View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Afe Babalola University Repository

**Open Science Journal of Pharmacy and Pharmacology**
2017; 5(6): 34-41
http://www.openscienceonline.com/journal/osjpp

Open Science

# Evaluation of the Antimicrobial Potential of *Gongronema latifolium* Extracts on Some Wound-Associated Pathogens

Enitan Seyi Samson[1, *], Omodiale Paul Ehizibue[1], Adejumo Esther Ngozi[1], Akele Yomi Richard[2], Owolabi Tolulope Omolara[1]

[1]Department of Medical Laboratory Science, Babcock University, Ilishan-Remo, Nigeria

[2]Department of Medical Laboratory Science, Afe Babalola University, Ado-Ekiti, Nigeria

## Email address

enitans@babcock.edu.ng (E. S. Samson), omodialep@babcock.edu.ng (O. P. Ehizibue), adejumoe@babcock.edu.ng (A. E. Ngozi), akeler@abuad.edu.ng (A. Y. Richard), owolabi.Lara@gmail.com (O. T. Omolara)

[*]Corresponding author

## To cite this article

Enitan Seyi Samson, Omodiale Paul Ehizibue, Adejumo Esther Ngozi, Akele Yomi Richard, Owolabi Tolulope Omolara. Evaluation of the Antimicrobial Potential of *Gongronema latifolium* Extracts on Some Wound-Associated Pathogens. *Open Science Journal of Pharmacy and Pharmacology*. Vol. 5, No. 6, 2017, pp. 34-41.

**Received**: August 8, 2017; **Accepted**: December 8, 2017; **Published**: January 16, 2018

## Abstract

This present study investigated the antimicrobial potential of the leaf and stem extracts of *Gongronema latifolium* against some selected wound-associated pathogens: *Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Klebsiella pneumonae* and *Candida albican* using the punch-hole agar diffusion method. The outcome of the study show that neither the aqueous nor the methanolic leaf and stem extracts of *Gongronema latifolium* showed any inhibitory activity against the bacterial and fungal isolates at all the concentrations (200, 150, 100 50 and 25 mg/mL) tested either singly or combined; whereas the positive control was effective against all of them; with zones diameter of inhibition ranging between 18-24 mm (*Pseudomonas aeruginosa*), 17-23 mm (*Escherichia coli*), 15-23 mm (*Proteus mirabilis*), 18-22 mm (*Staphylococcus auerus*) and 17-24 mm (*C. albicans*). On the other hand, as expected, the negative control (distilled water) did not show any zone of inhibition. The antibacterial activity of the positive control was statistically significant at P value <0.05 when compared against the extracts and negative control. Although the antimicrobial potential of extracts of *G. latifolium* have been previously demonstrated against various clinical isolates, the findings in this current study did not support claims made by different researchers in previously studies. All the test isolates were found to be resistant to the extracts. Lack of inhibitory activity by extracts of *G. latifolium* growing in Irolu, Ikenne Local Government Area of Ogun state in this present study is plausible and would require further investigation. Due to differences in topographical factors of various geographical locations, nutrient concentrations of the soil, chemical composition, age and time of harvest of the plant, extraction method as well as method used for antimicrobial study; it is therefore important that scientific protocols be clearly identified, adequately followed and reported.

## Keywords

Wounds, Pathogens, Infection, Medicinal Plant, *Gongronema latifolium*

## 1. Introduction

Wounds are injuries characterized by some types of piercing, cutting, or tearing of tissues, especially the one that is caused by physical means and with interruption of continuity [1]. Wounds can be clean, contaminated, colonized or infected. The skin is a formidable physical barrier. When the skin is broken, however, a portal of entry is created through which pathogens can reach interior tissues, establish themselves, and cause infection. Damaged tissues can supply nutrients for microbial growth. Besides, deep wounds provide the anaerobic environment needed for growth of anaerobic bacteria such as *clostridium perfringens* [2].

