European Journal of Botany, Plant Sciences and Phytology
Vol.5, No.1, pp.1-12, 2020
Print ISSN: 2055-8139(Print), Online ISSN: 2055-8147(Online)
Published by European Centre for Research Training and Development UK (www.eajournals.org)

ANTIBACTERIAL EFFECT OF *GONGRONEMA LATIFOLIUM* LEAF EXTRACTS ON SELECTED GRAM POSITIVE AND NEGATIVE CLINICAL BACTERIAL ISOLATES

***Ndubueze, C. W., Dike-Ndudim, J. N. and Udujih, H. I.**

Department of Medical Laboratory Science, Faculty of Health Sciences, Imo State University, Owerri, Nigeria.
Email: chizaramwinnersndubueze@gmail.com

ABSTRACT: *This work was aimed at assaying the in-vitro effect of aqueous and ethanolic leaf extracts of Gongronema latifolium on Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli. Six (6) milimetre sterile discs were impregnated with the aqueous and ethanolic extracts at different concentrations ranging from 6.25mg/mL to 100mg/mL. The test organisms were spread evenly on Mueller Hinton agar plate and the discs were aseptically placed on them. The sensitivity plates were incubated at 37ºC for 24 hours. All the test organisms showed sensitivity to both aqueous and ethanolic leaf extracts of Gongronema latifolium. The zones of inhibition were concentration dependent, ranging from 2.0mm to 10.8mm for aqueous extract and 2.0mm to 8.3mm for the ethanolic extract. Comparison of the zones of inhibition produced by the two extracts showed that there is no statistical difference (P > 0.05) between aqueous and ethanolic extracts. Staphylococcus aureus, Escherichia coli and Klebsiella pneumoniae had Minimum inhibitory concentration (MIC) of 6.25mg/mL, while Pseudomonas aeruginosa had MIC of 25mg/mL for the aqueous extract. The MIC was 3.125mg/mL, 6.25mg/mL, 6.25mg/mL and 25mg/mL for S. aureus, E. coli, K. pneumoniae and P. aeruginosa, respectively for the ethanolic extract. Gongronema latifolium extracts were also bactericidal in action. S. aureus, E. coli and P. aeruginosa all had Minimum bactericidal concentration (MBC) of 6.25mg/mL, while K. pneumoniae had MBC of 25mg/mL for the aqueous extract, while for the ethanolic extract, S. aureus, E. coli, K. pneumoniae and P. aeruginosa had MBC of 12.5mg/mL, 12.5mg/mL, 6.25mg/mL and 3.125mg/mL respectively. The data obtained from the study indicated that both the aqueous and ethanolic leaf extracts of Gongronema latifolium possess antibacterial properties. Therefore, the pharmaceutical industries should consider its usage for the production of novel antibiotics.*

KEYWORDS: antibiotic, resistance, medicinal, plants, Pseudomonas aeruginosa.

INTRODUCTION

The rapid emergence of resistant bacteria is a worldwide issue endangering the efficacy of antibiotics, which have transformed medicine and saved millions of lives (Golkar *et al.*, 2014). Many decades after the first patients were treated with antibiotics, bacterial infections have again become a threat (Spellberg and Gilbert, 2014). The antibiotic resistance crisis has been attributed to the overuse and misuse of these medications, as well as a lack of new drug development by the pharmaceutical industries due to reduced economic incentives and challenging regulatory requirements (Viswanathan, 2014).

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 (Iwu *et al.*, 1999). According to the World Health Organization (2002), medicinal plants would be the best source to obtain a variety of drugs.

Global prevalence of infectious diseases caused by bacteria is a major public health problem (Talib and Mahasneh, 2010). The bacterial agents including: *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Proteus vulgaris* cause several human infections (Peirano, 2008). Recent emergence of antibiotic resistance and related toxicity issues limit the use of antimicrobial agents (Eggleston *et al.*, 2010) and is prompting a revival in research of the antimicrobial role of plants against resistant strains due to comparable safety and efficacy (Alviano and Alviano, 2009).

