herbs, roots and seeds medically to satisfy their health needs (Ugoh and Nneji, 2013). Extraction and characterization of several phytochemicals from herbal products have led to the discovery of new high profile pharmaceuticals, healthcare products as well as preservatives (Ara, Buckhari, Solaiman and Bakir, 2012).

Piper nigrum, otherwise known as African black pepper is a West African spice plant which possesses some medicinal properties and is widely useful in traditional medicine for the treatment of various ailments. The leaves are used to treat respiratory infections, rheumatism, and syphilis. In Nigeria, the leaves have been shown to have antibacterial activity. *Piper nigrum* leaves are aseptic in nature and have the ability to relieve flatulence (Nwachukwu *et al.,* 2010). Anvam *et al.,* (2016) reported that the seeds of *Piper nigrum* are used to relieve discomfort in the stomach caused by excess gas and also used for the preparation of non-toxic insecticides and perfumes. Reports by researchers have also revealed that *Piper nigrum* finds use in the treatment of intestinal diseases, bronchitis, cough and rheumatism, infertility in woman and low sperm count in men (Nwachukwu, Ume, Obasi, Nzewuihe and Onyirioha, 2010). The research is aimed to evaluate phytochemical and antimicrobial activities of Piperine extract and essential oil of *Piper nigrum* leaves.

3 Methodology

Collection and Preparation of Sample

The leaves of *piper nigrum* used in this work were obtained from Eke Oko Market, Orumba-North L.G.A, Anambra state. The leaf was thoroughly and gently washed with tap water and rinsed with distilled water. The leaves of *Piper nigrum* leaves were air dried at room temperature. The dried leaves were ground into uniform powder using a commercial milling machine.

Extraction of the Essential Oil

The Clevenger apparatus was used for the extraction of the essential oil. 150 g of fresh sample was transferred into the distillation flask. 250 ml of water was poured into the 500 ml round bottom flask and the Clevenger apparatus was joined with the round bottom flask, which was placed on a heating mantle. The inlet and outlet jacket were also connected to aid circulation of cooling of vapour that pass through the condenser.

Solvent Extraction of Sample

The sample was prepared by soaking 50g of dried powered sample in 200 ml of ethanol for 24 hours. The extract was filtered using Whatman filter paper No.42. The extract was subjected to phytochemical screening.

Phytochemical Screening

The phytochemical screening was carried out to assess the presence or absence (qualitative analysis) and percentage of crude contents (Quantitative analysis) of alkaloids, flavonoids, alkaloid, tannin, saponins, phenol and cardiac glycosides.

Qualitative Screening

Chemical tests were carried out on the aqueous extract using standard procedures to identify the constituents. The methods used included those of Harborne (2015).

Test for Flavonoid

Exactly 1 ml of the extract was added to 1 ml of ferric chloride. The formation of a greenish-brown or black precipitate or color was positive result for the presence of flavonoids.

Test for Tannin

About 1 ml of extract was added in equal volume of bromine water. The formation of a greenish to red precipitate was taken as evidence for the presence of condensed tannins.

Test for Saponin

Exactly 1 ml of extract was boiled with 5 ml of distilled water for 5 minutes and discarded while still hot. The filtrate was used for the **Frothing Test:** Exactly 1 ml of the filtrate was diluted with 4 ml of distilled water, shaken vigorously and observed on standing for stable froth which was an evidence for the presence of saponin.

Test for Cardiac Glycosides

Accurately 1 ml of extract was added to 2 ml glacial acetic acid and a few drop of 5 % ferric chloride. 2 ml of conc. H_2SO_4, was carefully added by the side of the test tube. A red or reddish brown color at the interphase was taken as a positive test for steroid nucleus.

Test for Alkaloid

Exactly 1 ml of the extract was shaken with 5 ml of 2% Hcl on a steam bath and filtered; 1 ml of the filtrate was treated with Wagners reagent and observed for red brown precipitate.

Test for Phenol

About 1 ml of the extract was added to 2 ml distilled water followed by few drop of 10% ferric chloride.

Formation of blue / green color was observed which indicate the presence of phenol.

Quantitative Screening

Quantitative analyses of the chemical constituents were carried out to determine the crude content of the phytochamicals in the plant sample. The methods used included those of Harborne (2015).

