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Original Research Article

In-vitro antimicrobial potential of aqueous and methanolic extracts of *Piper guineense* and *Gongronema latifolium* on *Staphylococcus aureus* and *Escherichia coli*

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ABSTRACT

Plants are rich in different secondary metabolites, such as tannins, terpenoids and alkaloids which have been found in- vitro to have antimicrobial properties. The aim of this study was to determine the *in-vitro* antimicrobial potential of the methanolic and aqueous extracts of dried leaves of *Gongronema latifolium* and *Piper guineense* plants on S*taphylococcus aureus* and *Escherichia coli*. Soxhlet extractor was used for the methanolic solvent while cold maceration was used for the aqueous extraction. The phytochemical constituents of these extracts were screened thereafter the extracts were tested for their antimicrobial activity. The technique of agar well diffusion was employed. Wells was made on nutrient agar media and the extracts were placed in the well. Concentration of 1.25mg/ml, 2.5mg/ml, 5mg/ml and 10mg/ml were used. Surface of agar is inoculated (using sterile swab dipped into standardized broth culture of the test organism). Thereafter, the wells were bored into the inoculated agar and the extracts delivered into the well. After allowing for diffusion, the plates were incubated, after which the zones of inhibition around the well measured with the aid of vernier caliper. Activity of the extract against the test organism was expressed in terms of size (mm) of inhibition zone diameter around the well. The presence of alkaloids, flavonoids, tannins, saponins, and phenol in both plant extracts was observed. The aqueous extract of *P. guineense and G latifolium* showed more presence of phytochemical constituents than the methanolic extract and this is evidenced by higher zones of inhibition. Methanolic extracts of *Piper guineense* showed visible clearance suggestive of antibacterial activities by the extract only at 10mg/ml on *S. aureus*, but no form of inhibition on *E. coli.* Both extracts have concentration dependent antimicrobial effect on S*taphylococcus aureus* and *Escherichia coli, and* this may be due to phytochemicals found in them.

Keywords: Antimicrobial sensitivity, *Escherichi coli, Gongronema latifolium, Piper guineense, Staphylococcus aureus.*

INTRODUCTION

The use of medicinal plants (traditional medicine) in curing illnesses is as old as man (Abinu *et al*., 2007). Traditional medicine also known as indigenous medicine, can be defined as the health practices, approaches, knowledge and beliefs incorporating plants, animal and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illness or maintain well-being (Chikezie *et al.*, 2015).

Bacterial agents such as S*taphylococcus aureus* and *Escherichia coli* cause several human and animal infections (Peirano *et al.*, 2013). *Staphylococcus aureus* is a commensal organism that resides in skin and mucosa. Mild to life-threatening sepsis can occur if the organism enters into the body especially in an immuno-compromised or immunosuppressed individual. Routes of entry include broken skin or mucosa, and oral ingestion of contaminated food (Leung, 2014). It is an important pathogen that has a major impact on human health. Although it is notorious for causing skin and soft-tissue infections, it has the ability to infect nearly every organ in the human body, often with fatal consequences (Bose & Bayles, 2013).

Escherichia coli is commonly found in the lower intestine of warm-blooded organisms (endotherms) (Tenaillon *et al*., 2010). Most *E. coli* strains do not cause disease, but virulent strains can cause gastroenteritis, urinary tract infections, neonatal meningitis, hemorrhagic colitis

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and Crohn's disease (Todar, 2007). Common signs and symptoms include severe abdominal cramps, diarrhea, hemorrhagic colitis, vomiting, and sometimes fever (Lim *et al*., 2010). However, healthy populations of all ages are at risk to the severe consequences that may arise as a result of being infected with *E. coli* (CDC, 2018).

Recent emergence of antibiotic resistance and related toxicity issue limit the use of antimicrobial agents and is prompting a revival in research of the antimicrobial role of plants against resistant strains due to comparable safety and efficacy (Khameneh *et al.,* 2019). However, a vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds as alternatives that can potentially be effective in the treatment of these problematic bacterial infections (Okigbo & Mmeka, 2006). According to Palhares *et al*. (2015), medicinal plants would be the best source to obtain a variety of drugs. *Gongronema latifolium* is a tropical rainforest plant in South-eastern Nigeria, the Igbos call it "Utazi" and it is utilized for its medicinal and culinary properties as spice and vegetable for sauces and soups as well as having antimicrobial properties (Enyi-Idoh *et al*., 2017).