Gongronema latifolium is a tropical rainforest plant of the order Gentiales and the family Apocynaceae. It belongs to the Subfamily Asclepiadaceae, and genus *Gongronema* (Osuagwu *et al.*, 2013). 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 (Enyi-Idoh *et al.*, 2017).

It has been reported that *Gongronema latifolium* has an antimicrobial activity against some species of microorganisms (Enyi-Idoh *et al.*, 2012).

It is already known and documented that some of the strains of the aforementioned bacterial species are resistant to many antibiotics traditionally used to treat bacterial infections, such as penicillin and ampicillin. Therefore, this study aimed at assaying the in-vitro effect of aqueous and ethanolic leaf extracts of *Gongronema latifolium* on *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli.*

METHODOLOGY

Collection and Identification of Plant Samples and Test Organisms

Plant samples, *Gongronema latifolium* leaves were purchased from vegetable vendors at relief market, Owerri. Pure cultures of pathogenic bacteria such as *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were obtained from the Medical Microbiology Laboratory of the Department of Medical Laboratory Science, Imo State University. Before being used, the identities of the test organisms were confirmed from the slope culture and then used for the test on the plates. To confirm the test organisms, inocula were taken from the slant and inoculated onto the media. The inoculated plates were incubated appropriately at 37ºC for 24hours. The culture was examined for growth and conformity signifying purity. The pure isolates as shown by their colonial and microscopic features, and biochemical tests carried out, were certified.

Processing of the Leaves

The leaves of the shrub were carefully separated from the stalk. The leaves were cut into smaller pieces and were air dried. All the dried leaf samples were grinded in an electric grinding machine and sieved through a 1mm test sieve to obtain a powder processed sample used in the extraction. The extracts were subjected to analysis using different solvents. The samples were macerated in: Ethanol and Cold water.

Extraction with ethanol

After the grinding, 20g of the leaves was weighed and poured in a beaker; 200mLs of ethanol were added to it. The solution was shaken and allowed to stay at room temperature for 48hours. After that, it was filtered with Whatman's No. 1 filter paper. The filtrate was stored in the refrigerator until required.

Extraction with water

20grams of the grinded leaves were extracted and weighed. 200mL of distilled water was used for the experiment. The grinded leaves were poured into a beaker containing 200mL of distilled water and left at room temperature for 48hours after which it was filtered using Whatman's No. 1 filter paper. The filtrate was stored in the refrigerator until required for further analysis.

Preparation and Sterilization of Materials

The media used were prepared according to the manufacturers' instructions. The media were sterilized by autoclaving at 121ºC for 15minutes, allowed to cool for 45minutes and poured into sterile petri dishes.

The working bench was disinfected using cotton wool soaked in 70% alcohol. The clean glass wares were washed with detergent, rinsed properly in several changes of tap water and further rinsed with distilled water. They were then air dried and wrapped with aluminum foil and sterilized in the hot air oven at 170ºC for 2 hours. Aseptic technique was applied in the working environment by ensuring that all work was done near the naked flame of Bunsen burner (Ochei and Kolhaktar, 2008).

Preparation and sterilization of antibiotic discs

The antibiotics discs were produced from Whatman's filter paper. The 6mm diameter discs were sterilized in hot air oven at 160ºC for one hour. Each extract was individually used to impregnate the 6mm sterile discs at the concentrations of 100mg/mL, 50mg/mL, 25mg/mL, 12.5mg/mL and 6.25mg/mL so that each contained 20µL (0.02mL) of the extract and allowed to air dry at room temperature. The positive control, ciprofluoxacilin at concentration of 50mg/mL was also used to impregnate sterile 6mm discs and allowed to air dry at room temperature. The discs were later used in the sensitivity test.

