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# Phytochemical Screening and Antimicrobial Effects of Aqueous and Ethanol Leaf and Stem Extracts of *Gongronema latifolium* Benth.

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## ABSTRACT

Aqueous and ethanolic leaf and stem extracts of *Gongronema latifolium* were investigated for the presence and composition of these phytochemicals (alkaloid, flavonoid, phenol, saponin, sterol, terpenoid and cyanogenic glycoside) and their antimicrobial activities at various concentrations against some selected clinical microbes (fungal strains: *C. albicans* and *Aspergillus niger* and bacterial strains: *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi*) using standard methods. Analysis of variance (ANOVA) was employed in data analysis. Qualitative and percent quantitative phytochemical results showed that both the aqueous and ethanolic leaf and stem extracts contained these phytochemicals assayed but in varied quantities except cyanogenic glycoside. Antimicrobial studies indicated that both the aqueous and ethanolic leaf and stem extracts of *Gongronema latifolium* inhibited the growth of the microbes but at varied levels and the inhibition was extracts concentration dependent. The ethanolic extract showed significantly higher inhibition than the aqueous extract in all concentrations except at 150 mg $mL^{-1}$ where the reverse was the case. The extracts showed higher inhibition against the fungal strains than the bacterial strains. Inhibitory effect of the leaf extract was significantly higher than those of the stem extract. Antibiotics had a better activity when compared to the extracts at the same concentration. *Gongronema latifolium* extracts were biostatic in their action, when purified will give a product with higher activity. The data obtained from the study indicated that the plant possessed antimicrobial properties especially antifungal and could be used in the treatment of bacterial and fungal infections but more especially the latter.

**Key words:** Antimicrobial, ethanol, aqueous, *Gongronema latifolium*, phytochemicals, fungal strains, bacterial strains

## INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of this isolation were based on the uses of the agents in traditional medicine. Plants contain numerous biologically active compounds, many of which have antimicrobial activity (Cowan, 1999). This plant-based, traditional medicine system continues to play an essential role in health care with about 80% of the worlds' inhabitants relying mainly on traditional medicines for their primary health care (Owolabi *et al*., 2007). According to world health organization, medicinal plants would be the best source to obtain a

variety of drugs. Long before mankind discovered the existence of microbes, the idea that certain plants have healing potential, indeed, that they contained what we would currently characterized as antimicrobial properties was well accepted. An antimicrobial agent is a substance that kills or inhibits the growth of microorganism such as bacteria, fungi and protozoa.

The medicinal content of plant depends on its phytochemicals. Phytochemicals are bioactive, non-nutritive plant compounds in fruits, vegetables, grains and other plants food, that have been linked to reducing the risk of major degenerative diseases. It is well known that plants produce these chemicals to protect themselves, but research demonstrated that they protect humans against diseases (Bate-Smith and Swain, 1962). Antibiotics are one of the most important weapons in fighting bacterial and fungal infections and have greatly benefitted the health related quality of human life since their introduction. Synthetic antibiotics accumulate in the body causing liver damage and other tissue problems. Such problems are not seen when natural antibiotics extracted from plants are used. These extracts are safe and potentially effective. The success story of chemotherapy lies in the continuous search for new drug from natural source to counter the challenges posed by resistant strains of microorganisms. The investigation of certain indigenous plants for their antimicrobial properties may yield useful result. These plants emerged as compounds with potentially significant therapeutic application against human pathogens (El Astal *et al*., 2005).

Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds (Chah *et al*., 2006; Nair and Chanda, 2006; Parekh and Chanda, 2007). In an effort to expand the search for the new antimicrobial agents from natural sources *Gongronema latifolium* Benth. member of the family Asclepiadaceae has been evaluated in this study.

*Gongronema latifolium* Benth. is a climber with woody, hollow glabrous stem and it is characterized by greenish yellow flower. It is wide spread in tropical Africa and originated from Senegal East to Chad and South to DR Congo. In Nigeria, *Gongronema latifolium* is been traditionally used for the management and treatment of ailments such as diabetes, cough, high blood pressure etc.

The objective of this study was to evaluate the phytochemicals and antimicrobial effects of aqueous and ethanolic leaf and stem extracts of *Gongronema latifolium* against pathogenic bacteria and fungi to determine their potentials as antimicrobial agent.

## MATERIALS AND METHODS

**Collection of plant samples:** The plant samples were collected from Nibo in Awka South Local Government Area, Anambra State. The *Gongronema* species was authenticated at Department of Botany, Nnamdi Azikiwe University, Awka where the voucher specimen was deposited with the accession No. NAUH 315.

