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Profilistic Study of Bioactivities of Extracts of *Gongronema latifolium* Incorporated with Alum on some Clinical Bacteria

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ABSTRACT
Profilistic study of bioactivities of aqueous, ethanolic and methanolic leaf extracts of *Gongronema latifolium* in combination with potassium aluminium sulphate (Alum) against some clinical bacterial pathogens were investigated by disc diffusion (DD) and Agar well diffusion (AWD) methods respectively. The leaf extracts at concentrations of 0.1-0.3g were reconstituted in sterile distilled water as well as 1.0-3.0g of alum prior to application. In-vitro bioactivity of various concentrations of the extracts and in combination with alum were evaluated by measuring diameter of inhibition zones (DIZs) respectively. Methanolic leaf extract (MLE) had the largest mean DIZs of 14.5±0.5 and 11.5±0.0mm on *Escherichia coli* and *Salmonella typhi*, with enhanced bioactivity of 19.5±0.7 and 17.5±0.7mm with alum against *Bacillus subtilis*, *Sal. typhi* and *Pseudomonas aeruginosa* by DD and AWD methods respectively. However, aqueous leaf extract (ALE) and ethanolic leaf extract (ELE) and their combinations depicted appreciable antibacterial activity on the pathogens but incomparable to MLE. Generally, there was enhancement of bioactivties with the incorporation of Alum to the leaf extracts (irrespective of solvent of extraction) on a dose response fashion particularly by AWD method. Furthermore, the low MIC values of <0.05 to 0.2mg/ml on the bacteria with MLE and ALE, validates their potency and broad spectrum activity. In contrast, the very large DIZs of Ciprofloxacin (CP) reflects the beneficial impact of purified chemotherapeutics against pathogens. Thus, the improved efficacy of these extracts with alum would justify future application in ethnomedicine as well as in nutraceuticals/pharmaceuticals or in food systems as "green chemicals" or "biopreservatives".

***Key Words*:** Antibiogram, culture, flavonoids, minimum inhibitory concentration, nutraceutical, solvent.

INTRODUCTION

In Africa, phytomedicine has been in existence for several years and about 80% of the population depend on herbal medicine for its primary health care delivery (Okigbo and Mmeka, 2006; Okwu, 2007). Although modern medicine may be available but treatment of diseases with traditional medicine is more preferable, perhaps, for historical or cultural reasons (Balogun *et al*., 2016). In Nigeria, medicinal plants are potential sources of new antimicrobials and with renewed interest in phytochemicals, avalanche of researches have been undertaken to screen antimicrobial activities of these plants (Anyanwu and Okoye, 2017). Generally, medicinal effects of these plants stem from the interaction of a plethora of secondary metabolites such as alkaloids, tannins, phenols, saponins, flavonoids, essential oils and bioactive compounds. These plants have been used as drugs, foods and food additives, insecticides, fragrances and anticorrosion agents (Amnlou *et al*., 2005; van Vuuren, 2008; Okigbo *et al*., 2009; Oguzie *et al*., 2013). Many investigators, have suggested that studies on plants should be conducted on organisms related to the diseases managed with the plant in traditional medicine (Ncube *et al*., 2005; van Vuuren, 2008; Adebayo and Krettli, 2011; Osuagwu and Akomas, 2013) and these will however validate claims for their uses.

Several reports on antimicrobial screening studies on *G. latifolium* and/or with other plant leaves is well documented (Eleyinmi, 2007; Bankole *et al*., 2012; Ikegbunam *et al*., 2014; Morebise, 2015; Anyanwu and Okoye, 2017) because of the desire to provide cost effective and safe antimicrobial agents. However, the use of *G. latifolium* leaves in folklore medicine by different ethnic groups for treatment of diseases such as malaria, nausea, anorexia, diabetes, hypertension, constipation, dysentery, etc., had earlier been reported (Morebise *et al*., 2006; Nwinyi *et al*., 2008; Owu *et al*., 2012; Okpala, 2015; Usoh *et al*., 2016). *Gongronema latifolium* leaves are also eaten fresh, with pourage or roasted yam and/or plantain, spiced in soups, as gravy, etc., for its nutritional and medicinal benefits (Owu *et al*., 2012). The need to broaden raw material base and search for novel sources of antimicrobials is a global challenge preoccupying research institutes, nutraceutical, pharmaceutical industries and academia. This passion necessitated a further evaluation of readily available underutilised phytomedicinals to unravel their bioactivity and synergism with inorganic compounds or other species of plant extracts. In this study, *G. latifolium* leaf extracts was incorporated with alum to potentiate their bioactivity against bacterial pathogens.