Antimicrobial Screening

The agar diffusion disc method was used to determine the antibiotic sensitivity. The test organisms: *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were inoculated on Mueller Hinton agar in triplicates using streaking. With the help of a sterile forceps, the aqueous and ethanolic extracts impregnated discs were placed on the sensitivity plates, and incubated at 37ºC for 24hours. The zones of inhibition were measured using graduated metre rule after 24 hours incubation and recorded in milimetre (mm).

Determination of minimum inhibitory concentration (MIC)

A total of sixteen (16) sterile test tubes were placed in a test tube rack for each of the organisms. Two-fold dilutions were made using the different extracts, and tubes were mixed thoroughly.

Each tube was inoculated with the test organism and incubated at 37ºC for 24hours. Then, the MIC was taken to be the lowest dilution of the tube showing no visible growth (no turbidity).

Determination of minimum bactericidal concentration (MBC)

The minimum bactericidal concentration was determined. All tubes in minimum inhibitory concentration that showed no visible growth were sub-cultured on freshly prepared nutrient agar plates and incubated at 37ºC for 24hours. The minimum bactericidal concentration (MBC) was regarded as the lowest concentration of the extract that did not permit any visible growth on the nutrient agar plate after the period of incubation.

Statistical Analysis

Data were entered and analyzed using SPSS version 21. The results were statistically expressed as mean ± standard deviation and the P- value obtained. The results obtained were also represented graphically using Microsoft Excel.

RESULTS

Zones of Inhbition of Aqueous and Ethanolic Extracts on the Test Organisms

[Figure 1] below shows the zones of inhibition of aqueous extracts of *Gongronema latifolium* leaves on the test organisms. The zones of inhibition produced by the highest concentration (100mg/mL) of the aqueous extract proved to be the highest zones, while the least zone of inhibition was produced by the least concentration (6.25mg/mL).

Checking the sensitivity of the different organisms to the aqueous extract, *Staphylococcus aureus* was observed to be the most sensitive with zone of inhibition of 10.8 ± 0.02mm at 100mg/mL, followed by *Pseudomonas aeruginosa* with 8.5±0.00mm zone of inhibition at 100mg/mL, this is followed by *Klebsiella pneumoniae* which had a zone of inhibition of 7.4±0.30mm at the concentration of 100mg/mL. *Escherichia coli* proved to be least sensitivity to 100mg/mL of the aqueous extract with a zone of inhibition of 6.0±0.15mm.

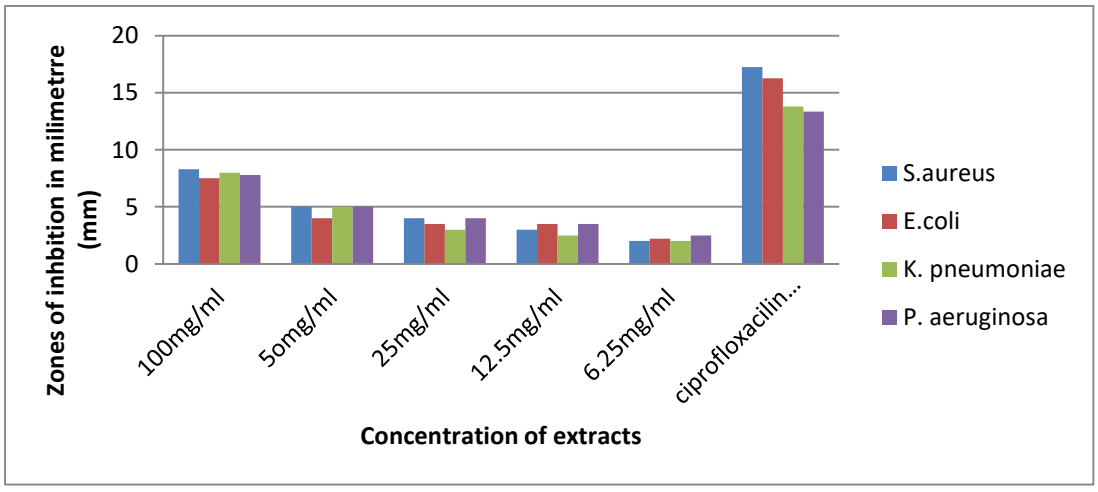

Figure 1: Zones of inhibition of aqueous extracts of *Gongronema latifolium* dried leaves