Alkaloid Determination

Accurately 5 g of the sample was weighed using sensitive balance into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for about 4 hours. This was filtered through a Whatmann No.42 filter paper and the extract was concentrated on water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide (NH_4OH) was then added drop-wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and

washed with dilute ammonium hydroxide and the residue was filtered. It was dried and weighed.

The weight of the alkaloid precipitated was expressed as a percentage of the weight of original sample used as shown below:

$$\% \text{ alkaloids} = \frac{W2 - W1}{WT}$$

Where WT= weight of the sample

W1= weight of the empty crucible

W2= weight of crucible +weight of sample.

Determination of Flavonoid

Exactly 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No.42. The filtrate was later transferred into a crucible and evaporates to dryness over a water bath and weigh to a constant weight.

$$\% \text{ flavonoid} = \frac{W2 - W1}{WT} \times \frac{100}{1}$$

W1 =weight of empty crucible

W2 = weight of crucible +sample

Wt = original weight of sample

Determination of Tannin

About 1 g of the sample was weighed into a conical flask. Then 100 ml of distilled water was added and boiled gently on a hot plate for 1 hour. This was filtered with whatmann filter paper into a 100ml volumetric flask and made up to the mark. It was allowed to cool, thus be the extract volume.

Color Development

10 ml of the filtrate and 80 ml of distilled water was pipette out into a 100 ml conical flask. 5 ml of folin denis reagent was added followed by 10 ml of saturated sodium carbonate. It was

diluted to volume with distilled water. The solution was mixed thoroughly and allowed to stand in a water bath (25c) for 30 minutes.

The absorbance of the solution was Measure at 700 nm with spectrophotometer and compare with standard tannic acid curve.

Note: the standard tannic acid containing 0.002 mg/ml of tannic acid is treated the same way the extract for color development.

Calculation

$$\text{Tannin (mg/100 g)} = \frac{\text{con. (mg)} * \text{extract volume}}{\text{Aliquot volume} * \text{sample weight}}$$

Determination of Saponin

Exactly 5 g of the dried sample was extracted in a soxhlet extractor with 200 ml of acetone to cause a reflux. It was extracted for 3hrs and the solvent was distilled off. This is the first extraction.

For the second extraction, a pre-weighed round bottom flask was fitted with a soxhlet apparatus containing the same sample. It was extracted exhaustively with methanol for 3hrs. The solvent was distilled off and the weight of the flask was weighed again. The difference between the final and initial weight of the flask represent the weight of saponin extracted.

$$\% \text{ saponin} = \frac{\text{weight of saponin} * 100}{\text{Weight of sample}}$$

W2= weight of filter + sample

W1=weight of filter paper

Wt= initial weight of sample

Determination of Cardiac Glycoside

Exactly 1 g of the sample was extracted with 20 ml of 70% alcohol and the mixture was filtered. (Note the extract was left overnight with occasional shaking). 8 ml of the filtrate was transferred to 100 ml volumetric flask and the volume was made to the mark with distilled water. 8 ml of the mixture was added to 8 ml of 12.5% lead acetate. The mixture was shaken and completed to the volume with distilled water and filtered. 50 ml of the filtrate was pipette into another 100 ml of volumetric flask and 8 ml of 4.7% disodium hydrogen phosphate to

precipitate excess lead. The mixture was made up to a volume and mixed and was filtered twice through the same filter paper. 10 ml of baljets reagent was added to 10 ml of the purified filtrate. A blank sample of 10 ml of distilled water was also added to 10 ml of baljets reagent. The two solutions were allowed to stand for one hour. The intensity of the color was read at 495 nm using spectrophotometer

$$\% \text{ glycoside} = \frac{A * 100}{17}$$

Determination of Phenol

Exactly 1 g of the sample was placed in a test tube and 10 ml of methanol was added to it and was shaken vigorously. It was allowed to stand for 15 mins and filtered using whatmann filter paper. 1 ml of the extract was placed in a test tube followed by folin denis reagent and 5 ml of distilled water. The color of the mixture was allowed to develop for 1 hour at room temperature. The absorbance was measured at 760 nm.

$$\text{Phenol} = \frac{\text{absorbance of unknown} * \text{con. Of standard}}{\text{Absorbance of standard}}$$

Determination of Antimicrobial Activities

Test Organism

The microorganisms used were *Staphylococus aureus*, *Esherichia coli*, *Salmonella typhi and Aspergilus niger*. They were obtained from Gunson Laboratory Awka.

