Original Research Article

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITIES OF POLAR AND NON-POLAR SOLVENT LEAF EXTRACTS OF *Gongronema latifolium*

ABSTRACT

Aim: This study was designed to assess the phytochemical constituents and antimicrobial activities of leaf extracts of *Gongronema latifolium* (Benth).

Methodology: The methods adopted were manual grinding of the air-dried leaves and maceration in polar and non-polar solvents (Ethanol and N-hexane) for 72 hrs. The resultant crude extracts were kept in dry, sterile airtight McCartney bottles and stored in the refrigerator. Thereafter, they were assayed for the presence of phytochemicals. Moreover, the plant extracts were screened for antimicrobial activities against *Bacillus subtilis, Staphylococcus aureus***,** *Salmonella typhii***,** *Escherichia coli* and **(**Fungi) *Candida albicans* and *Aspergillus fumigatus*.

Results: The results of the phytochemical screening revealed the presence of saponins, alkaloids, tannins, anthraquinones, steroids, flavonoids and terpenoids in the ethanol extracts while anthraquinones, steroids and terpenoids were absent in the N-hexane extract of the plant. Moreover, the results of the antimicrobial activity assay of the plant extracts revealed a concentration dependent trend as higher activities was observed as the concentration gradient increased. *S. aureus* (20.33±0.01) and *E. coli* (19.67±0.00) showed the highest susceptibility to the plant extracts while *B. subtilis* (12.67±0.01) showed the least susceptibility against the plant extracts at 300mg/ml. However, the plant extracts appeared not to have antifungal activity. The lowest MIC was observed in found in ethanol extract against *E. coli* (6.25mg/ml), while the highest was recorded in N-hexane extract against *B. subtilis* and *S. typhi* (100mg/ml). The ethanol extract of plant leaf was more active against the selected pathogens compared with N-hexane extract.

Conclusion: The outcome this investigation shows that the *G. latifolium* leaf extracts contain bioactive constituents such as saponins, alkaloids, tannins, anthraquinones, steroids, flavonoids and terpenoids which may account for the antibacterial activities recorded.

Keywords: *Gongronema; latifolium; Phytochemical; Antimicrobial; Pathogens*

INTRODUCTION

Traditional medicine also known as indigenous or (folk 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 (World Health Organization, 2012). Traditional medicine has been used for thousands of years with great contributions made by practitioners to human health, particularly as primary health care

providers at the community level. Countries in Africa, Asia, and Latin America use traditional medicine (TM) to help meet some of their primary healthcare needs. In Nigeria, for example, herbal medicine is the first line of treatment for 60% of children with high fever from malaria, while 85% of Nigerians use and consult traditional medicine for health care, social and psychological benefits (Amupitan, 2013). Herbal remedies have a therapeutic effect and are acceptable interventions for diseases and symptoms. Interestingly, demand for medicinal plants is progressively rising in industrialized nations as it is in developing countries (Abere *et al*, 2010). The world Health organization (WHO) has since urged developing countries to utilize the resources of traditional medicine for achieving the goals of Primary Healthcare. This has been due to the various advantages of traditional medicine namely: low cost, afford ability, accessibility, acceptability and perhaps low toxicity.

Gongronema latifolium (Benth) commonly called ‘‘utazi’’ and ‘‘arokeke’’ in South Eastern and South Western parts of Nigeria respectively is a perennial edible plant with soft and pliable stem, belonging to the family of *Asclepiadaceae* (Ugochukwu and Babady, 2002). It is widely used in the West African sub-region for a number of medicinal and nutritional purposes. It is a tropical rainforest plant primarily used as spice and vegetable in traditional folk medicine (Chinedu *et al.,* 2013). A range of pharmacological tests have shown promising hypoglycaemic activities, and also interesting antibacterial, antioxidant, anti-inflammatory, hepatoprotective, antiplasmodial, anti-asthmatic, anti-sickling, anti-ulcer, analgesic and antipyretic activities of *G. latifolium* (Burkill, 1998). The leaves of *G. Latifolium* are used as vegetables in preparation of soups to which they add a bitter-sweet flavor (Iwu, 1998).

There are little reports on the antimicrobial efficacy of this multi-dimensional potent medicinal plant therefore this study is aimed at assaying for the phytochemical and antimicrobial potency of the plant.