[Figure 2] below shows the zones of inhibition of ethanolic extract of *Gongronema latifolium* on the test organisms. The zones of inhibition produced by the highest

concentration (100mg/mL) of the ethanolic extract proved to be the highest zones, while the least zones of inhibition produced by the least concentration (6.25mg/mL).

Based on the sensitivity result of the different organisms to the ethanolic extract, *Staphylococcus aureus* was the most sensitive with zone of inhibition of 8.30±0.04mm at 100mg/mL, followed by *Klebsiella pneumoniae* with 8.00±0.01mm zone of inhibition at 100mg/mL, which was followed by *Pseudomonas aeruginosa* with inhibition of 7.80±0.20mm at the concentration of 100mg/mL. *Escherichia coli* had the least sensitivity to 100mg/dl with a zone of inhibition of 7.50±0.12mm.

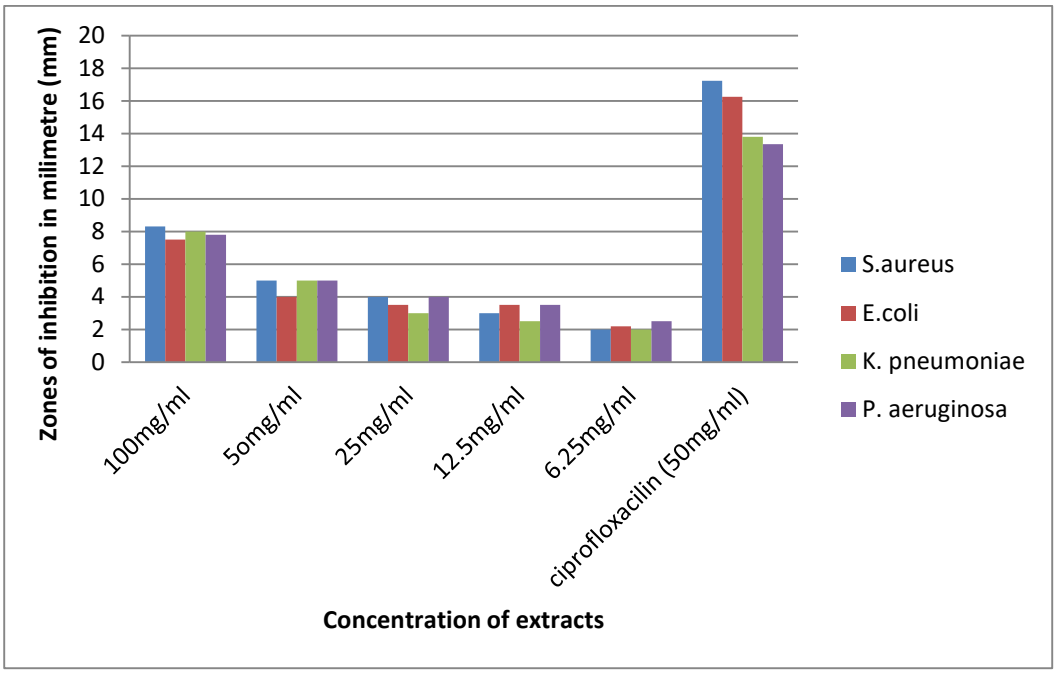

Figure 2: Zones of inhibition (in mm) of ethanolic extracts of *Gongronema latifolium* dried leaves

Minimum Inhibitory Concentration (MIC) of Aqueous and Ethanolic Extracts of *Gongronema Latifolium* on the Test Organisms

[Figure 3] shows the Minimum inhibitory concentration (MIC) of aqueous extract of *Gongronema latifolium* on the test organisms. *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* had 6.2mg/mL as the minimum inhibitory concentration that inhibited the growth of the organisms. This was quite different from *Pseudomonas aeruginosa* which had 25mg/mL as the minimum inhibitory concentration of the aqueous extract that inhibited the growth of the organism.