**Preparation of plant samples:** The leaves and stems of *Gongronema latifolium* were cut into bits with knife and oven dried at 70°C for 12 h to remove all moisture. The samples were then ground into fine powder.

### Extraction of plant material

**Aqueous extraction:** The aqueous extract of the plant was prepared by adding the ground sample of leaf and stem in 100 mL of distilled water. The concentration of each extract was determined by adding 50, 75, 100 and 150 g in 100 mL of distilled water. The experimental set-up was left for

24 h at room temperature and thereafter filtered using Whatman filter paper No.1. The extract was then concentrated by heating on water bath to 50 mL of the original volume of the extract.

**Ethanol extraction:** The ethanolic extract of the plant was prepared by soaking the ground sample of the leaf and stem in 100 mL of ethanol. The concentration of each extract was determined by adding 50, 75, 100 and 150 g in 100 mL of ethanol. The experimental set-up was left for 24 h at room temperature and thereafter filtered using Whatman filter paper No. 1. The extract was then concentrated to 50 mL of the original volume of the extract and stored in an air tight container in a refrigerator at 4°C until when needed.

**Preliminary phytochemical screening:** Qualitative phytochemical screening of the extracts was conducted to determine the presence of phytochemicals such as tannins, saponins, flavonoids, alkaloids, sterols, phenols and cyanogenic glycoside. This was done using standard procedure as described by Harborne (1973).

Quantitative phytochemical test of the extracts was conducted to determine the percent quantitative contents of above phytochemicals using standard procedure described by Harborne (1973), AOAC (1990) and Kirk and Sawyer (1998).

**Test microorganisms:** The following microorganisms: Bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*) and fungal strain (*Aspergillus niger* and *Candida albican*) were collected based on their clinical and pharmacological importance.

**Source of test microorganisms:** The pure cultures of the microorganisms were obtained from the pathology Department of National Root Crop Research Institute, Umudike, Abia State.

**Antimicrobial activity:** The zone of inhibition of the extracts was determined using agar diffusion method as described by ICMSF (1998a, b). Both bacteria and fungi pathogen were grown first in nutritional bath before use. The microorganisms were later sub-cultured in Mueller Hinton agar. Wells were then bored into the agar medium using a sterile 6 mm cork borer. The wells were then filled up with 0.02 mL of the extract and care was taken not to allow the solution to spill on the surface of the medium. The plates were allowed to stand on the laboratory bench for between 1-2 h for proper absorption of the solution into the medium. The plates were turned inside upside down and the wells labelled with a marker. The plates were incubated aerobically at 37°C for 24 h. Sensitivity of the organisms to the extract was recorded by measuring the zone of inhibition. The extent of inhibition was expressed in terms of the diameter of the inhibition zone as measured with a transparent metre rule. The effects of the extracts on bacteria and fungi pathogens were compared with those of the standard antibiotic ampicillin fungabacter for bacteria and fungi as standard control respectively.

**Statistical analysis:** The results were analyzed using ANOVA. The Duncan's multiple range test significance was use to test the difference among treatments. All analyses were carried out at 5% level of significance.

## RESULTS

The results of the study were shown in Table 1-10 and Fig. 1. Qualitative phytochemical screening of leaf and stem extracts of *Gongronema latifolium* in aqueous and ethanolic solvents is

Fig. 1: *Gongronema latifolium* species (Source: Self collection from farm)

Table 1: Qualitative phytochemical screening of aqueous and ethanol stem and leaf extracts of *Gongronema latifolium*

| | Phytochemicals | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Solvents extracts | Alkaloid | Flavonoid | Saponin | Phenol | Sterol | Tannin | Terpenoid | Cyanogenic glycoside |
| Aqueous leaf | + | + | + | + | + | + | + | - |
| Stem | + | + | + | + | + | + | + | - |
| Ethanol leaf | + | + | + | + | + | + | + | - |
| Stem | + | + | + | + | + | + | + | - |