Piper guineense is a medicinal plant growing in various parts of Africa and other parts of the world. It is commonly called Ashanti pepper or African black pepper, and also known with different vernacular names in Nigeria: Igbo (Uziza) and Yoruba (Iyere). It has been shown to be one of the most valuable spices with numerous health benefits (Ene-Obong *et al*., 2016).

These plants were chosen for this research because of so many reports on them to possess some medicinal properties. Ebana *et al*. (2016) and Ilodibia *et al*. (2015) reported that ethanol and aqueous leaf extracts of *Gongronema latifolium* have antimicrobial activities on bacterial organisms including *Staphylococcus aureus* and *Escherichia coli.* The veterinary importance of both infectious diseases includes: decreased weight gain in meat birds, drop in egg production in laying birds, mortality, cost of treatment, condemnation of carcass at slaughter, and economic loss (Landman & Van-Eck, 2015). The complaint by farmers of resistance to some antibiotics necessitated this study. The aim of the study is to determine whether extracts of *Piper guineensis* and *Gongronema latifolium* have antibiotic effects on *Staphylococcus aurues* and *Escherichia coli.*

MATERIALS AND METHODS

COLLECTION AND IDENTIFICATION OF PLANT SAMPLES

Gongronema latifolium was collected from local farms at Umuariga village in Ikwuano Local Government Area of Abia state, while *Piper guineense* was purchased from Isi-nweke local daily market in Ihitte-Uboma Local Government Area (LGA) of Imo State. These plants were identified and confirmed by Dr. Nkaa, F. A. of the Department of Botany, College of Natural and Applied Sciences, Michael Okpara University of Agriculture, Umudike, Abia State.

PREPARATION OF PLANT SAMPLES AND EXTRACTION PROCEDURE

The leaves were carefully separated from their stalk, washed and air dried for six weeks. The dried leaves were grounded with an electric grinding machine to a fine powder form for extraction which was stored in a sterile beaker at refrigeration temperature.

The two solvents, methanol and distilled water were used for the extraction of the grounded dry leaves because they have the potential to produce more yields and this method was also adopted by Fasakin *et al*. (2011).

The methanolic extraction was done with the use of Soxhlet extractor by reflux method as described by Zygler *et al*. (2012). Using electronic weighing balance, 248g and 257g of the stored dried powder *G. latifolium* and *P. guinense* leaves were collected differently into the Soxhlet apparatus with 250ml of methanol. External heat source was attached to the extractor with temperatures between 30^{o}C to 60^{o}C. The extraction ran for 2 days with anti-pumping silicon granules added each day before the apparatus was turned on. The extracts were collected into a clean beaker and filtered with a Whatman No. 1 filter paper. The methanol solvent was then evaporated *in vacuo* and later in a water bath at 40^{o}C until completely dry crude extract was obtained. The whole extracts were refrigerated at 4^{o}C until further use.

Cold maceration method as described by Dharajiya *et al*. (2017) was used. Two hundred grammes of the grinded leaves of each plant were poured into appropriately labelled containers each containing 1L of distilled water and left at room temperature for 48hours after which it was filtered using Whatman's No. 1 filter paper. Each filtrate was evaporated until dry crude extract was obtained and stored in the refrigerator until required for further analysis.

RECONSTITUTION OF THE EXTRACTS

The yield of the extracts were 20mg/ml, stored crude extracts were reconstituted using distilled water by 2 fold serial dilution to obtain the following concentrations 1.25. 2.5, 5.0 and 10.0mg/ml.

QUALITATIVE PHYTOCHEMICAL SCREENING OF *GONGRONEMA LATIFOLIUM* AND *PIPER GUINEENSE*

The extracts of the plant leaves were subjected to qualitative phytochemical analysis. The presence of tannins and phenols (Behlil *et al*., 2019), saponin (Ejikeme *et al*., 2014), flavonoids (Ezeonu & Ejikeme., 2016), alkaloids (Hikino *et*

al., 1984) were determined using the procedures of the cited authors.