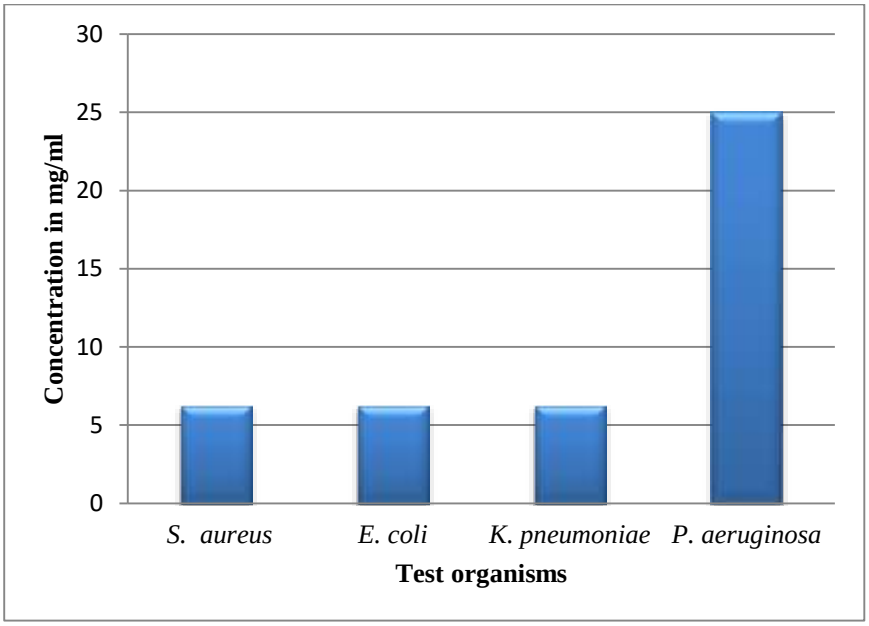

Figure 3: Minimum inhibitory concentration (MIC) of the aqueous extracts of *Gongronema latifolium* on the test bacteria.

[Figure 4] shows the Minimum inhibitory concentration of ethanolic extract of dried leaves of *Gongronema latifolium* on the test organisms. *Staphylococcus aureus* had 3.125mg/mL as the minimum concentration that inhibited its growth. *Escherichia coli* and *Klebsiella pneumoniae* had 6.25mg/mL as the lowest concentration of the ethanolic extract that inhibited their growth. 25.00mg/mL was the lowest concentration of the ethanolic extract that inhibited the growth of *Pseudomonas aeruginosa.*