+: Presence, -: Absence

Table 2: Percent quantitative phytochemical screening of aqueous and ethanol stem and leaf extracts of *Gongronema latifolium*

| | Phytochemicals | | | | | | |
|---|---|---|---|---|---|---|---|
| Solvents extracts | Alkaloid | Flavonoid | Saponin | Phenol | Sterol | Tannin | Terpenoid |
| Leaf | 0.78 | 0.45 | 0.63 | 0.03 | 0.05 | 0.71 | 0.37 |
| Aqueous | ±0.00$^{c}$ | ±0.00$^{c}$ | ±0.00$^{b}$ | ±0.00$^{a}$ | ±0.00$^{b}$ | ±0.02$^{c}$ | ±0.02$^{b}$ |
| Stem | 0.75 | 0.35 | 0.28 | 0.06 | 0.05 | 0.57 | 0.16 |
| | ±0.00$^{b}$ | ±0.01$^{b}$ | ±0.01$^{a}$ | ±0.00$^{b}$ | ±0.00$^{ab}$ | ±0.01$^{b}$ | ±0.00$^{a}$ |
| Leaf | 0.83 | 0.49 | 0.63 | 0.04 | 0.04 | 0.77 | 0.38 |
| Ethanol | ±0.00$^{d}$ | ±0.00$^{d}$ | ±0.01$^{b}$ | ±0.00$^{a}$ | ±0.00$^{a}$ | ±0.04$^{c}$ | ±0.00$^{b}$ |
| Stem | 0.72 | 0.29 | 0.31 | 0.07 | 0.05 | 0.46 | 0.22 |
| | ±0.00$^{a}$ | ±0.01$^{a}$ | ±0.01$^{a}$ | ±0.00$^{c}$ | ±0.00$^{ab}$ | ±0.00$^{a}$ | ±0.03$^{a}$ |
| Extract | ** | ** | ** | ** | ** | ** | ** |
| Solvents | ns | ns | ns | ** | ** | ns | ns |
| Extract* solvents | ** | ** | ns | ** | ns | ** | ns |

Results are in mean±standard deviation, *Column with the same alphabet are not significantly different. **There is significant different ($p<0.05$), ns: Not significant ($p>0.05$)

presented in Table 1. It was found from the result that all phytochemical (alkaloid, flavonoid, phenol, saponin, sterol, terpenoid and cyanogenic glycoside) assayed except cyanogenic glycoside was present in both solvent extracts of *Gongronema latifolium* (Table 1).

Quantitative phytochemical screening of leaf and stem extracts of *Gongronema latifolium* in aqueous and ethanolic solvents is presented in Table 2. The table shows that the mean alkaloid and

Table 3: Antimicrobial activity of aqueous and ethanol extracts of *Gongronema latifolium* at 50 mg $mL^{-1}$ of the extracts (zone of inhibition)

| Treatments | *Staphylococcus aureus* | *Salmonella typhi* | *Escherichia coli* | *Candida albican* | *Aspergillus niger* |
|---|---|---|---|---|---|
| **Zone of Inhibition of microbes in aqueous extract (mm)*** | | | | | |
| Stem | 0.75±0.00[a] | - | - | 3.74±0.00[a] | 4.20±0.00[a] |
| Leaf | 1.90±0.00[b] | 0.75±0.00[a] | 1.49±0.01[b] | 4.71±0.01[b] | 5.61±0.01[b] |
| Control | 12.71±0.01[c] | 8.68±0.04[b] | 9.46±0.09[b] | 14.78±0.03[c] | 15.32±0.03[c] |
| p-value | ** | ** | ** | ** | ** |
| **Zone of Inhibition of microbes in ethanol extract (mm)*** | | | | | |
| Stem | 1.20±0.00[a] | - | 0.64±0.01[a] | 4.32±0.03[a] | 5.50±0.00[a] |
| Leaf | 1.82±0.00[b] | 0.92±0.00[a] | 1.67±0.05[b] | 1.79±0.01[b] | 2.37±0.04[b] |
| Control | 12.71±0.01[c] | 8.68±0.03[b] | 9.46±0.09[c] | 14.78±0.03[c] | 15.32±0.03[c] |
| p-value | ** | ** | ** | ** | ** |

Results are in Mean±SD, *Columns followed by the same alphabet are not significantly different, **Significant difference exist p<0.05, Control: Ampicillin and fungabacter for bacteria and fungi, respectively

Table 4: Analysis of variance of the effect of aqueous and ethanol leaf and stem extracts of *Gongronema latifolium* at 50 mg $mL^{-1}$ on microbes' inhibition

| Sources | F-ratio | p-value |
|---|---|---|
| Microorganism | 39769.48 | 0.000 |
| Extracts | 6667.82 | 0.000 |
| Solvent | 1877.690 | 0.000 |
| Microbes' * extracts | 56.83 | 0.000 |
| Microbes * solvent | 275.90 | 0.000 |
| Extracts * solvent | 25.84 | 0.000 |
| Microbes * extract* Solvent | 60.76 | 0.000 |