2.0 MATERIAL AND METHODS

2.1 Collection, Identification and Extraction of the Extracts

Fresh *G. Latifolium* leaves were collected by plucking from the parent plants from Igbo-Oke forest in Ifon, Ondo state, Nigeria in November, 2016. The plant was then authenticated at the Herbarium section of the Department of Forest Resources Technology and a voucher specimen (XGL101) was deposited (in the same Department) Rufus Giwa polytechnic, Owo. The authenticated plant materials were washed and cleaned thoroughly with tap water and then air-dried under shade. The dried samples were then ground into coarse powder with the aid of a mechanical grinder and were stored in clean air- tight containers, and kept in a cool, dry place until required for use.

One hundred gram (100g) of the powdered sample was soaked in 300ml of different solvents (ethanol and N-hexane) for 72hr with intermittent stirring using sterile spatula. The plant extracts were then filtered through Whatman No1. filter paper into bijou bottles and then dried using rotary evaporator at a temperature of 50^0C to yield crude extracts [13]. Different concentrations of the extracts were prepared by diluting 0.50g, 1.00g, 2.00g and 3.00g of the extracts in 100ml of 0.01% Tween-20 to obtain concentrations of 50mg/ml, 100mg/ml, 200mg/ml and 300mg/ml respectively [14].

2.2 Test microorganisms

The test microorganisms used for this analysis include (Bacteria) *Bacillus subtilis, Staphylococcus aureus***,** *Salmonella typhii***,** *Escherichia coli* and **(**Fungi) *Candida albicans* and *Aspergillus fumigatus*. They were all collected from the Medical Microbiology Laboratory and Parasitology Department, Federal Medical Center, Owo, Ondo State, Nigeria in February, 2017. Organisms were sub-cultured and maintained on agar slants.

2.3 Qualitative phytochemical screening

The extracts of the different plant parts were subjected to qualitative phytochemical analysis for the presence of tannins, saponin, flavonoids, alkaloids and phenol were carried out on the extracts using standard procedures as described by [15 16]

2.3.1 Test for tannins

1ml of extract was boiled in 20ml of water in a test and then filtered. A few drops of 0.1% ferric chloride was added and observed green or a blue – black coloration which confirmed the presence of tannin.

2.3.2 Test for saponin

About 5ml of the extract was boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion which confirmed a positive presence of saponins.

2.3.3 Test for flavonoids

A 3ml portion of 1% Aluminum chloride solution was added to 5ml of each extract. A yellow coloration was observed indicating the presence of flavonoids. 5ml of dilute ammonia solution were added to the above mixture followed by addition of concentrated H_2SO_4. A yellow coloration disappeared on standing. The yellow coloration which disappeared on standing indicating a positive test for flavonoids.

2.3.4 Test for alkaloids

A 1ml portion of the extract was stirred with 5ml of 1% aqueous HCl on a steam bath and filtered while hot. Distilled water was added to the residue and 1ml of the filtrate was treated with a few drops of either Mayer's reagent (Potassium mercuric iodide- solution gave a positive test for alkaloids.

2.3.5 Test for steroids

A 2ml portion of acetic anhydride was added to 2ml extract of each sample followed by careful addition of 2ml H_2SO_4. The color changed from violet to blue or green indicating the presence of steroids.

2.3.6 Test for terpenoids (Salkowski test)

Five ml of each extract was mixed with 2ml of chloroform, and 3ml concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive result for the presence of terpenoids.

2.3.7 Test for anthraquinone

A 5ml portion of extract was mixed with 10ml Benzene, filtered and 5ml of 10% NH_3 solution was added to the filtrate. The mixture was shaken and the presence of violet colour in the ammoniac (lower) phase indicated the presence of anthraquinones

2.4 *In vitro* antibacterial susceptibility test

The extracts obtained from the test plants were screened against the test bacteria by agar well diffusion method [17]. A 25ml aliquot of Mueller-Hinton agar (Lab Oratorios Britania, Argentina) and Sabouraud Dextrose agar (Oxoid, UK) was poured into different Petri plate. When the agar solidified, each test organism was inoculated on the surface the appropriate plates (1×10^6 *cfu*/ml) using a sterile glass spreader and allowed to sink properly. Subsequently, the surface of the agar was punched with 6mm diameter cork borer into wells and a portion of 50µl of each of the extract concentrations was filled into the wells. Control wells containing the same volume of 30% Dimethyl sulphoxide (DMSO) served as negative control, while Chloramphenicol (50µg) and fungisol (100µg) was used as positive control for bacterial and fungal the plates respectively and the plates were incubated at 37^0C for 24 h (bacteria) and 27^0C for 72hrs (fungi). Each experiment was carried out in triplicate and the diameter of the zones of inhibition was then measured in millimeters.