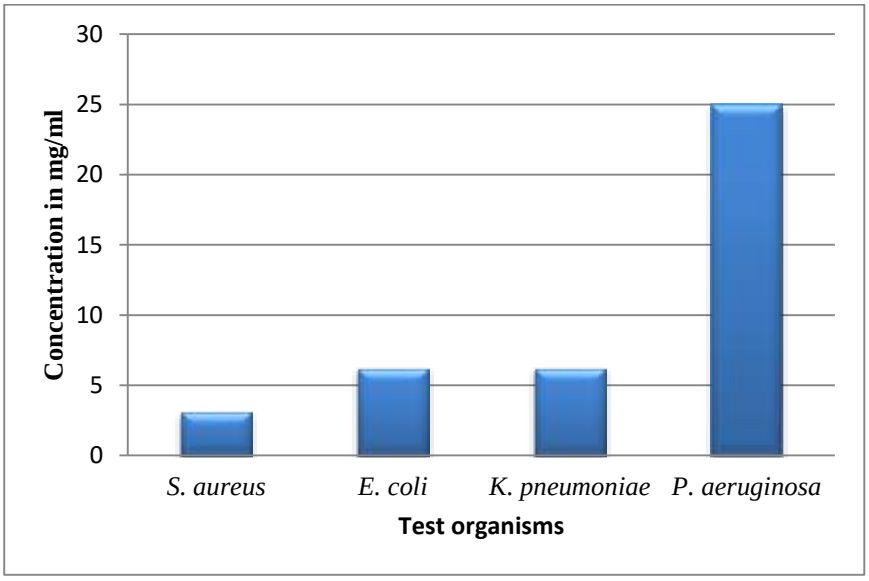

Figure 4: Minimum inhibitory concentration (MIC) of the ethanolic leaf extracts of *Gongronema latifolium* on the test bacteria.

Minimum Bactericidal Concentration (MBC) of the Aqueous and Ethanolic Extracts of *Gongronema Latifolium* on the Test Bacteria

[Figure 5] shows the minimum bactericidal concentration of the aqueous extract of *Gongronema latifolium* on the test bacteria.

The least concentration of the extract (6.25mg/mL) was the minimum concentration that resulted to the death of *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.* On the contrary, 25mg/mL of the aqueous extract was the minimum concentration that resulted to the death of *Klebsiella pneumoniae.*

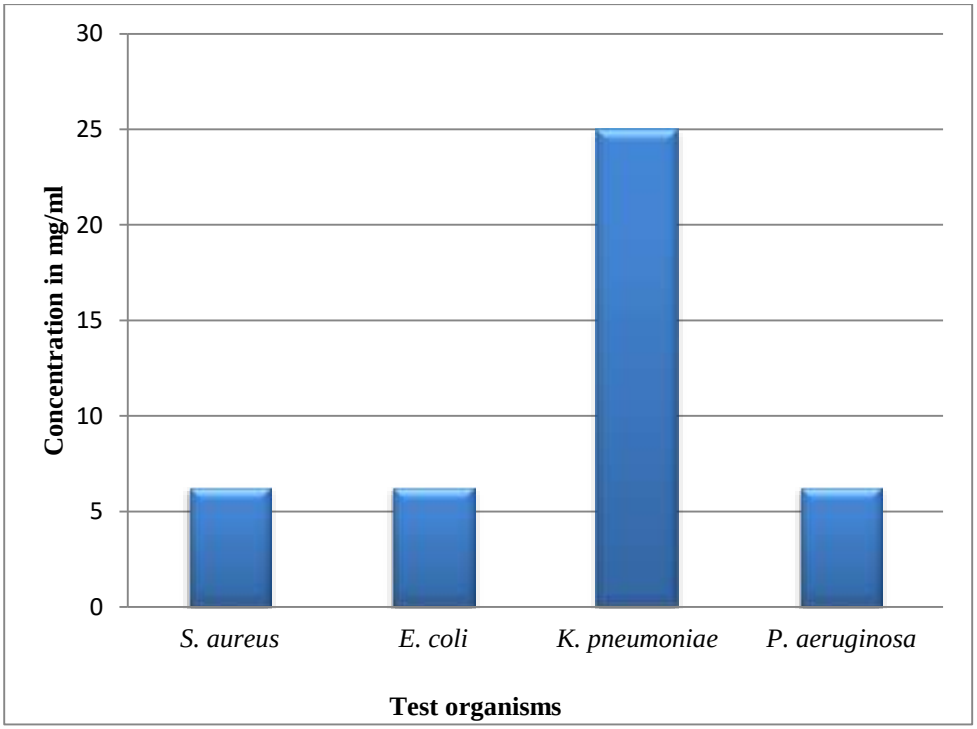

Figure 5: Minimum bactericidal concentration of aqueous extract of *Gongronema latifolium* on the test bacteria

[Figure 6] shows the Minimum Bactericidal Concentration (MBC) of the ethanolic extract of *Gongronema latifolium* on the organisms.