QUANTITATIVE DETERMINATION OF PHYTOCHEMICAL CONSTITUENTS OF THE PLANTS

DETERMINATION OF FLAVONOID

Flavonoid determination was by the method reported by Ejikeme *et al*. (2014). Exactly 50 ml of 80% aqueous methanol was added to 2.50 g of sample in a 250 ml beaker, covered, and allowed to stand for 24 hours at room temperature. After discarding the supernatant, the residue was re-extracted (three times) with the same volume of ethanol. Whatman filter paper number 42 (125 mm) was used to filter whole solution of each wood sample. Each sample filtrate was later transferred into a crucible and evaporated to dryness over a water bath. The content in the crucible was cooled in a desiccator and weighed until constant weight was obtained. The percentage of flavonoid was calculated as

$$\%\text{Flavonoid} = \frac{weight\ of\ Flavonoid}{weight\ of\ sample} \times 100$$

DETERMINATION OF TANNINS

Analytical method for quantitative determination of tannin was according to Ezeonu & Ejikeme, (2016). By dissolving 50 g of sodium tungstate (Na_2WO_4) in 37 ml of distilled water, Folin-Denis reagent was made. To the reagent prepared above, 10 g of phosphomolybdic acid ($H_3PMo_{12}O_{40}$) and 25 ml of orthophosphoric acid (H_3PO_4) were added. Two-hour reflux of the mixture was carried out, cooled, and diluted to 500 ml with distilled water. One gram of each powder (sample) in a conical flask was added to 100 cm^3 of distilled water. This was boiled gently for 1 hour on an electric hot plate and filtered using number 42 (125 mm) Whatman filter paper in a 100 ml volumetric flask. Addition of 5.0 ml Folin-Denis reagent and 10 ml of saturated Na_2CO_3 solution into 50 ml of distilled water and 10 ml of diluted extract was carried out after being pipette into a 100 ml conical flask for colour development. The solution was allowed to stand for 30 minutes in a water bath at a temperature of 25°C after thorough agitation. With the aid of a Spectrum Lab 23A spectrophotometer, optical density was measured at 700 nm and compared on a standard tannic acid curve. Dissolution of 0.20 g of tannic acid in distilled water and dilution to 200 ml mark (1 mg/cm^3) were used to obtain tannic standard curve. Varying concentrations (0.2–1.0 mg/ml) of the standard tannic acid solution were pipetted into five different test tubes to which Folin-Denis reagent (5 ml) and saturated Na_2CO_3 (10 ml) solution were added and made up to the 100 ml mark with distilled water. The solution was left to stand for 30 minutes in a water bath at 25°C. Optical density was ascertained at 700 nm with the aid of a Spectrum Lab 23A spectrophotometer. Optical density (absorbance) versus tannic acid concentration was plotted.

The following formula was used in the calculation:

$$\text{Tannic acid}\left(\frac{mg}{100g}\right) = \frac{C \times extract\ volume \times 100}{Aliquot\ volume \times weight\ of\ sample}$$

Where C is concentration of tannic acid read off the graph.

DETERMINATION OF SAPONIN

Saponin quantitative determination was carried out using the method reported by Ejikeme *et al*. (2014) Exactly 100 ml of 20% aqueous ethanol was added to 5 grams of each powder sample in a 250 ml conical flask. The mixture was heated over a hot water bath for 4 hours with continuous stirring at a temperature of 55°C. The residue of the mixture was re-extracted with another 100 ml of 20% aqueous ethanol after filtration and heated for 4 hours at a constant temperature of 55°C with constant stirring. The combined extract was evaporated to 40 ml over water bath at 90°C. Twenty (20 ml) of diethyl ether was added to the concentrate in a 250 ml separator funnel and vigorously agitated from which the aqueous layer was recovered while the ether layer was discarded. This purification process was repeated twice. 60 ml of n-butanol was added and extracted twice with 10 ml of 5% sodium chloride. After discarding the sodium chloride layer, the remaining solution was heated in a water bath for 30 minutes, after which the solution was transferred into a crucible and was dried in an oven to a constant weight. The saponin content was calculated as a percentage:

$$\%\text{Saponin} = \frac{weight\ of\ saponin}{weight\ of\ sample} \times 100$$

DETERMINATION OF ALKALOIDS

Quantitative determination of alkaloid was according to the methodology by Harborne (1973). Exactly 200 cm^3 of 10% acetic acid in ethanol was added to each powder sample (2.50 g) in a 250 cm^3 beaker and allowed to stand for 4 hours. The extract was concentrated on a water bath to one-quarter of the original volume followed by addition of 15 drops of concentrated ammonium hydroxide in a drop-wise to the extract. After 3 hours of mixture sedimentation, the supernatant was discarded and the precipitates were washed with 20 ml of 0.1 M of ammonium hydroxide and then filtered using Gem filter paper (12.5 cm). Using electronic weighing balance Model B-218, the residue weighed and the percentage of alkaloid is expressed mathematically as

$$\%\ \text{Alkaloid} = \frac{weight\ of\ alkaloid}{weight\ of\ sample} \times 100$$

DETERMINATION OF PHENOLS

The procedure according to Keay *et al*. (1964) was used. Defatting of 2 g powder sample was carried out for 2 hours in 100 ml of ether using a soxhlet apparatus. The defatted

sample (0.50 g) was boiled for 15 minutes with 50 ml of ether for the extraction of the phenolic components. Exactly 10 cm^3 of distilled water, 2 ml of 0.1 N ammonium hydroxide solution, and 5 ml of concentrated amyl alcohol were also added to 5 cm^3 of the extract and left to react for 30 minutes for colour development. The optical density was measured at 505 nm and 0.20 g of tannic acid was dissolved in distilled water and diluted to 200 mL mark (1 mg/ml) in preparation for phenol standard curve. Varying concentrations (0.2–1.0 mg/ml) of the standard tannic acid solution were pipetted into five different test tubes to which 2 ml of NH_3OH, 5 ml of amyl alcohol, and 10 ml of water were added. The solution was made up to 100 ml volume and left to react for 30 minutes for colour change. The optical density was then determined at 505nm.

TEST ORGANISMS

Stock cultures used (*S. aureus* and *E.coli*) were obtained from the Veterinary Microbiology Laboratory of Michael Okpara University of Agriculture, Umudike. The stored cultures were sub-cultured into mannitol salt agar (MSA) and eosin methylene blue (EMB), respectively, to confirm the identity of the isolates.

Concentrations used for the MIC 1.25, 2.5, 5.0, 10.0 and 20mg/ml and 2ml of each dilution was mixed with 18 ml of nutrient agar and poured into Petri-dishes and allowed to set. The test organisms (overnight culture) were standardized to 0.5 MacFarland turbidity standard, thereafter, streaked into the plates with the bacterial isolates and incubated at 37^{o}C for 24hours after which they were examined for the presence or absence of growth. The minimum concentration that completely inhibited macroscopic growth was regarded as the minimum inhibitory concentration of the respective extracts

IN-VITRO* ANTIMICROBIAL POTENTIAL OF *GONGRONEMA LATIFOLIUM* AND *PIPER GUINEESE

The stored extracts were reconstituted using distilled water to obtain stock solution. The concentrations of these stock solutions were 1.25, 2.5, 5.0 and 10.0mg/ml. Agar well diffusion test method as reported by Balouiri *et al*. (2016) was used to determine the *in-vitro* antibacterial activities of the extracts against the test bacteria by taken readings of the zones of inhibition of the extracts to the organism. Sterile swabs were used to inoculate the media and spread all over the surface of the nutrient agar plate and allowed set. Using a cork borer of 6mm in diameter, wells were made on each plate: two wells for the aqueous and two for the methanolic extract of each plant. Using a pipette, the volume delivered into each well will be known. Each plant extract as labeled was allowed to diffuse before incubated at 37^{o}C for 24 hours. The inhibition zone diameter (IZD) produce were measured using meter rule, and mean recorded to the nearest whole millimeter (mm)

MINIMUM INHIBITION CONCENTRATION:

The minimum Inhibition Concentration of the crude extract was carried out using the modified method of Akinpelu & Kolawole, (2004).