Table 5: Antimicrobial activity of aqueous and ethanol extracts of *Gongronema latifolium* at 75 mg $mL^{-1}$ of the extracts (zone of inhibition)

| Treatments | *Staphylococcus aureus* | *Salmonella typhi* | *Escherichia coli* | *Candida albican* | *Aspergillus niger* |
|---|---|---|---|---|---|
| **Zone of inhibition of microbes in aqueous extract (mm)*** | | | | | |
| Stem | 3.41±0.01[a] | 2.78±0.04[a] | 3.32±0.03[a] | 6.00±0.00[a] | 7.18±0.04[a] |
| Leaf | 5.22±0.03[b] | 4.93±0.01[b] | 4.47±0.02[b] | 7.49±0.01[b] | 8.23±0.06[b] |
| Control | 15.61±0.01[c] | 11.53±0.11[c] | 12.72±0.03[c] | 18.31±0.01[c] | 19.46±0.09[c] |
| p-value | ** | ** | ** | ** | ** |
| **Zone of inhibition of microbes in ethanol extract (mm)*** | | | | | |
| Stem | 6.43±0.04[b] | 4.71±0.01[a] | 4.78±0.04[a] | 7.44±0.09[a] | 7.71±0.13[a] |
| Leaf | 5.29±0.01[a] | 6.78±0.01[b] | 5.83±0.04[b] | 8.68±0.04[b] | 9.57±0.04[b] |
| Control | 15.61±0.01[c] | 11.53±0.11[c] | 12.72±0.03[c] | 18.31±0.01[c] | 19.46±0.09[c] |
| p-value | ** | ** | ** | ** | ** |

Results are in Mean±SD, *Columns followed by the same alphabet are not significantly different, **Significant difference exist p<0.05, Control: Ampicillin and fungabacter for bacteria and fungi, respectively

Table 6: Analysis of variance of the effect of aqueous and ethanol leaf and stem extracts of *Gongronema latifolium* at 75 mg $mL^{-1}$ on microbes' inhibition

| Sources | F-ratio | p-value |
|---|---|---|
| Microorganism | 16299.617 | 0.000 |
| Extracts | 6096.798 | 0.000 |
| Solvent | 7564.801 | 0.000 |
| Microbes' * extracts | 310.117 | 0.000 |
| Microbes * solvent | 90.370 | 0.000 |
| Extracts * solvent | 250.429 | 0.000 |
| Microbes * extract* Solvent | 379.605 | 0.000 |

flavonoid composition is highest in ethanol extract of the leaf (0.83±0.07 and 0.49±0.00) respectively and lowest in the ethanol extract of the stem (0.72±0.07 and 0.29±0.01) respectively (Table 2). Tannin is highest in both the aqueous and ethanol extract of the leaf (0.63±0.01) and lowest in the aqueous extract of the stem (0.28±0.01). Sterol is highest in the ethanol extract of the stem (0.07±0.00) and lowest in the aqueous extract of the leaf (0.03±0.00). Phenol is highest in the aqueous extract of the stem (0.05±0.00) and lowest in the aqueous extract of the leaf (0.04±0.00).

Table 7: Antimicrobial activity of aqueous and ethanol leaf and stem extracts of *Gongronema latifolium* at 100 mg $mL^{-1}$ of the extracts (zone of inhibition)

| Treatments | *Staphylococcus aureus* | *Salmonella typhi* | *Escherichia coli* | *Candida albican* | *Aspergillus niger* |
|---|---|---|---|---|---|
| **Zone of inhibition of microbes in aqueous extract (mm)*** | | | | | |
| Stem | 7.33±0.11[a] | 6.32±0.03[a] | 7.31±0.13[a] | 10.31±0.01[a] | 10.77±0.02[a] |
| Leaf | 8.38±0.03[b] | 7.85±0.00[b] | 7.51±0.13[a] | 10.32.±0.03[a] | 11.77±0.04[b] |
| Control | 18.48±0.18[c] | 15.32±0.03[c] | 17.54±0.09[b] | 21.77±0.09[b] | 23.61±0.01[c] |
| p-value | ** | ** | ** | ** | ** |
| **Zone of inhibition of microbes in ethanol extract (mm)*** | | | | | |
| Stem | 7.83±0.04[a] | 6.61±0.13[a] | 6.77±0.10[a] | 9.48±0.04[a] | 9.56±0.30[a] |
| Leaf | 8.38±0.12[b] | 9.28±0.04[b] | 8.18±0.02[b] | 11.74±0.00[b] | 12.71±0.13[b] |
| Control | 18.48±0.18[c] | 15.32±0.03[c] | 17.54±0.09[c] | 21.77±0.09[c] | 23.61±0.01[c] |
| p-value | ** | ** | ** | ** | ** |