According to Kingsley [3], the presence of microbes in a wound may result in: contamination (the bacteria do not increase in number or cause clinical problems), colonization (the bacteria multiply, but wound tissues are not damaged) or infection (the bacteria multiply, healing is disrupted and wound tissues are damaged). Most often, there is an increased susceptibility to infection after the occurrence of a wound, and this is dependent on the access of pathogens and the immunocompetency status of the individual [4]. A wound provides an environment which is conducive to microbial colonization and subsequent infection as it is an ideal medium for a wide variety of micro-organisms [5], [6].

So many bacteria and fungi have been implicated in different types of wound infections: *Staphylococcus aureus* is the commonest pathogen isolated from subcutaneous abscesses and skin wounds, while *Pseudomonas aeruginosa* is associated with infected burns. *Escherichia coli*, *Proteus* species and *Bacteroides* species are the pathogens most frequently isolated from abdominal abscesses and wounds. *Clostridium perfringens* is found mainly in deep wounds where anaerobic conditions exist. The toxins produced cause putrefactive decay of the infected tissue with gas production. The death and decay of tissue by *C. perfringens* is called gas gangrene. The commonest pathogens isolated from chronic leg ulceration are *Haemophilus ducreyi, Corynebacterium diphtheria, Bacillus anthracis, Streptococcus pyogenes, S. aureus*, *P. aeruginosa* and *Bacteroides* species. The commonest fungi implicated in burns are Candida species and Aspergillus species [7], [8].

Wound infection has continued to be a challenging problem and represents a considerable healthcare burden. Resistance to antibiotics by some wound pathogens had been reported and plants have been identified as the alternative in treating septic wounds with little or no microbial resistance. A special feature of higher plants is their capacity to produce a large number of organic chemicals of high structural diversity called secondary metabolites [9]. Active components of several plants are now being investigated, extracted and developed into drugs with little or no negative effects or contra-indications. Medicinal plants now serve as the starting point for the discovery of semi synthetic chemical compounds. The chemical structures derived from plant substances are now used as models for new synthetic compounds. The renewed interest in the used of medicinal plants may be attributed to cheapness, availability, and accessibility by the local populace, high incidence of side effects of synthetic medicines and environmental friendliness of plant extracts [10].

About 80% of the populations in Africa still use traditional medicine for their healthcare. In Nigeria for instance, many indigenous plants are used in herbal medicine to cure diseases and heal injuries [11]. *Gongronema latifolium* (Amaranth globe) is one of such medicinal plants, which is fast gaining recognition. The plant *Gongronema latifolium*, is a tropical rainforest plant primarily used as spice and vegetable in the traditional folk practice [12].

The plant belongs to the family Asclepiadaceae and genus *Gongronema* [13]. It is commonly grown in West Africa and is locally called "Utasi" by the Ibibios, Quas and Efiks; "Utazi" by the Igbos in South East and "Arokeke" or "Madunmaro" by the Yorubas in South Western part of Nigeria. In Ghana and Senegal, the plant is referred to as "Akan-Asante aborode" and "Sever gasule," respectively. It is a climber with woody, hollow glabrous stem that produces milky latex when cut. It is a perennial edible plant with green leaf and yellow flower. It has a characteristic sharp, bitter and slightly sweet taste, especially when eaten fresh [14].

*Gongronema latifolium* is widely believed to have strong nutritional and medicinal values. The leaf is rich in phytochemicals, fats, proteins, vitamins, minerals and essential amino acids [15]. According to Nwanjo *et al.* [16], phytochemical studies of *Gongronema latifolium* leaves show the presence of Glycosides, Alkaloids, Saponin, Tannin and Flavonoids. Egbung *et al.* [17] reported the presence of phytochemicals (tannins, saponins, alkaloids, flavonoids and hydrocyanide), mineral elements (Cr, Cu, Se, Zn and Fe) and vitamins (A, C, riboflavin, niacin and thiamine) in the root bark and twig extracts of *Gongronema latifolium*.