2.4.1 Minimum inhibitory concentration (MIC)

The MIC of the plants extracts were determined by double dilution broth methods of Ghosh et al. [18]. Twofold serial dilutions of the extracts were prepared in Mueller-Hilton broth (bacteria) and Saboraud Dextrose broth (fungi) to achieve a decreasing concentrations ranging from the least concentration that produced clear zone of inhibition (100mg/ml to 1.56mg/ml). All tubes with the controls were labeled accordingly. Each dilution was seeded with 1ml of standardized inoculums (1.0×10^6 *cfu*/ml) and incubated at 37^0C for 24 hr. A tube containing only seeded broth (i.e. without plant extract) was used as the positive control while the un-inoculated tube was used as negative control. The lowest concentration of each extract that showed a clear of inhibition was when compared with the controls was considered as the MIC.

2.6 Data Analysis

Data were presented as mean±standard error (SE). Significance difference between different groups was tested using two-way analysis of variance (ANOVA) and treatment means were compared with Duncan's New Multiple Range Test (DNMRT) using SSPS window 7 version17.0 software. The significance was determined at the level of $p\leq 0.05$.

3.0 RESULT AND DISCUSSION

3.1 Phytochemical constituents of *G. latifolium*

The phytochemical constituent screening of the *G. latifolium* leaf extracts revealed the presence of plant constituents such as saponins, alkaloids, tannins, anthraquinones, steroids, flavonoids and terpenoids which varied according to the extracting solvents (Table1). All the tested phytochemicals were detected in ethanol extract whereas anthraquinones, steroids and terpenoids were absent in N-hexane extract. The presence of various metabolites in the plant materials could justify its medical use [9]. Most of these compounds are also well known for their large spectrum of pharmacological properties, including antimicrobial (alkaloids and saponins) and antioxidant (tannins) activities [19, 20]. This result is also in line with the report of Afolabi and Eleyinmi (2007), who carried out the Chemical Composition and Antibacterial activity of methanolic extract of *G. latifolium* leaves and obtained similar results.

Table1: Phytochemical constituent of *G. latifolium* leaf

Phytochemical	Ethanol	N-hexane
Saponins	+++	++
Flavonoids	++	++
Alkaloids	++	+
Anthraquinones	+	-
Tannins	+	+
Steroids	+	-
Terpenoids	+	-

Key: +++= present in abundance, ++= present moderately, += present in trace amount, -= not detected

3.2 Antimicrobial Activities of *G. latifolium*

The results of the antimicrobial activities of the plant extracts were concentration dependent as higher activities was observed as the concentration gradient increased. The extracts exhibited different degrees of antimicrobial activity against tested organisms. Only *S. aureus* and *E. coli* were susceptible to the extracts at the lowest concentration used (50mg/ml), all the bacteria were susceptible at 100, 200 and 300mg/ml whereas none of the fungi was susceptible to the extracts at all the concentrations used. This is similar to the findings of Omodamiro and Ekeleme (2013) who reported that the ethanolic leaf extract show a significant dose dependent inhibition of *Staphylococcus aureus*, *Streptocollus pneumonia*, E. *coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa*.