Antimicrobial Assay

The antimicrobial activities were performed using Agar disc diffusion method. Mueller Hinton agar plates was used to study the test, organisms were grown in nutrient broth for 24 hours, the 24 hours broth culture contain approximately 25 cfm/ml, 50 cfm/ml, and 75 cfm/ml. For *Staphylococus aureus*, *Esherichia coli, Salmonella typhi,* and *Aspergilus spp.* respectively as determined by plate count method were serially diluted to 10^{-2} dilution. Sterile Swab Stick was used to inoculate the media by dipping it in the diluted Culture and spreading all the Surfaces of the agar plate. Wells of 5 mm size were made with sterile borer into agar plates containing the bacterial inoculums. 2mg of piperine was completely dissolved in 2ml of ampiclox. Antimicrobial activity was measured at different concentrations of extract ranging from 25 cfm/ml, 50 cfm/ml, and 75 cfm/ml was poured into a well of inoculated

plates. Ampiclox served as control. The bacteria Culture were then incubated for 24 hours at 37 ^{0}C and the fungi for three days at room temperature. After incubation the diameter zone of inhibition was measured using millimeter rule.

RESULTS

Table 1: Qualitative Phytochemical Composition of *Piper nigrum*

Parameter	inference
Alkaloid	+
Tannin	+++
Saponin	+
Flavonoid	++
Glycoside	++
Phenol	++

Keys	
+++	Present in high concentration
++	Present in moderate concentration
+	Slightly or sparingly present
_	Absent

Table 2 Mean Quantitative Phytochemical Composition of *Piper nigrum*

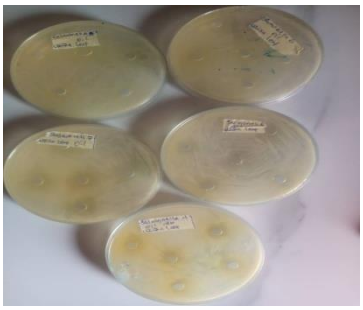
Parameters	Mean Conc.	Standard deviation
Tannin (mg/100g)	71.58	±0.1
Saponin (%)	1.4	±0.4
Alkanoid (%)	1.5	±0.3
Glycoside (%)	4.73	±0.03
Phenol (mg)	7.59	±0.1
Flavoniod (%)	1.1	±0.1

Table 3 Zone of Inhibition of piperine of *Piper nigrum* fresh Leaves

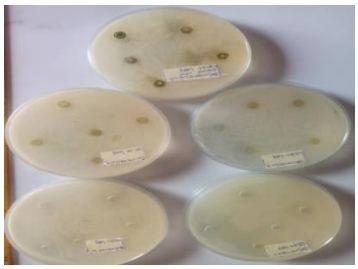
Organism	Zone of Inhibition (mm)	
	Mean Conc	control
Staphylococcus aureus	18	22
Salmonella spp	19	21
E.*coli*	21	26
Aspergillus spp	18	24

Table 4 Zone of Inhibition of Essential Oil of *Piper nigrum* leaves

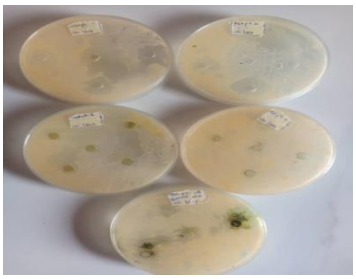
Organism	Zone Of Inhibition (mm)	
	Mean Conc	control
Staphylococcus aureus	33	48
Salmonella spp	40	49
E.*coli*	47	35
Aspergillus spp	8	12

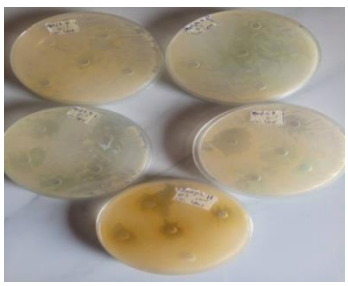


Salmonella (extract)

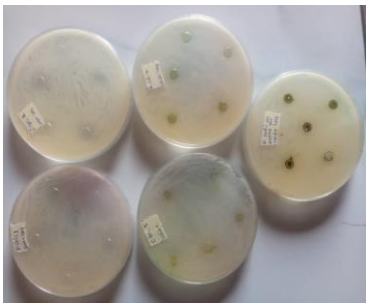


Salmonella (Eo)