Results are in Mean±SD, *Columns followed by the same alphabet are not significantly different, **Significant difference exist $p<0.05$ , Control: Ampicillin and fungabacter for bacteria and fungi, respectively

Table 8: Analysis of variance of the effect of aqueous and ethanol leaf and stem extracts of *Gongronema latifolium* at 100 mg $mL^{-1}$ on microbes' inhibition

| Sources | F-ratio | p-value |
|---|---|---|
| Microorganism | 4789.538 | 0.000 |
| Extracts | 1984.106 | 0.000 |
| Solvent | 73.292 | 0.000 |
| Microbes' * extracts | 89.294 | 0.000 |
| Microbes * solvent | 28.749 | 0.000 |
| Extracts * solvent | 403.331 | 0.000 |
| Microbes * extract* Solvent | 63.635 | 0.000 |

Table 9: Antimicrobial activity of aqueous and ethanol leaf and stem extracts of *Gongronema latifolium* at 150 mg $mL^{-1}$ of the extracts (zone of inhibition)

| Treatments | *Staphylococcus aureus* | *Salmonella typhi* | *Escherichia coli* | *Candida albican* | *Aspergillus niger* |
|---|---|---|---|---|---|
| **Zone of inhibition of microbes in aqueous extract (mm)*** | | | | | |
| Stem | 9.72±0.12[a] | 9.30±0.00[a] | 9.53±0.11[a] | 11.73±0.07[a] | 11.91±0.01[a] |
| Leaf | 11.44±0.23[b] | 10.82±0.11[b] | 10.82±0.03[b] | 13.72±0.17[b] | 13.75±0.07[b] |
| Control | 21.60±0.00[c] | 19.52±0.03[c] | 20.48±0.04[c] | 23.72±0.17[c] | 24.46±0.23[c] |
| p-value | ** | ** | ** | ** | ** |
| **Zone of inhibition of microbes in ethanol extract (mm)*** | | | | | |
| Stem | 9.53±0.32[a] | 8.66±0.06[a] | 8.52±0.26[a] | 11.56±0.08[a] | 11.67±0.10[a] |
| Leaf | 11.56±0.09[b] | 12.45±0.07[b] | 10.78±0.04[b] | 12.68±0.11[b] | 13.63±0.25[b] |
| Control | 21.60±0.00[c] | 19.52±0.03[c] | 20.48±0.04[c] | 23.72±0.17[c] | 24.46±0.23[c] |
| p-value | ** | ** | ** | ** | ** |

Results are in Mean±SD, *Columns followed by the same alphabet are not significantly different, **Significant difference exist $p<0.05$, Control: Ampicillin and fungabacter for bacteria and fungi, respectively

Table 10: Analysis of variance of the effect of aqueous and ethanol leaf and stem extracts of *Gongronema latifolium* at 150 mg $mL^{-1}$ on microbes' inhibition

| Sources | F-ratio | p-value |
|---|---|---|
| Microorganism | 1121.783 | 0.000 |
| Extracts | 1969.149 | 0.000 |
| Solvent | 15.112 | 0.001 |
| Microbes' * extracts | 17.915 | 0.000 |
| Microbes * solvent | 20.104 | 0.000 |
| Extracts * solvent | 39.940 | 0.000 |
| Microbes * extract* Solvent | 34.908 | 0.000 |

Saponin is highest in the ethanol extract of the leaf (0.77±0.04) and lowest in the ethanol extract of the stem (0.46±0.00). Finally, terpenoid is highest in the ethanol extract of the leaf (0.38±0.00) and lowest in the aqueous extract of the stem (0.16±0.00) (Table 2). Generally, the leaf extract has higher composition of alkaloid, flavonoid, tannin, saponin and terpenoid while the stem extract has

only higher composition of phenol and sterol. Ethanol solvent gave higher yield of alkaloid, tannin, sterol and terpenoid while aqueous solvent gave higher yield of flavonoid, phenol and saponin (Table 2).

**Antimicrobial result:** Antimicrobial activity of aqueous and ethanol extracts (leaf and stem) of *Gongronema latifolium* were studied at different concentrations ( 50, 75, 100, 150 mg $mL^{-1}$ ) against three pathogenic bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*) and two fungal strains (*Aspergillus niger* and *Candida albican).* Antimicrobial potential of extracts were assessed in terms of zone of inhibition of microorganisms' growth.

Antimicrobial activity of aqueous and ethanolic leaf and stem extracts of *Gongronema latifolium* at 50 mg $mL^{-1}$ of the extracts (zone of inhibition) are presented in Table 3.