RESULTS

Qualitative phytochemical qualitative analysis showed the presence of yellow coloration confirmatory of flavonoids, blue-black coloration for tannins, formation of bulky precipitates for phenols, formation of emulsions for saponins and alkaloids was positive with the formation of white precipitates for both the aqueous and methanolic extracts of *P. guineense* and *G. latifolium* as shown in Table 1. Tannins and phenols were most present of all other phytochemical components in methanolic and aqueous extract of *G. latifolium*

The quantitative phytochemical quantitative analysis showed that aqueous extracts of *P. guineense* had alkaloids (0.21±0.01mg/g), flavonoid (0.21±0.03mg/g), tannins (0.01±0.02mg/g), saponin (0.25±0.3mg/g) and phenol (0.19±0.02mg/g) Table 2 while, methanolic extracts had alkaloids (0.41±0.02mg/g), flavonoid (0.28±0.04mg/g), tannins (0.15±0.02mg/g), saponin (0.35±0.01mg/g) and phenol (0.33±0.03mg/g) Table 2. At 10 mg/ml, methanolic extract of *P. guineense* produced an inhibition zone diameter of 1o mm against *Staphylococcus aureus* while there was no zone of inhibition produced against *Escherichia coli (*Figure I). At 10mg/ml, aqueous extract of *P. guineense* produced an inhibition zone diameter of 13mm against *S. auerus*, but 8mm against *Escherichia coli* (Figure II).

The quantitative phytochemical analysis also showed that aqueous extract of *G. latifolium* had alkaloids (1.28 ±0.03mg/g), flavonoids (0.26±0.06mg/g), tannins (0.17±0.03mg/g), saponin (2.08±0.03mg/g) and phenol (7.28±0.08mg/g) Table 3 while, methanolic extract had alkaloid (1.38±0.03mg/g), flavonoid (5.66mg/g), tannins (11.27±0.07mg/g), saponin (3.10±mg/g) and phenol (15.68±0.11mg/g) (Table 3). Methanolic extract of *G. latifolium* at 7mg/ml exhibited a zone of inhibition of 9mm for *S. aureus* but no form of inhibition zones for *Escherichia coli* 0mm. However, at 10mg/ml, *G. latifolium* exhibited inhibition zone of 10mm for *S. aureus,* while for *Escherichia coli*, the zone of inhibition was 8mm (Figure III). Aqueous extract of *G. latifolium* at 7mg/ml exhibit an inhibition zone diameter of 10mm for *S. aureus* but no form of inhibition for *Esherichia coli* 0mm. At 10mg/ml, the inhibition zones shown by *Staphylococcus aureus* was 13mm, while *E.coli* had an inhibition zone of 12mm (Figure IV). The minimum inhibitory concentration of aqueous extract of *Piper guineense* for *Staphylococcus aureus* and *E. coli* was 10

mg/ml and 20mg/ml, while the MIC of the methanolic extract for *Staphylococcus aureus* was 7mg/ml and 10mg/ml respectively. The MIC of methanolic extract of *Gongronema latifolium* for *Staphylococcus aureus* was 7mg/ml while the aqueous had an MIC of 10 mg/ml for both test organisms.

Table 1; Phytochemical Qualitative analysis for methanol and aqueous extract of *G. latifolium*

Extracts	Methanol	aqueous (mg/100g)
Alkaloids	white precipitates (+)	(+)
Flavonoid	yellow color (+)	(+)
Tannins	blue-black color (+++)	(++)
Saponin	emulsion formed (++)	(+)
Phenol	bulky precipitate (+++)	(+)

Present (+), more present (++), most present (+++)

Table II; Phytochemical Quantitative analysis for methanol and aqueous extract of *P. guineense*

Extracts	methanol	Aqueous (mg/100g)
Alkaloids	0.41±0.02	0.21±0.01
Flavonoid	0.28±0.04	0.21±0.03
Tannins	0.15±0.02	0.10 ± 0.02
Saponin	0.35±0.01	0.25± 0.03
Phenol	0.33±.030	0.19 ± 0.02

Table III: Phytochemical Quantitative analysis for methanol and aqueous extract of *Gongronema latifolium*