Potassium Aluminium sulphate or potash alum or alum as a hygroscopic inorganic salt has many applications in the pharmaceutical, cosmetics, water and food industries because of its astringency and acidifying properties (Ahmed, 2011; Alzomor, *et al*., 2014; Efiuvwevwere and Amadi, 2015). It is used as an adjuvant in immunological studies, antibacterial agent and as a drug (Doherty and Anderson, 2005; Bnyan *et al*., 2014; Al-Talib *et al*., 2016; Amadi and Ngerebara, 2017; Ali *et al*., 2017). The prevalence of antimicrobial resistance (AMR) has heightened the search at every ecological niche including soil, plant, animal and marine as well as from inorganic sources for potentially new and safe antimicrobial agents (Laport *et al*., 2009; Osuagwu and Edeoga, 2010; Nasir *et al*., 2015; Amadi *et al*., 2017). Despite the growing interest in the study of phytomedicinals

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Figure 1. Freshly harvested *G. latifolium* leaves: broad, heart shaped and slightly oval in appearance with chordate base. (A). Roasted yam and plantain, spiced and garnished with the sliced leaves prior to consumption (B).

and possibility of enhancing potency with organic or inorganic substances, there is paucity of literatures on the use of *G. latifolium* with alum to boost antibacterial efficacy. In view of this, a profilistic bioactivies of leaf extracts of *Gongronema latifolium* incorporated with Alum against some clinical bacterial pathogens was initiated.

MATERIALS AND METHODS

Collection of *Gongronema latifolium* leaf samples

Fresh leafy *G. latifolium* were purchased from Bori market in Khana Local Government Area, Rivers State, Nigeria. The sample were taken to the Department of Science Laboratory for identification and further analysis using analytical grade reagents. Figure 1 (A and B). Freshly harvested *G. latifolium* used to garnish roasted yam and plantain prior to consumption.

Preparation of Leaf Extracts

Leafy parts of freshly harvested *G. latifolium* leaves were rinsed to remove dirt and extraneous debris. The leaves were allowed to drain and pounded (with sterile porcelain pestle and mortar) to facilitate the rate of solvent extraction. The resulting mash was packaged in sterile polyethylene from which six hundred grammes (600g) was dissolved in 1.2litres of 90% ethanol and filtered with muslin cloth and Whatman No.1 filter paper. The filtrate was maintained in water bath at 80ºC for 48-72h to evaporate to obtain ethanolic leaf extract (ELE). This procedure was repeated with methanol and water to obtain methanolic leaf extract (MLE) and aqueous leaf extract (ALE) respectively and stored in a refrigerator at 4°C for use.

Preparation of concentrations of leaf **extracts and alum**

A mass of 0.10g of the leaf extract was reconstituted in 99.9ml sterile distilled water to obtain 0.1% (w/v) concentration of ALE, ELE and MLE and 1.0% for alum (Analytical grade. Vickers Laboratories, Ltd, England) respectively.

Test Bacteria and confirmation

Bacterial pathogens used for susceptibility test were four (4) Gram negative bacterial species; *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Pseudomonas aeruginosa* and two (2) Gram positive bacteria; *Bacillus subtilis* and *Staphylococcus aureus* were obtained from Microbiology Department, University of Port Harcourt Teaching Hospital (UPTH), Choba, Rivers state, Nigeria. These cultures were confirmed by carrying out inter-laboratory tests (at the Department of Science Laboratory Technology, Ken Saro-Wiwa Polytechnic, Bori.) such as Gram staining and biochemical tests, etc.

Sensitivity test of leaf extracts with or without alum

Various concentrations of ELE, MLE and ALE were used and in combination with alum at a ratio of 1:1 respectively (Amadi *et al*., 2017). Antibiogram efficacy were compared with standard antibiotics Ciprofloxacin (CP) as control. Sensitivity test was performed by discs and agar well methods (CLSI, 2011). The 18h overnight culture of each bacterial species was adjusted to 0.5 McFarland turbidity standard before spread-plating on Mueller Hinton agar (MHA (Titan Biotech, Ltd. Bhiwadi-301019, Rajasthan, India) and dried for 2 to 5min. The paper discs were made from Whatman No. 1 absorbent filter paper as described by Ochei and Kolhatkar (2008) and were dispensed in batches of 40 in Petri-dishes and sterilized at 160ºC for 1hour. A 0.2mL of each of the concentration was added into the plate of 40 discs, each containing 0.005mL (5µL) of the various concentrations of aqueous, ethanolic and methanolic leaf extracts with or without alum (Taiwo *et al*., 2007) respectively. These combinations and discs of commercially supplied Ciprofloxacin (CP = 10µg, Abtek Biologicals Ltd., UK) were placed on the surface-dried inoculated MHA with sterile forceps. Agar well diffusion (AWD) was performed by spread-plating equal volume of the bacterial suspensions into four (4) wells of 6mm diameter equidistant (c. 2cm) from one another made on agar-solidified plates using sterile Cork borer. Equal volumes of extracts with or without alum concentrations were dispensed into the wells and duplicate plates were incubated at 37ºC for 24hours. The diameter of inhibition zones (DIZ) were measured with a transparent ruler and expressed in millimeters (mm). The mean and standard deviation values of DIZ were calculated and compared with CP. Interpretation of results was based on the zones of inhibition (Cheesbrough, 2006; Forbes *et al*., 2007). The minimum inhibitory concentration (MIC), i.e., the lowest concentration

able to inhibit any visible bacterial growth, was determined by measuring cell growth after 18-24h incubation at 37ºC with the various concentrations used (Forbes *et al*., 2007; Harvey, 2012).