The result in Table 3 indicates that both the aqueous and ethanolic leaf extract of *Gongronema latifolium* showed inhibitory effect against all microbes (*S. aureus*, *S. typhi*, *E. coli*, *C. albicans* and *A. niger*). The aqueous and ethanolic stem extract had no inhibition against *S. typhi*, while the aqueous extract of stem showed no inhibition against *E. coli*. Moreover, in the aqueous extract, the leaf showed significantly higher inhibition against *S. aureus* (1.90±0.00), *C. albicans* (4.71±0.01) and *A. niger* (5.61±0.01) than the stem extract and (4.20±0.00) which had least zone of inhibition of (0.75±0.01) for *S. aureus*, (3.74±0.00) for *C. albicans* and for *A. niger*. In the ethanol extract, the leaf showed significantly higher inhibition against *S. aureus*, (1.82±0.00), *E. coli* (1.67±0.05), *C. albicans* (5.53±0.11) and *A. niger* (6.78±0.04) than the stem extract which had least zone of inhibition (1.20±0.00) for *S. aureus*, (0.64±0.01) for *E. coli*, (4.32±0.03) for *C. albicans* and (5.50±0.00) for *A. niger*. Generally, *S. aureus*, *S. typhi*, *C. albicans* and *A. niger* showed higher susceptibility in the ethanol extract while, *E. coli* showed higher susceptibility in the aqueous extract. However, in comparison with the control, the inhibition of the microbe's is significantly higher in the control than in plant extract (both aqueous and ethanol) (Table 3).

Analysis of variance of the effect of aqueous and ethanolic leaf and stem extracts of *Gongronema latifolium* at 50 mg $mL^{-1}$ on microbes' inhibition is presented in Table 4.

As can be noticed from Table 4, there is a significant difference in the antimicrobial activity of stem and leaf extracts and between ethanol and aqueous extracts ($p<0.05$). The susceptibility of the microbes to the extracts also differs significantly ($p<0.05$) (Table 4). However, there is interaction between microbes and solvent and between microbes, extracts and solvents ($p<0.05$) (Table 4).

Antimicrobial activity of aqueous and ethanolic leaf and stem extracts of *Gongronema latifolium* at 75 mg $mL^{-1}$ of the extracts (zone of inhibition) are presented in Table 5.

As shown in Table 5, the aqueous and ethanolic leaf and stem extracts showed inhibition against all the microbes. In particular, the aqueous extract of the leaf showed a significantly higher inhibition against *S. aureus*, (5.22±0.03), *S. typhi* (4.93±0.01), *E. coli* (4.47±0.02), *C. albicans* (7.49±0.01) and *A. niger* (8.23±0.10) than the stem extract with least zone of inhibition of (3.41±0.01) for *S. aureus*, (2.78±0.04) for *S. typhi*, (3.32±0.03) for *E. coli*, (6.00±0.00) for *C. albicans* and (7.18±0.04) for *A. niger*. Similarly, the ethanol extract of the leaf showed a significantly higher inhibition against *S. typhi*, (6.78±0.04), *E. coli* (5.83±0.04), *C. albicans* (8.68±0.04) and *A. niger* (9.57±0.04) than stem extract with least zone of inhibition of (4.71±0.01) for *S. typhi*, (4.78±0.04) for *E. coli*, (7.44±0.09) for *C. albicans* and (7.71±0.13) for *A. niger*. The stem showed only significantly higher activity against *S. aureus* (6.43±0.04) than the leaf extract (5.29±0.01). However, in comparison with the control, the inhibition of the microbes is significantly higher in the control than in plant extract (both aqueous and ethanol). Generally, the susceptibility of the microbes was all higher in the ethanol extract than in the aqueous extract (Table 5).

Analysis of variance of the effect of aqueous and ethanolic leaf and stem extracts of *Gongronema latifolium* at 75 mg $mL^{-1}$ on microbes' inhibition is presented in Table 6.

As shown in Table 6, there is a significant difference in the antimicrobial activity of stem and leaf extracts and in ethanol and aqueous extracts ($p<0.05$). The susceptibility of the microbes to the extracts also differs significantly ($p<0.05$) (Table 6). However, there is interaction between microbes, extracts and solvents ($p<0.05$) (Table 6).

Antimicrobial activity of aqueous and ethanolic leaf and stem extracts of *Gongronema latifolium* at 100 mg $mL^{-1}$ of the extracts (zone of inhibition) are presented in Table 7.