Staph (extract) *Staph (Eo)*



E.coli (extract) E.coli (Eo)

Eo = essential oil

Figure 1. Image showing the zone of inhibition of piperine and essential oil of *Piper nigrum* leaves

4 Discussion

The active ingredient tannins are compounds with proline-rich proteins that help to inhibit the absorption of iron when present in the gastrointestinal lumen thus reducing the bioavailability of iron due to the presence of compounds that help in the treatment of diseases like enteritis, gastritis, and esophagitis (Echo *et al.*, 2012). This work found tannin to be (71.58 ± 0.1). The findings of this study have some similarity with previous works on *Piper* species reported by Al-Tememy (2013) which shows aqueous extract of *Piper cubeba* leaves to be (71.56 ± 0.1).

Phenol has been reported to protect proteins, DNA and lipids from oxidative stress thus exerting anticancer properties (Delmas *et al.*, 2016) and this work showed the presence of phenol to be 7.59 ± 0.1) which correspond to the findings of Dai and Mumper, (2010) on the leaves of *Talinum triangulare* (7.59 ± 0.1).

Saponins have anti-carcinogenic properties and may also play an important role in antimalarial activity of plants (Trease and Evans 2002). This work revel the presence of saponin to be (4.73 ± 0.03) which is highly difference from the value obtain by Shalom *et al.*, (2011) on leaves of *Solanum macrocapon* (10.56 ± 0.02). The difference in values may be attributed to the laboratory conditions.

Glycosides are useful in the management of diseases associated with the heart (Dada *et al.*, 2013). This work found glycoside to be (1.4 ± 0.4) which is in accordance with the value obtained by Synder, (2013) on the leaves of *Allamanda cathertica* (1.4 ± 0.4).

The flavonoids possess antioxidant, anti-inflammatory, anti-tumor and anti-allergic properties. They are also found to have cholesterol lowering ability (Galeotti *et al.*,2008). This work showed the presence of flavonoids to be (1.1 ± 0.1) which is slightly difference from the value obtained by Sailage *et al.*, (2016) on the leaves of *Carica papaya* (1.2 ± 0.1).

Alkaloids are being used as central nervous system (CNS) stimulant, powerful pain relievers, topical anesthetic in ophthalmology among others (*Aremu et al.*, 2018). This study found the presence of alkaloids to be (1.5 ± 0.3). Nahak and Sahu (2011) reported the presence of Alkaloid in the leaves of *Canarium schweinfurthii* Gnn to be (1.6 ± 0.1) which is slightly difference from this study.

The occurrences of some phytochemical constituents in this plant suggest their pharmacological properties (Epidi *et al*., 2016).

The effect of leaves extracts and essential oil of *Piper nigrum* on the zone of inhibition of some selected microbial isolates is presented in table 3 and 4 and figure 1.

Mean zone of inhibition exhibited by the extracts were 18 mm for *staphylococcus spp*, 19 mm for *salmonella spp*, 21 mm for *E.coli* and 18 mm for *Aspergillus spp* for 1% concentration of the ampiclox used as control (AMP), while the Mean zone of inhibition exhibited by the essential oil were 33 mm for *staphylococcus spp*, 40 mm for *salmonella spp*, 47 mm for *E.coli* and 8 mm for *Aspergillus spp* for 1% concentration of the ampiclox used as control (AMP).

The presence of insoluble active compound found in extracts could be the contributing factor why there is low zone of inhibition among the various test organisms compared to that of the essential oil. The findings of this study validate the traditional use of *Piper nigurm* by indigenous people of Anambra state. Pepper soup is one of the common delicacies prepared with it especially for post-partum women as a stimulant to relieve constipation, aid lactation, prevent post- partum contraction and control passive uterine hemorrhage. The lower values of known antibiotics used as positive control could be due to the concentration applied (1%) in the cause of the experiment. This values obtained is close to the values previously for *E.coli* (50 mm) and *S.aureus* (37 mm) reported by Kigigha *et al*. (2015), Kigigha *et al*. (2016), Kigigha and Onyema (2015), Kigigha and Charlie (2012), Kigigha and Atuzie (2012).