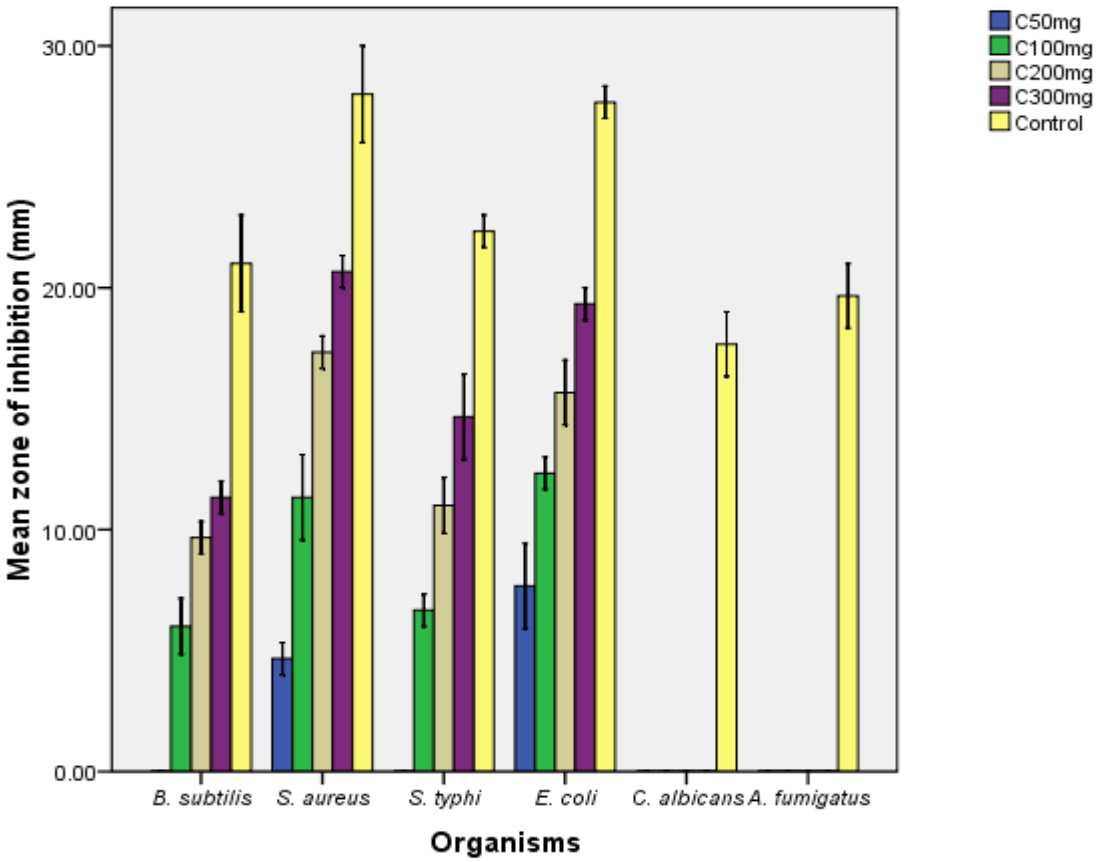

Fig 1: Antimicrobial activity of ethanol extract of *G. latifolium* against some pathogens

In all, ethanol extract of the plant showed higher antimicrobial activities than the N-hexane extracts. At the highest concentration used (300mg/ml), the ethanol extracts of the plant showed comparable activities with the pure commercial antibiotic (chloramphenicol) used as positive control against *S. aureus* and *E. coli* compared with N-hexane extract which recorded a rather low activity against all the tested organisms. This research work is in consonance with the findings of Eja *et al.* (2011) who reported the antimicrobial activity of Utazi (*G. latifolium)* on *E. coli* and *S. aureus*. It might also be due to the differences in the concentration of the phytocompounds of various secondary metabolites present in the extracts as well as the extracting ability of the solvents. It therefore implies that polar solvent (ethanol) may be a better extraction solvent for the leaf of this plant than non-polar (N-hexane) solvent. This corroborates the observations of Abo and Ashidi [26].