Statistical analysis

Means of duplicate measurements and standard deviations (SD) were determined for each sample using Microsoft Excel® 2016.

RESULTS

Bioactivity of different concentrations of *G. latifolium* extracts without alum and standard antibiotic, Ciprofloxacin (Control) using disc diffusion method are displayed in Figure 2. Of all the extracts, methanolic leaf extract (MLE) exhibited the largest DIZs on the test bacteria, except on *Klebsiella pneumonia* and *Pseudomonas aeruginosa,* followed by ELE whereas ALE was ineffective. Comparatively, Ciprofloxacin depicted the largest inhibition zones on all the bacterial species (Figure 2).

Figure 3 Represents diameter of inhibition zones (DIZs) of various concentrations of *G. latifolium* extracts with alum using disc diffusion methods. MLE + Alum and ELE + Alum exhibited appreciable bioactivity on all the bacterial species. In contrast, ALE + Alum showed strong bioactivity on few species such as *Bacillus subtilis* (largest DIZ), *Escherichia coli* and *Staphylococcus aureus* with no effect on others. The largest DIZ (24.0mm) was observed with ALE + Alum against *B. subtilis* whereas *P. aeruginosa* and *Salmonella typhi* were resistant with *K. pneumoniae* being more consistent (Figures 2-4).

Largest DIZs (sensitivity) was observed with MLE against *Sal. typhi* and *K. pneumoniae*, followed by *St. aureus* at 0.3g concentration whereas ALE displayed high sensitivity to *St. aureus* and *P. aeruginosa*, followed by *B. subtilis* and *Sal. typhi* but no activity on *K. pneumoniae* (Figure 4). The same scenario was exhibited in Figures 2 and 3 using DD method.

All the bacterial species were sensitive to various extracts incorporated with alum and at different concentrations used (Figure 5). The largest DIZs occurred with MLE + Alum at 0.3g concentration against *B. subtilis*, *P. aeruginosa* and followed by *Sal. typhi*. ELE + Alum exhibited appreciable DIZs against *P. aeruginosa* and *E. coli* whilst *P. aeruginosa, Sal. typhi B. subtilis* and *St. aureus* were inhibited by ALE + Alum. Generally, the least DIZs were observed with extracts without incorporation of Alum irrespective of the method applied, DD or AWD (Figures 2 and 4). Enhanced bioactivity due to synergism of *G. latifolium* extracts and alum were apparently demonstrated and visible in Figures 3 and 5.

The minimum inhibitory concentration (MIC) against all the test bacterial pathogens occurred at 0.1mg/ml except *Sal. typhi* (MIC < 0.05mg/ml) with ALE and MLE respectively (Table 1). With ELE, only the growth of *K. pneumoniae* and *B. subtilis* were not inhibited at 0.1mg/ml but at 0.2mg/ml. This indicates the beneficial effect of ALE and MLE over ELE.

DISCUSSION

Medicinal effects of Nigerian plants are attributed to interactions of phytochemicals (such as flavonoids, saponins, essential oils, etc.) and bioactive compounds contained in their tissues (Morebise and Fafunso, 1998; Anyanwu and Okoye, 2017). This revealed apparent bioactivity with all the extracts on the test pathogens using both susceptibility tests but much better results were obtained with the incorporation of alum by agar well diffusion (AWD) method. Although, the compounds and mechanism for the strong