The plant is commonly used in soup as vegetable, or dried and applied as powdery spice. It is also consumed fresh and can be used in salad preparations [18] - [20]. Apart from its nutritional values, *G. latifolium* is believed to possess strong medicinal qualities due to its composition of different active chemicals. In the southern part of Nigeria for instance, it is used traditionally in the management and treatment of a wide array of unrelated ailments. The leaves is used for dysentery, catarrh, congested chest, running nose, cough, viral hepatitis, bilharzias, malaria, hypertension, diabetes, asthma, constipation, nausea, and anorexia among others. The roots on the other hand are used for Sickle cell anemia and relieve wheezing associated with asthma. In Sierra Leone, the root and stem are used as chewing stick or liquor. The liquor is obtained by boiling the sliced plant with lime juice or infused in water for over 3 days. It is then taken as a purgative for colic and stomach pains as well as to treat symptoms of worm infection [12], [20], [21].

This plant is well known for its: hepato- and nephro-protective effect [22] - [24], immunomodulatory effect [25], hematological effect [26], hypolipidemic activity [27], anti-ulcer activity [28], hypoglycemic activity [29], antioxidant activity [16], anti-inflammatory activity [19] and antimicrobial activity [15], among several others.

Even though the antimicrobial potential of *Gongronema latifolium* have been investigated by different researchers, very little is known about the sensitivity of wound-associated pathogens to the leaf and stem extracts. To the best of our knowledge, no work has been done to investigate the single and combined effects of the leaf and stem extracts of *Gongronema latifolium* growing in Irolu community, Ikenne Local Government Area of Ogun state on wound-associated pathogens. It therefore appears that there is dearth of information on the scientific use of *Gongronema latifolium* extracts as an economic and accessible treatment option for wound infection, hence the reason for this study.

## 2. Materials and Methods

### 2.1. Study Area

The study was carried out at the Medical Microbiology and Parasitology Unit of the Department of Medical Laboratory Science, Babcock University, Ilishan-Remo, Ogun State, Nigeria: a Seventh Day Adventist Institution of higher learning located in the Southwest region of Nigeria, coordinates: 6.8862°N, 3.7055°E.

### 2.2. Study Duration

The study was carried out between the period of May and June, 2016.

### 2.3. Research Design

#### 2.3.1. Collection of Plant Materials

Fresh Plants of *Gongronena latifolium* were harvested from a local farm in Irolu community, Ikenne Local Government Area of Ilishan, Ogun state, Nigeria.

#### 2.3.2. Plant Authentication

The plant materials were authenticated by Dr. J. S. Ashidi of the Department of Plant sciences, Olabisi Onabanjo University, Ago Iwoye, Ogun state, Nigeria.

***Figure 1.*** *A photograph showing the leaves of G. latifolium.*

***Figure 2.*** *A photograph showing the stems of G. latifolium.*

#### 2.3.3. Preparation of Crude Aqueous and Methanolic Extracts

Fresh leaf and stem of *Gongronema latifolium* about 500g each were shredded, washed gently with distilled water, without squeezing, to remove dirt and sun-dried for 4 consecutive days. The dried leaf and stem were pulverized in a mortar with a pestle and further ground to powder using an electric blender. Extractions were done as follows: Coarse powder (25g) of the leaf and stem were soaked in 250ml distilled water and methanol in separate beakers and the mixture were allowed to stand for 24hrs before filtration according to the method described by Bagavan and Rahuman [30]. The mixture were allowed to pass through a muslin cloth and later filtered with a Whatman No. 1 filter paper (110 mm). The liquid filtrate obtained were poured into a pre-weighed beaker and evaporated to dryness in a vacuum oven at 45°C to obtain a solid residue. The residues obtained were stored in the desiccator until use.

#### 2.3.4. Preparation of Various Concentrations of Extract

After preparation of the crude extracts as described; the double dilution procedure were done on the fresh filtrates (neat) to obtained lower concentrations (100 mg/ml, 50 mg/ml and 25 mg/ml) of the extract using sterile distilled water. While 2 g of the dried extracts were reconstituted in 10 ml of distilled water to obtain a 200 mg/ml extract solution. The double dilution procedure was also done to obtained lower concentrations of the extracts.

#### 2.3.5. Test Isolates

Stock cultures of *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis, Escherichia coli* and *Candida albicans* isolated from wound infections already characterized by: Gram stain, Sugar fermentation tests and Biochemical tests were obtained from the Medical Microbiology Laboratory of Olabisi Onabanjo University Teaching Hospital (OOUTH), Shagamu, Ogun state.