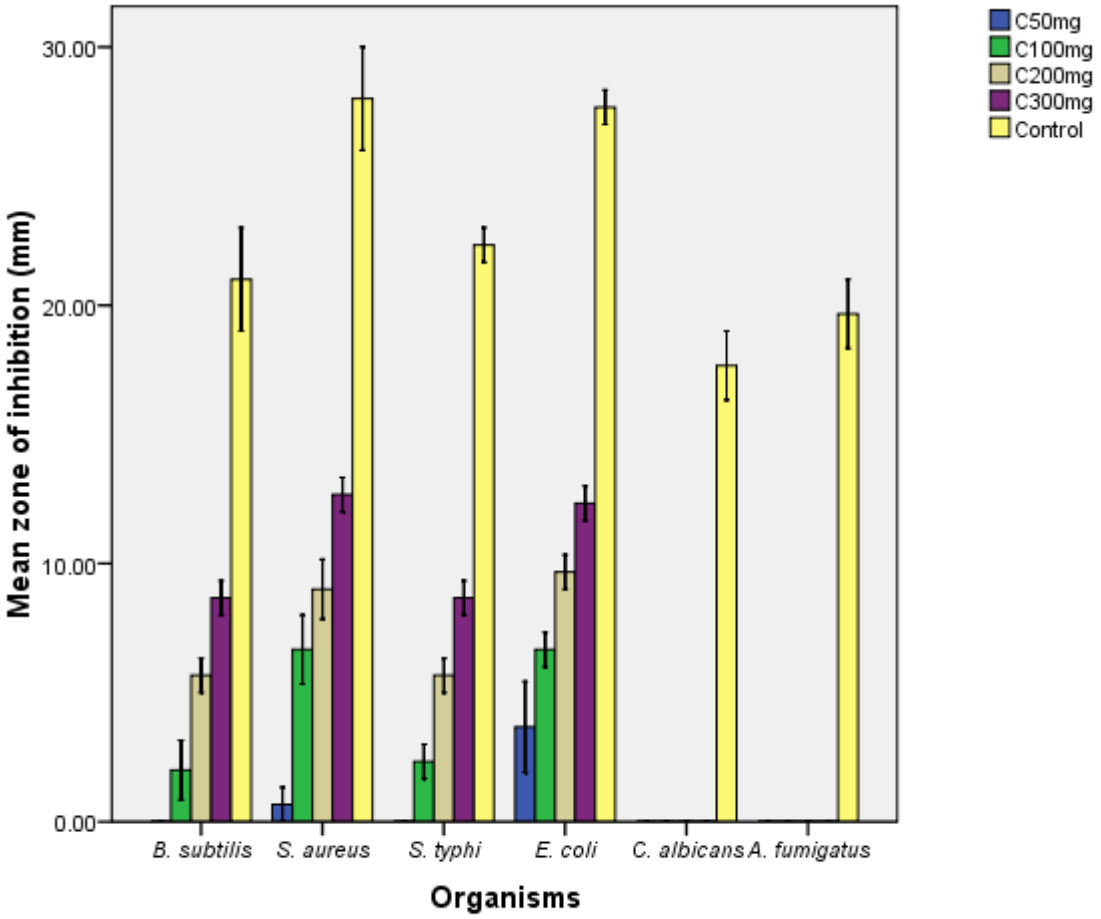

Fig 2: Antimicrobial activity of N-hexane extract of *G. latifolium* against some pathogens.

Table2: Minimum inhibitory concentration of extract of *G. latifolium* against some pathogens

Microorganism	Ethanol (mg/ml)	N-hexane (mg/ml)
Bacillus subtili	75	100
Staphylococcus aureus	12.5	25
Salmonella typhii	75	100
Escherichia coli	6.25	25
Candida albicans	ND	ND
***Aspergillus fumigatus*.**	ND	ND

Key: ND= not detected

The minimum inhibitory concentration (MIC) is the least concentration of the extracts that inhibit growth of organisms. It is an important diagnostic tool since helps in confirming resistance of microorganisms to antimicrobial agents. The lowest MIC was observed in found

in ethanol extract against *E. coli* (6.25mg/ml), while the highest was recorded in N-hexane extract against *B. subtilis* and *S. typhi* (100mg/ml). This disagrees with the report of Nwinyi *et al.* (2008) who reported zones of inhibition between 6 and 10mm while minimum inhibitory concentrations (MIC) were 10.0 and 2.5mg/ml respectively for the aqueous and ethanolic extracts of *G. latifolium* against *E. coli* and *S. aureus* respectively. This suggests that this plant may be useful in the management of intestinal pathogens especially the *Enterobacteriaceae* and to treat some related microbial infection.

Conclusion

From the results obtained in this study, ethanol and N-hexane extracts of *G. latifolium* leaves contain bioactive phytochemicals like saponins, alkaloids, tannins, anthraquinones, steroids, flavonoids and terpenoids. Moreover, the extracts possess antibacterial activity at higher concentrations against the test bacterial pathogens while it was not active against any of the fungus tested. Finally, polar solvent (ethanol) is a better extraction solvent for this plant than non-polar (N-hexane) solvent.