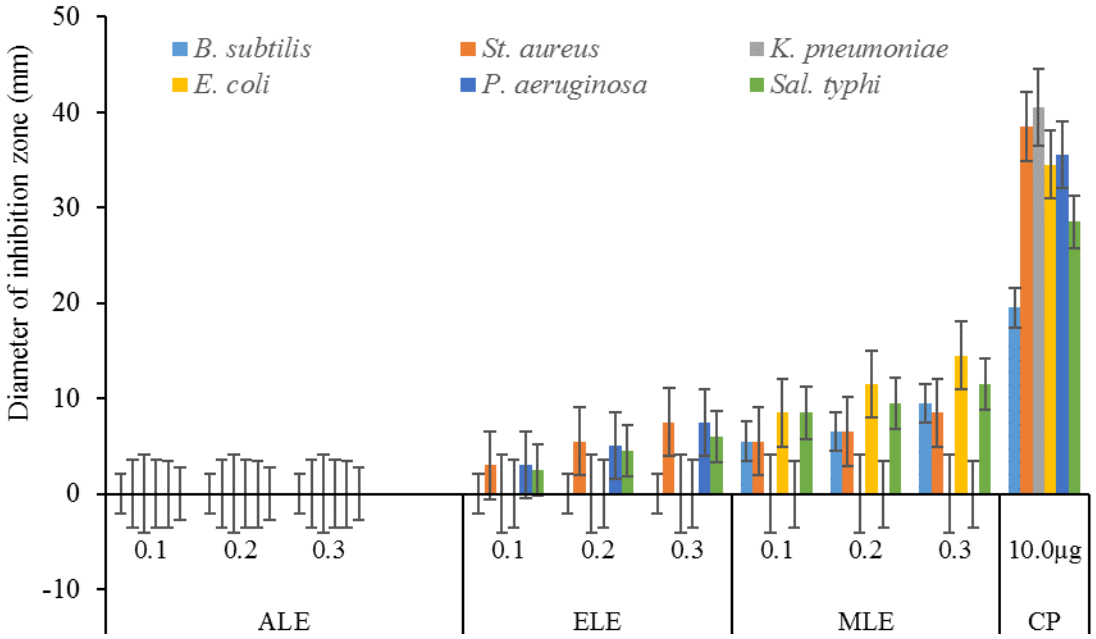

Figure 2. Inhibitory activity of different concentrations (0.1-0.3g) of Aqueous Leaf Extract (ALE), Ethanolic Leaf Extract (ELE), Methanolic Leaf Extract (MLE) of *G. latifolium* and Ciprofloxacin (CP) as control respectively using Disc Diffusion (DD) Method. Each data point and error bars show the mean and ± standard deviation of duplicate samples.

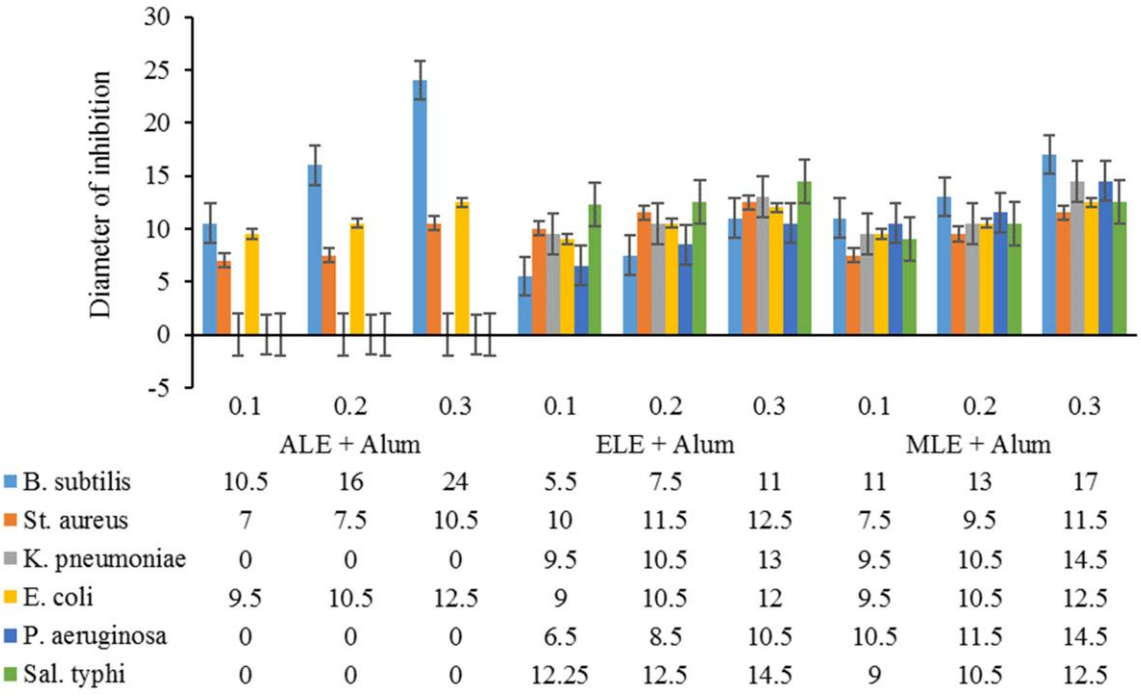

Figure 3. Inhibitory activity of different concentrations (0.1-0.3g) of Aqueous Leaf Extract (ALE), Ethanolic Leaf Extract (ELE), Methanolic Leaf Extract (MLE) with Alum (1:1 ratio) respectively using Disc Diffusion (DD) Method. Each data point and error bars show the mean and ± standard deviation of duplicate samples.