The figure has 3.125mg/ml as the least concentration of the ethanolic extract that was able to kill one of the test organisms, *Pseudomonas aeruginosa*. 6.25mg/ml was the least concentration of the extract that resulted to death of *Klebsiella pneumoniae*. *Staphylococcus aureus* and *Echerichia coli* both had 12.5mg/ml as the least concentration of the ethanolic extract that resulted to their death.

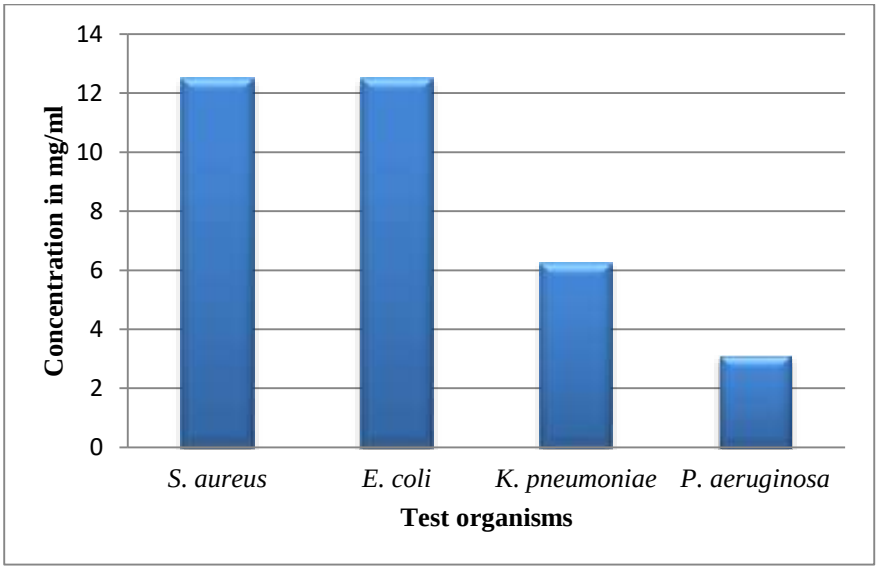

Figure 6: Minimum bactericidal concentration of the ethanolic extract of *Gongronema latifolium* on the test bacteria.

Comparison of Potency of Aqueous and Ethanolic Extracts of *Gongronema latifolium* on the Test Bacteria Based on the Zones of Inhibition

[Table 1] shows the mean ± standard deviation of the zones of inhibition produced by the different concentrations (100mg/mL, 50mg/mL, 25mg/mL, 12.5mg/mL and 6.25mg/mL) of aqueous and ethanolic extracts of *Gongronema latifolium* on the test organisms. The result showed that there is no statistical difference between the zones of inhibition produced by aqueous and ethanolic extracts.

Test organisms	**Zones of inhibition (mm)**		***P*-value**
	Aqueous Extract	**Ethanolic Extract**	
S. aureus	5.32±3.25	4.46±2.42	.65
E. coli	3.80±1.52	4.14±1.99	.77
K. pneumoniae	4.80±1.91	4.10±2.46	.63
P. aeruginosa	4.68±2.60	4.56±2.02	.94

Table 1: Mean ± standard deviation of the zones of inhibition produced by the different concentrations of aqueous and ethanolic extracts on the test organisms and the *P*-values

DISCUSSION

The increasing incidence of antibiotic resistant pathogens has drawn the attention of the pharmaceutical/scientific communities towards research on the antimicrobial potentials/activities of plant-derived substances (Gamil *et al.,* 2016). The increase in bacterial resistance to conventional antibiotics has necessitated the search for new and cost effective ways for the control of infectious diseases. Many studies have shown that medicinal plants constitute a great source for the isolation of active antimicrobials (Bessong *et al.,* 2006).