References

1. *World Health Organization.* Traditional medicine. *Health topics. 2012; Retrieved 20th Jan., 2017.*
2. Onike R. A survey of medicinal values of *Gongronema latifolium* (madumaro) in African Alternative Medicine. Nig. J. Physiol. Sci. 2010; 24(1): 79-83.
3. Abere TA, Okolo PE, Agoreyo FO. Antidiarrhoea and toxicological evaluation of the leaf extract of *Dissotis rotundifolia* and *Triana melastomatacea. BMC Complement Alternative Medicine,* 2010; 10:71.
4. Ugochukwu NH, Babady NE. Antihyperglycemic effect of aqueous and ethanolic extracts of *Gongronema latifolium* leaves on glucose and glycogen metabolism in livers of normal and streptozotocin-induced diabetic rats. Life Sci. 2003; 73: 1925-1938.
5. Chinedu I, Uhegbu FO, Imo CK, Ifeanacho NG. Ameliorating effect and haematological activities of methanolic leaf extract of *Gongronema latifolium* in acetaminophen- induced hepatic toxicity in Wistar albino rats. Intern. J. Biol. Sci. 2013; 3(11): 183-188.
6. Oliver-Bever B. Medicinal plants in tropical West Africa. Camb. Univ. Press., London. 1986; pp. 89-90.
7. Agbo CU, Baiyeri KP, Obi IU. Indigenous knowledge and utilization of *Gongronema latifolium* Benth: a case study of women in university of Nigeria Nsukka. Biol. Res. J. 2005; 3(2): 66-69.
8. Meyer BN, Ferrigni NR, Putma JE, Jacobson LB, Nicholas DE and McLaughlin JL. Brine Shrimp: a convenient general bioassay for active plant constituents. Planta Medicine, 1982; 45:31-34.
9. Vashit H, Jundal A. Antimicrobial Activities of Medicinal Plants- Reviews. International Journal of Research Pharmaceutical and Biomedical Science, 2012; 3: 222-230.
10. Harborne JB. Phytochemical methods- A guide to Modern Techniques of plant Analysis. Springer Pvt Ltd, India. 1978.
11. Sofowora A. *Medicinal Plants and Traditional Medicines in Africa*. Chischester john Willey & Sons New York. 1993.

12. Perez C, Pauli M, Bazerque P. An antibiotic assay by the agar-well diffusion method. Actabiologiaeet Medicine Experimentalis. 1990; 15:113-115.
13. Ghosh G, Subudhi BB, Badajena LD, Ray J, Mishra MK, Mishra SK. Antibacterial activity of polyalthia longifolia var. angustifolia stembark extract. International Journal of PharmTech Research, 2011; 3(1): 256-260.
14. Kubmarawa D, Ajoku GA, Enwerem NM, Okorie DA. Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. Afr. J Biotechnol. 2007; 6(14):1690-1696
15. Hag IU, Mannan A, Ahmed I, Hussain I, Jamul M, Mirza B. Antimicrobial activity and Brine Shrimp toxicity of *Artemisia dubia* extract. Pakistan Journal of Botany. 2012; 44(4):1487-1490.
16. Ngbolua KN, Fatiany PR, Robijaona B, Randrianirina AYO, Rajaonariveto PJ, Rasondratoro B, et al. Ethnobotanical survey, chemical composition and *in vitro* Antimicrobial activity of essential oils from the root bark of *Hazomakinia voyroni* (Jum.) Capuron (Hernandiaceae). Journal of Advancement in Medical and Life Sciences. 2014; 1(1):1-6.
17. Eleyinmi AF. Chemical composition and antibacterial activity of *Gongronema latifolium*. J. Zhejiang Universal Sci. 2007; 8: 352-358.
18. Omodamiro OD, Ekeleme CM. Comparative study of *in vitro* antioxidant and antimicrobical activities of *Piper guineense*, *Curmuma longa*, *Gongronema latifolium*, *Allium sativum*, *Ocimum gratissimum*. World J. Med. Med. Sci. 2013; 1(4): 51-69.
19. Morebise O, Fafunso MA. Antimicrobial and phytotoxic activities of saponin extracts from two Nigerian edible medicinal plants. Biokemistri. 1998; 8(2): 69-77.
20. Cushnie T, Cushnie B, Lamb A. Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. Int J Antimicrob Agents, 2014; 44(5):377–386
21. Nwinyi OC, Chinedu NS, Ajani OO. Evaluation of antibacterial activity of *Pisidum guajava* and *Gongronema latifolium*. J. Med. Plant Res. 2008; 2: 189-192.