#### 2.3.6. Preparation of Test Isolates

The isolates were sub-cultured from preserved agar slants onto selective and differential solid media and re-identified biochemically using standard methods as described by Cheesbrough [31], [32].

#### 2.3.7. Standardization of Innoculum

Five colonies from the pure culture of each isolate were inoculated into nutrient broth (NB) and incubated at 37°C for 18 to 24 h. Surface viable counts were carried out as described by [33]. Turbidity of the bacterial suspension (*i.e* overnight nutrient broth with population density of $10^7$ CFU/ml), was adjusted to match that of 0.5 McFarland standard ($10^5$ CFU/ml) by making a dilution of 1:100 in sterile nutrient broth.

### 2.4. Antimicrobial Susceptibility Testing

Agar diffusion test using punch-hole method described by Slack [34] was used to determine the susceptibility of the test isolates to the plant extracts. Sterile semi-solid Nutrient Agar (NA) and Sabouraud Dextrose Agar (SDA) plates were prepared. 1 ml of 24 h old standardized cultures of bacteria broths was separately used to flood the surface of NA plates, while 1 ml of 24 h old broth culture of *C. albicans* was used to flood the surface of SDA plates. The plates were swirled, allowing the inoculums to spread on the surface of the agar, and the excess were drained off, in a disinfectant jar. With the aid of

a sterile standard cork borer of 5 mm diameter, six ditches (wells) were bored at equal distances around the plates. The bottoms of the wells were sealed with one drop of the sterile molten agar; to prevent diffusion of the extracts under the agar.

A 0.1 ml of reconstituted extract of various concentrations equivalent to 20 mg, 10 mg, 5 mg and 2.5 mg of the extract were aseptically dropped into each appropriately labeled well on the plate (wells, 1-4). The 5th and 6th wells served as negative and positive controls, and were filled with sterile distilled water and Ciprofloxacin® (used at tissue concentration- 10µg/ml), respectively. Ketoconazole was used as positive control for *C. albicans*. The inoculated plates were left on the table for 1 hr to allow pre-diffusion of the extract into the agar. The NA plates were incubated aerobically at 37°C for 24 h.

### 2.5. Data Collection

The resulting zones of inhibition were measured using a ruler calibrated in millimetres and recorded. Data obtained were presented as means of 3 replicates of zones diameter of inhibition using tables.

### 2.6. Data Analysis

Data were analyzed using Chi-Square with SPSS-18.0 (Statistical packages for social Scientists – version 18.0) software. P values <0.05 was considered significant [35].

### 2.7. Disposition of Clinical Isolates

Used clinical isolates were autoclaved at 121°C for 15 minutes before been discarded.

### 2.8. Ethical Approval

Ethical approval for the study was obtained from the Babcock University Health Research Ethics Committee (BUHREC).

## 3. Results and Discussion

The outcome of this study shows that neither the aqueous nor the methanolic leaf and stem extracts of *Gongronema latifolium* showed any inhibitory activity against the bacterial isolates at all the concentrations (200, 150, 100 50 and 25 mg/mL) tested either singly or combined; whereas the positive control (Ciprofloxacin) was effective against all of them; with zones diameter of inhibition between 18-24 mm (*Pseudomonas aeruginosa*), 17-23 mm (*Escherichia coli*), 15-23 mm *(Proteus mirabilis*) and 18-22 mm (*Staphylococcus auerus*). On the other hand, as expected, the negative control (distilled water) did not show any zone of inhibition. The antibacterial activity of the positive control was statistically significant at P value <0.05 when compared against the extracts and negative control (Table 1, 2, 3 and 4).