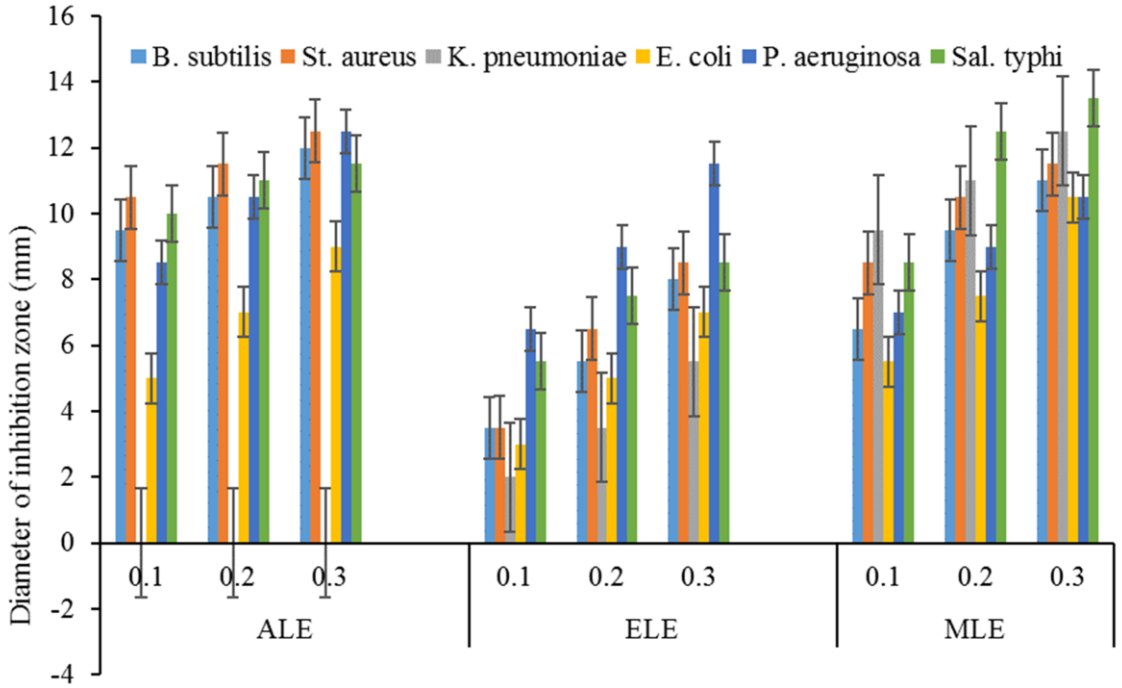

Figure 4. Inhibitory activity of different concentrations (0.1-0.3g) of Aqueous Leaf Extract (ALE), Ethanolic Leaf Extract (ELE), Methanolic Leaf Extract (MLE) with Alum (1:1 ratio) respectively using Agar Well Diffusion (AWD) Method. Each data point and error bars show the mean and ± standard deviation of duplicate samples.

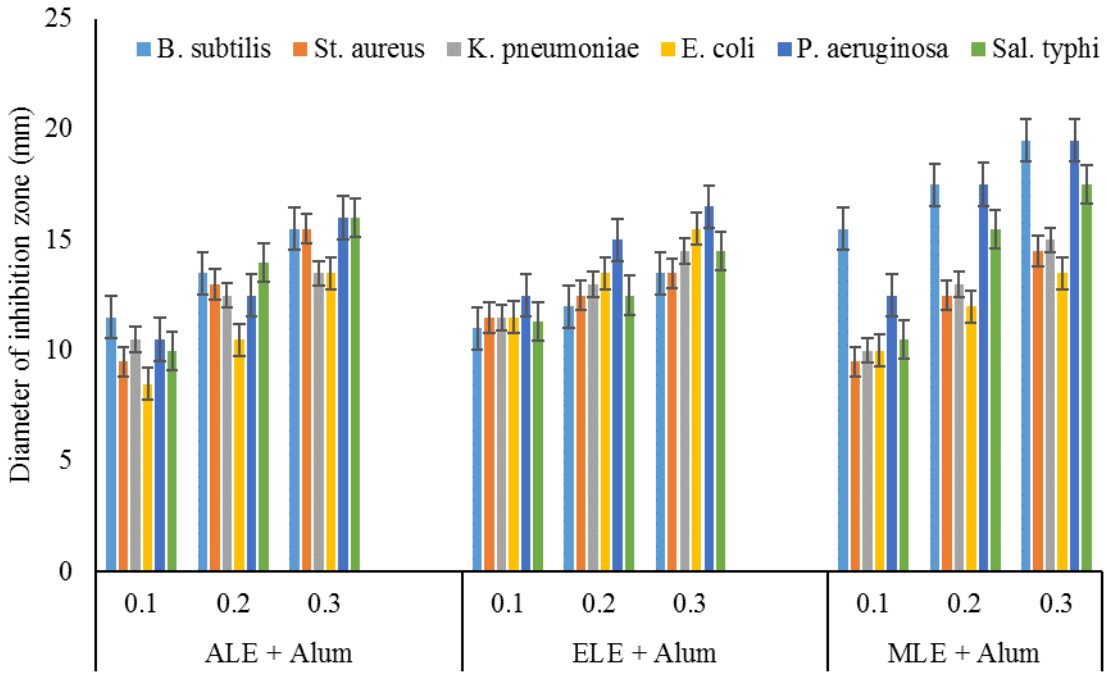

Figure 5. Inhibitory activity of different concentrations (g) of Aqueous Leaf Extract (ALE), Ethanolic Leaf Extract (ELE), Methanolic Leaf Extract (MLE) with Alum (1:1 ratio) respectively using Agar Well Diffusion (AWD) Method. Each data point and error bars show the mean and ± standard deviation of duplicate samples.