As can seen in Table 7, in the aqueous extract, the leaf showed significantly higher inhibition against *S. aureus*, (8.38±0.03), *S.typhi* (7.85±0.01), *E. coli* (7.51±0.13), *C. albicans* (10.32±0.03) and *A. niger* (11.77±0.04) than the stem extract with least zone of inhibition of (7.33±0.11) for *S. aureus*, (6.32±0.03) for *S. typhi*, (7.31±0.13) for *E. coli*, (10.31±0.01) for *C. albicans* and (10.77±0.02) for *A. niger*.

In the ethanol extract, the leaf showed significantly higher inhibition against *S. aureus*, (8.38±0.11), *S. typhi* (9.28±0.04), *E. coli* (8.18±0.02), *C. albicans* (11.74±0.00) and *A. niger* (12.71±0.13) than the stem extract with least zone of inhibition of (7.83±0.04) for *S. aureus*, (6.61±0.13) for *S. typhi*, (6.77±0.10) for *E. coli*, (9.48±0.04) for *C. albicans* and (9.56±0.30) for *A. niger*. However, in comparison with the control, the inhibition of the microbes is significantly higher in the control than in plant extract (both aqueous and ethanol) (Table 7).

Analysis of variance of the effect of aqueous and ethanolic leaf and stem extracts of *Gongronema latifolium* at 100 mg $mL^{-1}$ on microbes' inhibition is presented in Table 8.

The table indicates a significant difference in the antimicrobial activity of stem and leaf extracts and between ethanol and aqueous extracts ($p<0.05$). The susceptibility of the microbes to the extracts also differs significantly ($p<0.05$) (Table 8). However, there is interaction between microbes, extracts and solvents ($p<0.05$) (Table 8).

Antimicrobial activity of aqueous and ethanolic leaf and stem extracts of *Gongronema latifolium* at 150 mg $mL^{-1}$ of the extracts (zone of inhibition) are presented in Table 9.

In the aqueous extract, as can be seen in the table, the leaf showed significantly higher inhibition against *S. aureus*, (11.44±0.23), *S.typhi* (10.82±0.11), *E. coli* (10.82±0.03), *C. albicans* (13.72±0.17) and *A. niger* (13.75±0.07) than the stem extract with least zone of inhibition of (9.72±0.12) for *S. aureus*, (9.30±0.00) for *S. typhi*, (9.53±0.11) for *E. coli*, (11.73±0.07) for *C. albicans* and (11.91±0.01) for *A. niger*. Similarly, in the ethanol extract, the leaf extract showed significantly higher inhibition against S. *aureus*, (11.56±0.09), *S. typhi* (12.4582±0.07), *E. coli* (10.78±0.04), *C. albicans* (12.68±0.11) and *A. niger* (13.63±0.25) than the stem extract with least zone of inhibition of (9.53±0.32) for *S. aureus*, (8.66±0.06) for *S. typhi*, (8.52±0.36) for *E. coli*, (11.56±0.08) for *C. albicans* and (11.67±0.10) for *A. niger*. However, in comparison with the control, the inhibition of the microbes is significantly higher in the control than in plant extract (both aqueous and ethanol) (Table 9).

Analysis of variance of the effect of aqueous and ethanolic leaf and stem extracts of *Gongronema latifolium* at 150 mg $mL^{-1}$ on microbes' inhibition is presented in Table 10.

The table indicates a significant difference in the antimicrobial activity of stem and leaf extracts and between ethanol and aqueous extracts ($p<0.05$). The susceptibility of the microbes to the extracts also differs significantly ($p<0.05$) (Table 10). However, there is interaction between microbes, extracts and solvents ($p<0.05$) (Table 10).