***Table 1.*** *Effect of Leaf and Stem Extracts of Gongronema latifolium on Pseudomonas aeruginosa.*

| Extracts | Mean Zone Diameter of Inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| ALEGL | 0 | 0 | 0 | 0 | 0 | 0 | 24 |
| MLEGL | 0 | 0 | 0 | 0 | 0 | 0 | 22 |
| AREGL | 0 | 0 | 0 | 0 | 0 | 0 | 21 |
| MREGL | 0 | 0 | 0 | 0 | 0 | 0 | 23 |
| CAL+REGL | 0 | 0 | 0 | 0 | 0 | 0 | 24 |
| CML+REGL | 0 | 0 | 0 | 0 | 0 | 0 | 18 |
| CONCENTRATION OF THE EXTRACTS (mg/ml) | 200 | 150 | 100 | 50 | 25 | -C | +C |

KEY: ALEGL – Aqueous leaf extract of *Gongronema latifolium,* MLEGL – Methanolic leaf extract of *Gongronema latifolium*
AREGL – Aqueous stem extract of *Gongronema latifolium,* MREGL – Methanolic stem extract of *Gongronema latifolium,* CAL+REGL – Combined aqueous leaf and stem extracts of *Gongronema latifolium,* CML+REGL – Combined methanolic leaf and stem extracts of *Gongronema latifolium,* - C Negative control, + C Positive control

Similarly, the aqueous and methnolic leaf and stem extracts of *Gongronema latifolium* showed no antifungal activities against the fungal isolate tested. *Candida albicans* was found to be resistant to the extracts just like the bacterial isolates when tested both singly and combined even at the highest concentration (200 mg/mL). However it was significantly sensitive to the positive control (Ketoconazole) with zones diameter of inhibition between 17-24 mm at P value <0.05 when compared to the extracts and negative control (Table 5).

***Table 2.*** *Effect of Leaf and Stem Extracts of Gongronema latifolium on Escherichia coli.*

| Extracts | Mean Zone Diameter of Inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| ALEGL | 0 | 0 | 0 | 0 | 0 | 0 | 17 |
| MLEGL | 0 | 0 | 0 | 0 | 0 | 0 | 23 |
| AREGL | 0 | 0 | 0 | 0 | 0 | 0 | 22 |
| MREGL | 0 | 0 | 0 | 0 | 0 | 0 | 20 |
| CAL+REGL | 0 | 0 | 0 | 0 | 0 | 0 | 23 |
| CML+REGL | 0 | 0 | 0 | 0 | 0 | 0 | 23 |
| Concentration of the extracts (mg/ml) | 200 | 150 | 100 | 50 | 25 | -C | +C |

KEY: ALEGL – Aqueous leaf extract of *Gongronema latifolium,* MLEGL – Methanolic leaf extract of *Gongronema latifolium*
AREGL – Aqueous stem extract of *Gongronema latifolium,* MREGL – Methanolic stem extract of *Gongronema latifolium,* CAL+REGL – Combined aqueous leaf and stem extracts of *Gongronema latifolium,* CML+REGL – Combined methanolic leaf and stem extracts of *Gongronema latifolium,* - C Negative control, + C Positive control

***Table 3.*** *Effect of leaf and stem extracts of Gongronema latifolium on Proteus mirabilis.*

| Extracts | Mean Zone Diameter of Inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| ALEGL | 0 | 0 | 0 | 0 | 0 | 0 | 23 |
| MLEGL | 0 | 0 | 0 | 0 | 0 | 0 | 21 |
| AREGL | 0 | 0 | 0 | 0 | 0 | 0 | 19 |
| MREGL | 0 | 0 | 0 | 0 | 0 | 0 | 15 |
| CAL+REGL | 0 | 0 | 0 | 0 | 0 | 0 | 18 |
| CML+REGL | 0 | 0 | 0 | 0 | 0 | 0 | 19 |
| Concentration of the extracts (mg/ml) | 200 | 150 | 100 | 50 | 25 | -C | +C |

KEY: ALEGL – Aqueous leaf extract of *Gongronema latifolium,* MLEGL – Methanolic leaf extract of *Gongronema latifolium*
AREGL – Aqueous stem extract of *Gongronema latifolium,* MREGL – Methanolic stem extract of *Gongronema latifolium,* CAL+REGL – Combined aqueous leaf and stem extracts of *Gongronema latifolium,* CML+REGL – Combined methanolic leaf and stem extracts of *Gongronema latifolium,* - C Negative control, + C Positive control

This present study examines the potential antimicrobial activities of aqueous and methanolic leaf and stems extracts of *G. latifolium* growing in Irolu, Ikenne Local Government Area of Ogun State. The single and combined extracts tested separately were observed to have no inhibitory activity against all the selected wound-associated test isolates studied. The absence of zones diameter of inhibition on the seeded agar plates show lack of sensitivity by the test isolates and non-inhibitory activity of the extracts at the different concentrations tested. This lack of *in vitro* antimicrobial activity of the leaf and stem extracts of *G. latifolium* in this present study contradicted several previously findings on the antimicrobial potential of the plant.