Table 1. Minimum inhibitory concentrations (MICs) of *G. latifolium* leaf extracts.

Bacteria	**Diameter of inhibition zone (DIZ) in mm**								
	ALE			**ELE**			**MLE (mg/ml)**		
	0.05	**0.1**	**0.2**	**0.05**	**0.1**	**0.2**	**0.05**	**0.1**	0.2
B. subtilis	-	+	+	-	-	+	-	+	+
St. aureus	-	+	+	-	+	+	-	+	+
K. pneumoniae	-	+	+	-	-	+	-	+	+
E. coli	-	+	+	-	+	+	-	+	+
P. aeruginosa	-	+	+	-	+	+	-	+	+
Sal. typhi	+	+	+	-	+	+	+	+	+

+ = Positive (Growth); - = Negative (No growth). ALE = Aqueous leaf extract; ELE = Ethanolic leaf extract; MLE = Methanolic leaf extract.

bioactivity of methanolic and aqueous leaf extracts are unknown, but reports indicate that methanol and water were more effective for obtaining flavonoids, saponins and phenolic compounds which had been associated with antimicrobial activity of *G. latifolium* (Hernandez *et al*., 2000; Eleyinmi, 2007; Ikegbunam *et al*., 2014; Pham *et al*., 2015; Anyanwu and Okoye, 2017). Virtually, all the extracts showed strong activity on a dose response fashion on the bacterial pathogens but much more enhanced with alum, and methanolic extract with or without alum being the most beneficial. In this study, lack of activity with aqueous extract on *Staphylococcus aureus* agrees with earlier reports by Oshodi *et al*., 2004 but was limited to susceptibility test by disc diffusion and not with agar well diffusion method. Methanolic extract exhibited the strongest bioactivity on *B. subtilis* (a human axillary malodour-producing bacterium (Al-Talib *et al*., 2016), *Sal. typhi*, *P. aeruginosa, St. aureus* and *K. pneumoniae*. Similar findings on antibacterial activity against these bacteria had been previously reported (Eleyinmi, 2007; Nwinyi *et al*., 2008; Enyi-Idoh *et al*., 2017). The relatively low MIC levels of the crude extracts (between < 0.05 and 0.2mg/ml) that caused growth inhibition of test bacteria underscores the need to further explore the antimicrobial potentials in *G. latifolium*. Bioactivity of alum on a variety of microorganisms and as drug has been well documented (Kolaei *et al*., 2013; Al-Talib *et al*., 2016; Amadi and Ngerebara, 2017; Ali *et al*., 2017). The synergistic effect of alum with various extracts was phenomenal and apparently visible by enhancement of bioactivity which corroborates earlier studies on other medicinal plants (Bnyan *et al*., 2014; Amadi *et al*., 2016; 2017). The properties of alum such as astringency and acidification in solution has potentiated its activity not only on bacteria but on fungi as well (Ahmed, 2011; Kolaei *et al*., 2013; Amadi and Ngerebara, 2017).

The sensitivity of these pathogens to these extracts validates their use for traditional medicine and herbal medicinal products. However, the extraction, isolation, purification and identification of bioactive compounds in *G. latifolium* is cardinal for further utilization in the nutraceutical, pharmaceutical and food industries.

CONCLUSION

G. latifolium is a plant with great prospects and the profiles of its extracts with or without alum suggest strong bioactivity and broad spectrum efficacy. The large DIZs and low MIC values point to the fact that this plant is a potential resource of cost effective and safe antimicrobials. Therefore, further research to unravel the effective ingredients and mechanisms of bioactivity would consolidate their application in nutraceutical, pharmaceutical and in food industries as "green chemicals" or "biopreservatives".

CONFLICT OF INTEREST

None

REFERENCES

Adebayo, J.O., Krettli, A.U., 2011. Potential antimalarials from Nigerian plants: A review. *J Ethnopharmacol.*,133:289-302.

Ahmed, K.T., 2011. Inhibition of swarming in *Proteus mirabilis* by Alum (Hydrated Aluminum Potassium Sulphate). *J Univ. Anbar for Pure Science*, **5**(2):20-24.

Ali, Akhtar., Hamiduddin., Zaigham, M., 2017. Shibb-E-Yamani (Alum) A unque drug and its utilization in Unani Medicine: A physicochemical and pharmacological review. *Int. J. Res. Ayurveda Pharm*., **8**(2):17-22. [DOI]

Al-Talib, H., Nasir, M.S.I.N., Yaziz, H., Zulkafli, F.N., Adani, A.N., Rashidi, N.I.A., Murugaiah, C., Shaari, A.S., 2016. Potassium aluminium sulphate (Alum) inhibits growth of Human axillary malodour-producing skin flora in vitro. *J. Clin. Health Sciences*, **1**(1):59-63.