E.coli which have superior or higher zone of inhibition could be due to biochemistry, nutrition, physiology and metabolism compared to other isolates (*S.aureus* and *Aspergillus spp*). This trend in zone of inhibition (for *E.coli* > *S.aureus*) on the antimicrobial activity of *P.nigrum* against some bacteria isolates have been previously reported by Ganesh *et al.* (2014). The zone of inhibition found in this study has some similarity with values previously reported plants parts including *Alstonia boonei* (Epidi *et al.*, 2016), *Vitex grandifolia* (Epidi *et al.*, 2016), *Aframomum melegueta* (Kigigha *et al.*, 2015), *Musanga cecropioides* (Kigigha *et al.*, 2016).

5 Conclusion

This study on Piperine extract and essential oil from the leaves of *Piper nigrum* showed the presence of flavonoid, alkaloid, phenol, glycoside, tannin and saponin in *Piper nigrum* leaves. The Piperine extract and essential oil of *Piper nigrum* shows high medicinal value but that of essential oil is more effective because it shows higher zone of inhibition than the piperine extract. Conclusively essential oil from this *Piper nigrum* leaves could be used as cheap alternative antimicrobial drugs which could be useful to cure and control human infectious diseases.

Recommendations

From the findings of the study, the following recommendations were made:

- The leaves of *Piper nigrum* is also recommended for drugs production and in herbal medicine.
- Further research should be carried out on the plant to determine its safety and the side effects, the active ingredients they contain and also to verify it feasibility as food supplement.
- Government should encourage farmers to cultivate more of this plant because of its medicinal values.

References:

Al-Tememy, T.M.K. (2013). Antibacterial Activity of *Piper Cubeba* Linn. Fruit Extracts against Selected Bacterial Pathogens in Basrah city. *Bas. J. Vet. Res*., 12(1), 142 – 151.

Anvam, Z.P.H., Biyiti, L., Tchoumbougang, F., Menut, C., and Lamaty, G (2016). Aromatic Plants of Tropical and Central Africa. Part Xxx11. Chemical Composition and Antifungal Activity of Thirteen Essential Oil from Aromatic Plants of Cameroon. *Flavor and Fragrana J.*, 13(2):107-114.

Ara I., Bukhari, N.A., Solaiman, D. and Bakir, M.A (2012). Antimicrobial Effect on Local Medicinal Plant Extracts in the Kingdom Of Saudi Arabia and Search for Their Metabolites by Gas Chromatography-Mass Spectrometric (Gc-Ms) Analysis. *Journal of Medicinal Plant Research* 6(45), 5688-5694.

Aremu, M.O., Ibrahim, H.O. and Akinyeye, R.V. (2018). Chemical and Phytochemical Compositions of Wonderful Kola (*Buccholzia coriaceae). Bangladesh Journal of Scientific and Industrial Research*, 53 (2): 125.

Bjeldanes L.ana Shibamot T. (2010). *Introduction to Food Toxicology* (2nd Ed), Burlington: Elsevier .p 124.S

Dada, A.A, Ifesan, B.O.T and Fashakin, J.F (2013). Antimicrobial and Antioxidant Properties of Selected Local Spices used in "Kunun" Beverage in Nigeria. *Journal of Appl.Environ. Chem.* 4 (12): 374.

Dai, J and Mumper, R.J (2010). Plant Phenolics: Extraction, Analysis and their Phytochemical and Anticancer Properties of *Talinum triangulare*. *Molecules Journal of Chemical Science*.; 15: 7313-7352.

Delmas D, Lançon A, Colin D, and Latruffe N. (2016) Resveratrol as a Chemopreventive Agent: A Promising Molecule for Fighting Cancer. *Current Drug Targets*. 7: 423-442.

Echo, I.A., Osuagwu, A.N., and Ekanem, B.E (2012). Phytochemical Composition of Aframomun Melegueta and Piper *nigrum* Seeds. World *Journal of Appl.Environ. chem*. 2 (1) :17-21.

Epidi, J.O, Izah, S.C. and Ohimain, E.I. (2016). Antibacterial and Synergistic Efficacy of Extracts of *Alstonia boonei* Tissues. *British Journal of Applied Research*, 1(1), 0021-0026.

Epidi, J.O, Izah, S.C., Ohimain, E.I. and Epidi, T.T. (2016). Antibacterial and Synergistic Potency of Tissues of *Vitex Grandifolia. Biotechnological Research*, 2(2), 69-76.