The majority of traditional healers use water to extract active compounds from this plant, because water is not harmful to humans and is generally cheap and easily acquired. However, successful isolation of compounds from plant material is largely dependent on the type of solvent used in the extraction process (Masoko *et al.,* 2008)

In this study, aqueous and ethanolic extracts of dried leaves of *Gongronema latifolium* were tested for their antibacterial activity against four species of bacteria: *Staphylococcus aureus* (Gram-positive), *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Gram-negative); after which MIC and MBC were determined to establish the potency of the extract(s).

The result of the study showed that aqueous and ethanolic extracts of *Gongronema latifolium* have concentration dependent inhibitory effect on the test organisms, *Staphylococcus aureus, Escherichichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* This is in agreement with the work of Chinyere *et al.,* (2015) which also reported that both aqueous and ethanolic extracts of *G. latifolium* possess antibacterial activities. In this study, the results obtained from the aqueous extract sensitivity test [figure 1] showed that all the organisms were sensitive to the extract at different concentrations. [Figure 2] also showed that the organisms were sensitive to different concentrations of the ethanolic extracts. Although much, research has not been carried out on the antimicrobial activity of *G. latifolium* which has been used for ages by the people of West Africa particularly Nigerians for dietary and medicinal purposes (Eja *et al.,* 2011). The antimicrobial activity revealed in this study agrees with the work reported by Nwanyi *et al.,* (2009) in which they said that *G. latifolium* has antimicrobial activities against *S. aureus and E.coli.* The aqueous extract proved to be effective against *S. aureus* and does not agree with the report of Oshodi *et al*., (2004) which has it that the aqueous extract possesses no antimicrobial activity. The ethanolic extract of *Gongronema latifolium* inhibited all the test organisms. This corroborates with the report of Adeleye *et al.,* (2011).

In this study, antimicrobial activity of aqueous and ethanolic extracts of *Gongronema latifolium* were studied at different concentrations (100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml) against four pathogenic bacterial stains (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). Antimicrobial potential of extracts were assessed in terms of zone of inhibition of microorganisms. The minimum inhibitory concentration and the minimum bactericidal concentration of both the aqueous and ethanolic extracts against the test organisms were also assessed.

The results in [figures 1 to 6] indicate that both the aqueous and the ethanolic leaf extract of *Gongronema latifolium* showed inhibitory effect against all the organisms tested. The inhibition of *Staphylococcus aureus* and *Escherichia coli* suggests that the plant possesses broad spectrum antibacterial properties which could be used in the treatment of some

infections due to these organisms. In the present study, the Gram-positive bacterium (*Staphylococcus aureus*) was more susceptible than the Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). This is because Gram-negative bacteria are known to be more resistant to inactivation by medium and long chain fatty acids than Gram-positive bacteria because of their impermeability to hydrophobic compounds as reported by Kabara, (1981).

Comparison of the zones of inhibition produced by the aqueous and ethanolic extracts revealed that there was no statistical difference between the two extracts. Therefore, both ethanol and water can be efficiently used for the extraction. This is in disagreement with the report of Nduche *et al.,* (2018).

CONCLUSION

Conclusively, this study revealed that both the aqueous and ethanolic leaf extracts of *Gongronema latifolium* have activity against multiple bacterial isolates. Therefore, the extracts can be effectively used in the eradication/treatment of clinical infections caused by these test organisms, (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). There was no statistical difference between the aqueous and ethanolic extracts. Therefore, any of the solvents can be efficiently used in extraction process. Antibacterial effect of *G. latifolium* which is evident from this study explains the long history of the use of these plants in traditional medicine for the treatment of different bacterial infections such as the use in the treatment of stomach pains/infections.

RECOMMENDATION

This work suggests further research in the use of the leaf extract of *Gongronema latifolium* and other herbs in the treatment of infections caused by different bacterial organisms (both Gram-positive and Gram-negative). Pharmaceutical industries are encouraged to consider extracting and purifying the active ingredients of *G. latifolium* in the production of novel antibiotics which could be of help in curbing the menace of antibiotic resistance.

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