Alzormor, A. K., Moharram, A.S., Al Absi, N.M., 2014. Formulation and Evaluation of Potash alum as deodorant lotion and after shaving astringent as cream and gel. *Int. Current Pharm J.*, 2: 228-233.

Amadi, L.O., Ngerebara, N.N., 2017. Susceptibility Profiles of Alum on Bacteria Isolated from Shellfish Bivalve Oyster. *International Journal of Current Microbiology and Applied Sciences*, **6**(1):941-947. [DOI]

Amadi, L.O., Osiagor, M.C., Christopher, C.R., 2017. In-vitro Antibiogram of *Bryophyllum pinnatum* extracts with or without Potassium Aluminum Sulphate against some Pathogenic Bacteria. *European Journal of Biotechnology and Bioscience*, **5**(1):32-35.

Amadi, L.O., Wanabia, D., Amadi, V., 2016. Synergistic effects of Alum and Guava (*Psidium guajava*) leaf extract on some Pathogens from Clinical samples. *Int. J. Current Research*, **8**(05):31354-31358.

Amnlou, M., Ariae, S., Farsam, H., 2005. Allergies, Antiviral, Antimalarial, Antioxidant, Anti-Inflammatory and Anti carcinogenic Properties of *Bryophyllum Pinnatum* Leaf Extract. *Journal of Medical Aromat*. 27: 469-475.

Anyanwu, M.U., Okoye, R.C., 2017. Antimicrobial activity of Nigerian plants. *J. Intercult Ethnopharmacol.*, **6**(2):240-259. [DOI]

Balogun, M. E., Besong, E. E., Obimma., J. N., Mbamalu, O. S., Djobissie, S. F. A., 2016. *Gongronema latifolium*: A phytochemical, Nutritional and pharmacological review. *Journal of Physiology and Pharmacology Advances*, **6**(1):811-824.

Bankole, H. A., Anjorin, A. A., Kazeem, M.I., Ogbeche, M.E and Agbafor,U. 2012. Antibacterial activity of *Ocimum gratissimum* and *Gongronema latifolium* on *Staphylococcus aureus* and *Salmonella typhi*. *East African Journal of Science and Technology*, **2**(1):114-128.

Bnyan, I.A., Alta'ee, A.H., Kadhum, N. H., 2014. Antibacterial Activity of AluminumPotassium Sulphate and *Syzygium Aromaticum* extract against Pathogenic microorganisms. *J Natural Sciences Research*, **4**(15):11-14.

Cheesbrough, M., 2006. District Laboratory Practices in Topical Countries. Vol., 2 part 2 Cambridge University Press.

Clinical and Laboratory Standards Institute (CLSI), 2012. Performance Standards for Antimicrobial Susceptibility testing; Twenty second Informational Supplement M100-22. Vol. 32. Wayne. Pennsylvania. USA.

Doherty, T.M., Anderson, P., 2005. Vaccines for tuberculosis: Novel concepts and recent progress. *Clin Microbiol. Reviews*, **18**, 687-702.

Efiuvwevwere, B.J.O., Amadi, L.O., 2015. Effects of Preservatives on the Bacteriological, chemical and sensory qualities of mangrove oyster (*Crassostrea gasar*) harvested from the Niger Delta Region, Nigeria. *British Journal of Applied Science and Technology*, **5**(1):76-84. [DOI]

Eleyinmi. A.F., 2007. Chemical composition and antibacterial activity of *Gongronema latifolium*. *Journal of Zhejiang University Science B.*, **8**(5):352-358. [DOI]

Enyi-Idoh, K.H., Njoku, C.C., Idim, V.D., Bassey, I.U and Egeonu, S.U. 2017. Invivo studies of antibacterial activity of *G. latifolium* and antibiotics on *Staphylococcus aureus* infected Albino rats. *International Journal of current Microbiology and Applied Science*, **6**(1):811-818. [DOI].

Forbes, B.A., Sahm, D.E., Weissfeld, A.S., 2007. Bailey and Scott's Diagnostic Microbiology. International edition. 12th edn. Mosby, Inc., an affiliate of Elsevier, Inc., USA.

Harvey, R., 2012. Lippincott's Illustrated Reviews: Pharmacology. 5th Edition. Lippincott and Williams & Wilkins, Philadelphia, USA.

Hernández, N.E., Tereschuk, M.L., Abdala, L.R., 2000. Antimicrobial activity of flavonoids in medicinal plants from Tafi del Valle (Tucuman, Argentina). *J. Ethnopharmacol.*, **73**(1-2):317-322. [DOI]

Ikegbunam, M.N., Anagu, I.O., Nwakile, D.C., Afunwa, A.R., Esimone, C.O., 2014. Antimicrobial activity of selected medicinal plants of South-Eastern Nigeria on *Pseudomonas* species expressing extended spectrum beta-lactamase (ESBL). *Eur J Med Plants*, 4:1367-7.