## DISCUSSION

The study demonstrated that both aqueous and ethanolic leaf and stem extracts of *Gongronema latifolium* contained all the phytochemical assayed (alkaloid, tannin, saponin, sterol, flavonoid, terpenoid and cyanogenic glycoside) except cyanogenic glycoside. The phytochemical were present in the extracts in varied quantities (Table 1 and 2). Tannins are astringent in taste and help in healing of wounds and inflamed mucous membrane (Njoku and Akumefula, 2007). Tannins is potential metal ion chelator, proton precipitating agents and biological antioxidant (Okonkwo, 2009). Flavonoids are most commonly known for their antioxidant activity and act as transformers which modify the body's reactions to carcinogens, viruses and allergens. They possess anti-cancerous, anti-inflammatory, anti-microbial and anti-allergic activity (Balch and Balch, 2000) and may, therefore be useful in therapeutic roles (Jisika *et al*., 1992). Terpenoids are antifungal and antibacterial which is attributed to their membrane disruption action and inhibitory action on bacterial cell or fungus (Cichewicz and Thorpe, 1996). Many alkaloids for example are known to have effect on the central nervous system and act as antipyretic such as morphine, a painkiller. Similarly, saponins which are a special class of glycosides have been found to possess antifungal activity (Ogu *et al*., 2012). Saponins have been reported to have a wide range of pharmacological and medicinal activities. Interestingly, they have been indicated to usually have low oral toxicity in humans (Sparg *et al*., 2004). Plants containing saponins are used to heal wounds (Okwu and Josiah, 2006) because saponins have the ability to precipitate and coagulate Red Blood Cells (RBCs) (Sood *et al*., 2012). Sterols have been used in medicine to treat variety of conditions ranging from endocrine hormonal alteration to coronary insufficiency (Clifford *et al*., 1973). Phenols are known to inhibit the mutagenicity of cell DNA and neutralize free radicals (Heinonen *et al*., 1998). They also function as antimicrobial compounds produced by some plants to protect them from pathogens.

Result also showed that the leaf extract of *Gongronema latifolium* contained significantly higher composition of the phytochemicals than the stem except in phenol and sterol where the stem had higher composition than the leaf (Table 2). This indicated that these extracts could be a better source of these phytochemicals for medicinal purposes. The ethanol solvent had higher yields of alkaloid, tannin, sterol and Terpenoid while aqueous solvents had higher yield of flavonoid, phenol and saponin (Table 2). According to Cheremisinoff (2003), the reason for this could be attributed to the fact that both ethanol and water are classified as polar solvent, although ethanol is not very polar as water.

Antimicrobial studies indicated that both the aqueous and ethanol leaf and stem extracts of *Gongronema latifolium* inhibited the growth of the microbes but at varied levels and the inhibition was extracts concentration dependent (Table 5, 7 and 9). The stem and leaf extracts of *Gongronema latifolium*, both showed inhibition against test microbes indicating that the plant possesses antimicrobial properties. This could be attributed to the presence of chemical compounds in the extracts. These phytochemicals are known to have medicinal properties. The inhibition of bacterial strains (*Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli*) suggests that the plant possesses broad spectrum of antibacterial properties which could be used in the treatment of skin diseases and food poisoning of which the microbes are commonly implicated. The inhibition of the fungal strain (*Candida albicans*) suggests also that the plant possesses antifungal property and could be used for the treatment of refractory candidacies (oral) that has a global challenge with HIV/AIDS patients.

The leaf extract showed higher antimicrobial activity against the microbes than the stem extract. This according to Hassan *et al*. (2009), could be attributed to presence of higher bioactive compounds in leaf extracts. Furthermore, the sensitivity and susceptibility of the microbes to the plant extracts varied. The fungal strains were highly sensitive and susceptible to the plant extracts than the bacterial strains. The difference according to Karaman *et al*. (2003) is due to the fact that gram positive bacteria such as *Escherichia coli* develop resistant to inhibition caused by plant extract except when the extracts are used at higher concentration.

Generally, the ethanol extracts are more effective than the aqueous extract though the reverse was the case at higher (150 mg $mL^{-1}$) concentrations. The findings conform to the study of Thabile (2008), who observed higher microbial activity of aqueous extract of lemon grass against human pathogen at higher concentration of plant extracts. Cheremisinoff (2003) reported that at increasing concentration of extract, differences in interaction between phytochemicals and solvent do exist and that this may account for differences in microbial activity of extracts of different solvents. Mada *et al*. (2013) reported that antimicrobial activity is solvent dependent with ethanol extract being most potent than aqueous extract.

## CONCLUSION

Many plants emerged as compounds with potentially significant therapeutic application against human pathogens. It is only through research efforts that these potentials could be discovered for eradication of these resistant strains of microbes. This study revealed that the plant extracts possessed bioactive compounds that have antibacterial and antifungal activities against some human pathogens, which justified their use in ethnomedicine for treatment of infectious diseases. The ethanol extract showed significantly higher inhibition than the aqueous extract in all concentrations except at 150 mg $mL^{-1}$ where the reverse was the case suggesting its potential as a better extraction medium for *Gongronema latifolium* than the aqueous extract except at 150 mg $mL^{-1}$. The *Gongronema latifolium* extracts both showed antimicrobial activities however, the leaf extract showed better inhibitions than the stem extract indicating that it is a better antimicrobial agent than the stem. The data obtained from the study indicated that the plant possessed antimicrobial properties.

Furthermore, before use in human being isolation of pure compound, toxicological study and pharmacological activity should be carried out thereafter.

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