Galeotti, F., Barile, E., and Lanzotti, V (2014). *"Flavonoids from Carnation (Dianthus Caryophyllus) and their Antifungal Activity"*. London: Vintage Books 1: 44–48.

Ganesh, P., Kumar, R.S. and Saranraj, P. (2014). Phytochemical Analysis and Antibacterial Activity of Pepper (*Piper nigrum* L.) Against Some Human Pathogens. *Central European Journal of Experimental Biology*, 3 (2), 36-41.

Harborne, J.B. (2015).*Phytochemical methods*. London. Chapman and Hall Ltd.

Kigigha, L.T and Charlie, E.J. (2012). Effect of Pepper-Soup Cooking on the Antibacterial Activity of Monodora Myristica. *Continental J. Food Science and Technology*, 6 (1), 8 – 11.

Kigigha, L.T. and Atuzie, M.N. (2012). Assessment of Traditional Medicinal Application of *Alchornea Cordifolia*. *African Journal of Biotechnology*, 11(8), 2083 – 2086.

Kigigha, L.T. and Onyema, E. (2015). Antibacterial Activity of Bitter Leaf (*Vernonia amygdalina*) Soup *on Staphylococcus aureus and Escherichia coli). Sky Journal of Microbiology Research*, 3(4), 041- 045.

Kigigha, L.T., Apreala, A. and Izah, S.C. (2016b). Effect of Cooking on the Climbing Pepper (*Piper nigrum*) on Antibacterial Activity. *Journal of Environmental Treatment Techniques*, 4(1), 6 – 9.

Kigigha, L.T., Biye, S.E. and Izah, S.C. (2016a). Phytochemical and Antibacterial Activities of *Musanga cecropioides* Tissues against *Escherichia coli, Pseudomonas aeruginosa Staphylococcus aureus, Proteus and Bacillus species*. *International Journal of Applied Research and Technology*, 5(1), 100 – 107.

Kigigha, L.T., Izah, S.C. and Ehizibue, M. (2015a). Activities of *Aframomum melegueta* Seed *Against Escherichia coli, S. aureus and Bacillus species. Point Journal of Botany and Microbiology Research*, 1(2), 23 – 29.

Kigigha, L.T., Izah, S.C. and Prebo, P.E. (2015c). Effect of Residual Antibiotics in Snacks against *Escherichia coli and Staphylococcus aureus. Journal of Environmental Treatment Techniques*, 3(4), 201 – 203.

Kigigha, L.T., Izah, S.C. and Sika, B.A. (2015b). Effect of Residual Antibiotics in Frozen Cray Fish (Pleoticus muelleri) against *Staphylococcus aureus and Escherichia coli. International Journal of Applied Research and Technology*, 4(12): 21-25.

Nahak, G. and Sahu, R.K. (2011). Phytochemical Evaluation and Antioxidant Activity of *Piper cubeba* and *Piper nigrum*. *Journal of Applied Pharmaceutical Science*, 01 (08), 153-157.

Nwachukwu, C.U., Ume, N.C., and Onyirioha C. (2010), The Qualitative uses of some Medicinal Plants in Ikeduru L.G.A of Imo State, Nigeria, *New York Science Journal*,11(3):132.

Nwachukwu, C.U., Ume, N.C., and Onyirioha C. (2010), The Qualitative uses of some Medicinal Plants in Ikeduru L.G.A of Imo State, Nigeria, *New York Science Journal*,11(3):132.

Sailage, V., Madhu, M. and Neeraga, V. (2016). Quantitative Phytochemical Analysis of *Carica papaya*. *Journal of Pharmacognosy 3(3):172 – 178.*

Shalom, N., Abayomi, C., and Olajumoke, K. (2011). Proximate and Phytochemical Analysis of *Solanum macrocapon*. *Research Journal of Chemical Science, 1(3): 23-35.*

Synder, H. (2013). Method of Determination of Glycosides in Leaves of *Allamanda cathertica*. *Journal of the American Chemistry 3(20):60 – 65.*

Trease, G. and Evans, S.M. (2002) *Pharmacognosy*. 16th edition. Edinburg: Harciut Publishers Limited.

Ugoh, S.C. and Nneji, L.M (2013). Evaluation of the Phytochemical Composition and Antibacterial Activities of Methanolic and Aqueous Leaf Extract of *Psidium Guajava* (L.) *Report and Opinion*, 5(9): 14-20