Kolaei, E.A., Cenatus, C., Tweddell, R.J., Avis, T.J., 2013. Antifungal activity of aluminium-containing salts against the development of carrot cavity spot and potato dry rot. *Annals of Applied Biology*, 163:311-317. [DOI]

Laport, M.S., Santos, O.C., Muricy, G., 2009. Marine sponges: Potential sources of new antimicrobial drugs. *Curr Pharm Biotechnol*., 10:86-105.

Morebise, O., 2015. A review on *Gongronema latifolium*, an extremely useful plant with great prospects. *European Journal of Medicinal Plants*, **10**(1):1-9. [DOI]

Morebise, O and Fafunso, M., A., 1998. Microbial and phytotoxic activities of saponin extracts from two Nigerian edible medicinal plants. *Biokemistri, 8(2)*:69-77.

Morebise, O., Fafunso, M. A., Makinde, J. M., Olajide, O. A., 2006. Evaluation of the bioactivity of *Gongronema latifolium* leaf extract in rodents. *Science Focus*, **11**(1):27-30.

Nasir, B., Fatima, H., Ahmed, M., Haq, I.U., 2015. Recent trends and methods in antimicrobial drug discovery from plant sources, *Austin J Microbiol*., 1:1002.

Ncube, N.S., Afolayan, A.J., Okoh, A.I., 2005. Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. *J Biotechnol.*, 7:1797-806.

Nwinyi, O.C., Chinedu, N.S., Ajani, O.O., 2008. Evaluation of antibacterial activity of *Psidium guajava* and *Gongronema latifolium*. *J. Med. Plants Research*, **2**(8):189-192.

Okpala, B. 2015. Benefits of *Gongronema latifolium* (utazi). Global food book. [Link]

Ochei, J., Kolthatkar, A., 2008. Medical Laboratory Science Theory and Practice. 7th Edn. Tata MacGraw-Hill Publishing Ltd., New Delhi, India.

Oguzie, E.E., Oguzie, K.L., Akalezi, C.o., Udeze, I.O., Ogbulie, J.N., Njoku, V.O., 2013. Natural products for materials protection: Corrosion and Microbial growth inhibition using *Capsicum frutescens* biomass extraction. Chemical Eng., 1:214-224.

Okigbo, R.N., Anuagasi, C.L., Amadi, J.E., 2009. Advances in selected medicinal and aromatic plants indigenous to Africa. *J Med Plants Res*., 3:86-95.

Okigbo, R.N., Mmeka, E.C., 2008. Antimicrobial effects of three tropical plant extracts on *Staphylococcus aureus, Escherichia coli* and *Candida albicans*. *Afr J Trad Complement Altern Med*., 5:226-9.

Okwu, D. E., 2007. Nigerian Medicinal Plant II. *Medical Aromatic Plant Science and Biotechnology*, 1:97-102.

Oshodi, A.A., Amoo, I.A., Eleyinmi, A.F., 2004. Antimicrobial activity of aqueous extracts of *Vernonia amygdalina*, *Garcinia kola* and *Gongronema latifolium* and their blends on some beer spoilage organisms. *Technical Quarterly of the Master Brewers' Association of the Americas*, **41**(4):398-402.

Osuagwu, G.G., Akomas, C.B., 2013. Antimicrobial activity of three species of Nigerian *Pterocarpus* (Jacq). *Int J Med Arom Plants*, **3**:178-83.

Osuagwu, GGE and Edeoga HO. 2010. Effect of fertilizer treatment on the antimicrobial activity of the leaves of *Ocimum gratissimum* (L) and *Gongronema latifolium* (Benth). *Afri. J. Biotechnol*. **9**(52):8918-8922.

Owu, D. U., Nwokocha, C. R., Obembe, A. O., Essien, A. D., Ikpi, D. E., Osim, E. E., 2012. Effect of *Gongronema latifolium* ethanol leaf extract on gastric acid secretion and cytoprotection in streptozocin-induced diabetic rats. *West Indian Med. J.,* **6**(9):853-860.

Pham, H.N.T., Nguyen, V.T., Vuong, Q.V., Bowyer, M.C., Scarlett, C.J., 2015. Effect of extraction solvents and drying methods on the physicochemical and antioxidant properties of *Helicteres hirsuta* Lour. Leaves. *Technologies*, 3, 285-301. [DOI]

Taiwo, S.S., Oyekanmi, B. A., Adesiji, Y.O., Opaleye, O.O., Adeyeba, O.O., 2007. In vitro Antimicrobial Activity of Crude extracts of *Citrus aurantifolia* Linn and *Tithonia diversifolia* Poacea on Clinical Bacterial Isolates. *Int. J. Tropical Medicine*, **2**(4):113-117.

Usoh, I.F., Akpan, H.D., Akpanyung, E.O., 2016.Nephroprotection against streptozotocin diabetes is more effective in combined than single leaves extracts of *Gongronema latifolium* and *Ocimum gratissimum L. Journal of innovations in Pharmaceutical and Biological Sciences*. 3 (1), 001-016, 2016.

van Vuuren, S.F., 2008. Antimicrobial activity of South African medicinal plants. *J Ethnopharmacol.,*119:462-72.