Extracts	methanol	aqueous (mg/100g)
Alkaloids	1.38±0.03	1.28±0.03
Flavonoid	5.66±0.065	0.26 ±0.06
Tannins	11.27±0.07	0.17 ±0.03
Saponin	3.10± 0.05	2.08 ±0.03
Phenol	15.68±0.11	7.38±0.08

DISCUSSION

In this study, aqueous and methanolic extracts of dried leaves of *Gongronema latifolium* and *Piper guineense* were tested for their antimicrobial potential against two bacterial organisms; *Staphylococcus aureus* and *Escherichia coli* at different concentrations of the extracts. From the study, methanolic and aqueous extracts of *Piper guineense* and *Gongronema latifolium* have concentration dependent antimicrobial activity on the test organisms; *Staphylococcus aureus* and *Escherichia coli.* This is in agreement with the findings of Mgbeahuruike *et al*. (2018).The organisms (*Staphylococcus aureus*) were sensitive to methanolic extract of *Piper guineense* at the highest concentration of 10mg/ml with inhibitory zone of 10mm but had no inhibitory effect on *Escherichia coli* at same concentrations. This observation is consistent with a previous research by Mgbeahuruike (2019), who reported the methanolic extract of *Piper guineense* to have inhibitory effect of 14.7mm on *Staphylococcus aureus*. The aqueous extract of *Piper guineense* was able to produce some inhibitory effect on the growth of *Staphylococcus aureus* and *Escherichia coli* at the highest concentration of 10mg/ml with inhibitory zones of 13mm and 8mm respectively while other lower concentrations had no inhibitory effect. This supports the earlier study of Edada *et al*. (2018) who reported *Staphylococcus aureus* and *Escherichia coli* being sensitive to aqueous extract of *Piper guineense* with inhibitory zones of 16.5mm and 12.0mm respectively. Previous studies showed that *Staphylococcus aureus* was more sensitive to the methanolic and aqueous leaf extract of *Piper guineense than Escherichia coli.* From the study, these two organisms were sensitive to methanolic extract of *Gongronema latifolium.* This sensitivity is directly proportional to the concentration of the extract in which an increase in concentration leads to increased sensitivity. This corresponds with the report of Etta *et al*. (2012) and Ilodibia

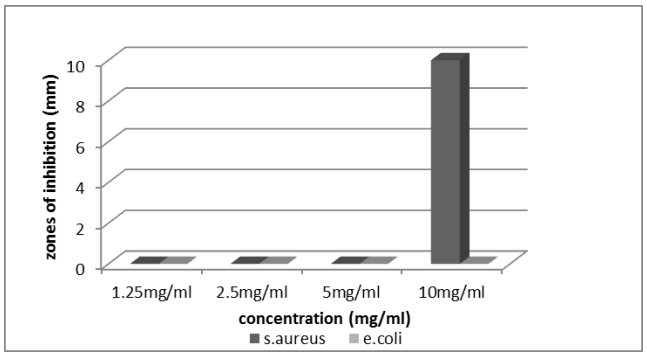

Figure1: Zones of inhibition of methanolic extracts of *Piper guineense* dried leaves.

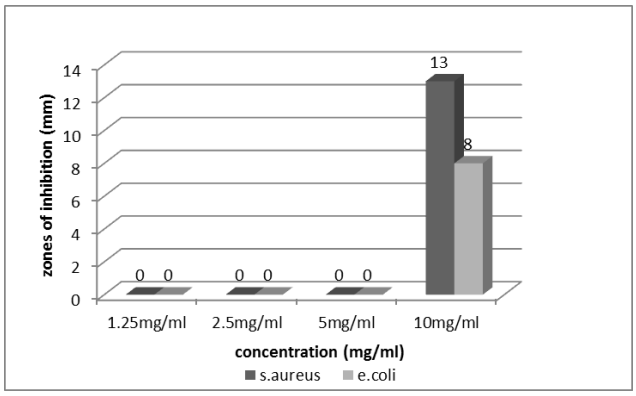

Figure II: Sensitivity pattern of test organism to aqueous extract of *Piper guineense* and their zones of inhibition