Morebise and Fafunso [36] for instance, reported that the saponin fraction obtained from the methanolic extract of *G. latifolium* leaves strongly inhibited the human pathogenic microbes that were tested, including *Bacillus cereus, Staphylococcus aureus, Candida albicans* and *Aspergilus niger*.

***Table 4.*** *Effect of leaf and stem extracts of Gongronema latifolium on Staphylococcus aureus.*

| Extracts | Mean Zone Diameter of Inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| ALEGL | 0 | 0 | 0 | 0 | 0 | 0 | 20 |
| MLEGL | 0 | 0 | 0 | 0 | 0 | 0 | 18 |
| AREGL | 0 | 0 | 0 | 0 | 0 | 0 | 22 |
| MREGL | 0 | 0 | 0 | 0 | 0 | 0 | 22 |
| CAL+REGL | 0 | 0 | 0 | 0 | 0 | 0 | 21 |
| CML+REGL | 0 | 0 | 0 | 0 | 0 | 0 | 19 |
| Concentration of the extracts (mg/ml) | 200 | 150 | 100 | 50 | 25 | -C | +C |

KEY: ALEGL – Aqueous leaf extract of *Gongronema latifolium,* MLEGL – Methanolic leaf extract of *Gongronema latifolium*
AREGL – Aqueous stem extract of *Gongronema latifolium,* MREGL – Methanolic stem extract of *Gongronema latifolium,* CAL+REGL – Combined aqueous leaf and stem extracts of *Gongronema latifolium,* CML+REGL – Combined methanolic leaf and stem extracts of *Gongronema latifolium,* - C Negative control, + C Positive control.

***Table 5.*** *Effect of leaf and stem extracts of Gongronema latifolium on Candida albicans.*

| Extracts | Mean Zone Diameter of Inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| ALEGL | 0 | 0 | 0 | 0 | 0 | 0 | 23 |
| MLEGL | 0 | 0 | 0 | 0 | 0 | 0 | 20 |
| AREGL | 0 | 0 | 0 | 0 | 0 | 0 | 23 |
| MREGL | 0 | 0 | 0 | 0 | 0 | 0 | 17 |
| CAL+REGL | 0 | 0 | 0 | 0 | 0 | 0 | 24 |
| CML+REGL | 0 | 0 | 0 | 0 | 0 | 0 | 21 |
| Concentration of the extracts (mg/ml) | 200 | 150 | 100 | 50 | 25 | -C | +C |

KEY: ALEGL – Aqueous leaf extract of *Gongronema latifolium,* MLEGL – Methanolic leaf extract of *Gongronema latifolium*
AREGL – Aqueous stem extract of *Gongronema latifolium,* MREGL – Methanolic stem extract of *Gongronema latifolium,* CAL+REGL – Combined aqueous leaf and stem extracts of *Gongronema latifolium,* CML+REGL – Combined methanolic leaf and stem extracts of *Gongronema latifolium,* - C Negative control, + C Positive control.

It also fails to agree with the work of Eleyinmi [15], who demonstrated the inhibitory activity of the methanolic leaf extract of G. *latifolium* against *salmonella enteritidis*, *salmonella cholerasius ser typhimurium*, *Pseudomonas aeruginosa* and *Listeria monocytogenes* and that of the aqueous leaf extract against *E. coli* and *P. aeruginosa*.

And contrary to the findings by Nwinyi *et al.* [37] who reported that the ethanolic leaf extracts show more inhibitory effect than aqueous extracts against *Escherichia coli* and *staphylococcus aureus*; neither the methanolic nor the aqueous extract was effective against the test isolates in this present study.