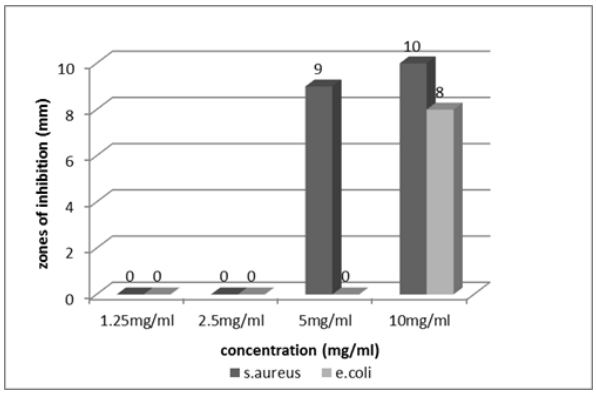

Figure III: Sensitivity pattern of test organisms to methanol extract of *Gongronema latifolium*

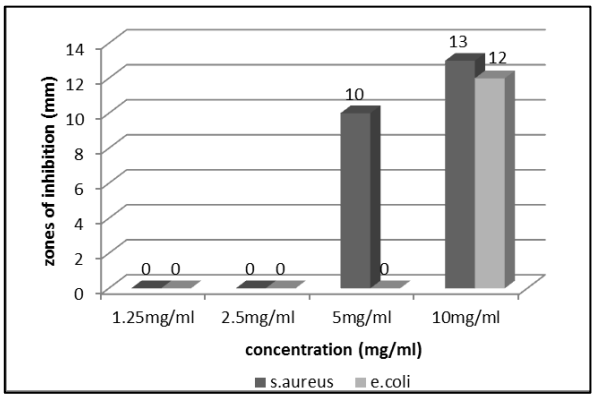

Figure IV: sensitivity pattern of test organisms to aqueous extract of *Gongronema latifolium*

et al. (2015), they reported methanolic extract of *G. latifolium* to have concentration dependent effect on *Staphylococcus aureus.* However, in a study by Ebana *et al*. (2016), they reported aqueous extract of *G. latifolium* to be inactive on *Escherichia coli*, but sensitive against *Staphylococcus aureus* with an inhibition zone of 14.0mm. The finding in the study is in agreement with Nwinyi *et al*. (2008). Nwinyi and his co-workers reported that, aqueous extract of *Gongronema latifolium* was observed to be sensitive against *Staphylococcus aureus* and *Escherichia coli*. However, Malachy *et al.* (2017) reported that aqueous and methanolic extracts of *Gongronema latifolium* does not support this research. In their separate reports, aqueous and methanolic extracts of *Gongronema latifolium* had no antimicrobial activity on *Staphylococcus aureus* and *Escherichia coli*. Based on the result of the sensitivity test, *Staphylococcus aureus* was observed to be more sensitive to the methanolic extract of *Piper guineense* at 10mg/ml concentration.

The antibiotic activities of the extracts can be attributed to the phytochemical constituents present in them such as flavonoids, alkaloids, saponins, tannins, and phenols and this agrees with the findings of Chukwuma & Chigozie (2016).

From the results of the phytochemical analysis, these secondary metabolites were found to be more abundant in *Gongronema latifolium* than in *Piper guineense* which explains its higher exertion of antimicrobial effect on the test organisms than that of *Piper guineense*.

CONCLUSION

Methanolic and aqueous extracts of *Gongronema latifolium* and *Piper guineense* dried leaves have antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. Aqueoeus extract (distilled water) showed higher antimicrobial activities than methanolic extract, hence, should be the preferred solvent. This study supports the long history of the use of these plants in traditional medicine for the treatment of local and systemic disease. Further research on the effect of the use of other solvents for plant extraction on the antimicrobial potential of the plant extracts is suggested. This will provide information on the best solvent to use that will yield the most desired result. Pharmaceutical industries and research based organization should conduct more work in this area so that the safe dosage for use of herbs would be determined and use of natural plants in place of synthetic medicines as antibiotics would be guaranteed and the issue of resistance by the synthetic antibiotics will be eliminated.

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CONFLICT OF INTEREST

There was no conflict of interest to declare.

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