Furthermore, this current study also disagrees with the work of Adeleye *et al.* [38], who reported that the aqueous and ethanolic extracts together with the essential oil from *G. latifolium* leaves showed moderate inhibitory activity against some bacterial (*Staphylococcus* sp., *Escherichia coli, Shigella* sp., *Salmonella* sp., *Klebsiella pneumoniae* and

*Pseudomonas aeruginosa)* and fungal pathogens (*Candida albicans)* isolated from HIV patients in Lagos, Nigeria. They also found out that the inhibitory effects of the extracts were comparable to those of Ampicillin but less than those of Ciprofloxacin and Chloramphenicol; whereas in this study, the extracts failed to show any inhibitory activity against the test isolates when compared to the standard drugs used (Ciprofloxacin and Ketoconazole). Other previous works that reported otherwise include that of Edim *et al.* [39]; Enyi-Idor *et al.* [40]; Omodamiro and Ekeleme [41].

Lack of inhibitory activity by extracts of *G. latifolium* harvested from a private farm in Irolu, Ikenne Local Government Area of Ogun state, Nigeria in this present study is plausible and would require further investigation. It is worthy of note that antimicrobial activity results of the same plant part tested most of the time varied from researcher to researcher. Plausible reasons could be due to genetic differences between the microbial strains and the plant used.

On one hand, it is possible that the clinical isolates used in this present study were drug-resistant strains and as a result, were not sensitive to the extracts. Although, some medicinal plants have been reported to be active against drug-resistant pathogens [42] - [44]; but not *G. latifolium* growing in Irolu, Ikenne Local Government Area of Ogun state, Nigeria in this case.

On the other hand, the concentration of plant constituents of the same plant organ can vary from one geographical location to another depending on the age and time of harvest of the plant, differences in topographical factors, the nutrient concentrations of the soil, extraction method as well as method used for antimicrobial study. The relationship between chemical composition of plants and geographical location has been documented. Rao and Rout [45] reported a variation in the composition of essential oils of *Jasminum sambac. (L.)* collected from different parts of India. The composition of bee propolis has also been found to depend on geographical source [46]. It contains flavonoids and phenolic esters in temperate regions but these compounds are absent in propolis obtained from tropical regions. Although the phytochemical analysis of the test plant was not carried out in this present study, it is possible that certain active principles were present in very low concentrations or completely absent in this particular test plant obtained from Irolu in Ikenne Local Government Area of Ogun state, Nigeria, hence the lack of inhibitory activity. This could explain the reason behind the differences observed in this study when compared to that of Eleyinmi [15], Omodamiro and Ekeleme [41] and Ilodibia *et al.* [47] who obtained their plant materials from different geographical locations in the country: Akure, Ondo State, UmuahiaAlaocha, Abia State and Nibo, Anambra State, respectively. It is therefore hope that future researchers will carry out both qualitative and quantitative phytochemical screening of *G. latifolium* growing in Irolu, Ogun state to further elucidate the reason behind the lack of inhibitory activity as recorded in this current study.

Furthermore, it has long been known that extraction method can eliminate or modify the characteristics and potency of medicinal plants [48]. Another plausible reason for the non-inhibitory activity of *G. latifolium* in this present study, may be due to the fact that the plant extract is crude and may contain other constituents that do not possess antimicrobial property, and also may be due to the inability of the extract to diffuse through the gel because of its large molecules (stearic hinderance), against standard processed and purified antibacterial (Ciprofloxacin) and antifungal (Ketoconazole) agents with small and readily diffusible molecules.

## 4. Conclusion

Although the antimicrobial potential of extracts of *G. latifolium* have been demonstrated against various clinical isolates, the findings in this current study do not support claims made by different researchers in previously studies. Neither the aqueous and methanolic leaf extracts nor the stem extracts showed any inhibitory activity against the test isolates. Lack of inhibitory activity by extracts of *G. latifolium* growing in Irolu, Ikenne Local Government Area in this present study is plausible and would require further investigation. The findings here, also suggest that infection caused by these test pathogens will not respond favourably *in vivo* upon ingestion or topical application of the test plant extracts. This therefore suggests that, at the concentration tested, treatment failure and persistence of infection should be expected. Finally, due to differences in topographical factors of various geographical locations, nutrient concentrations of the soil, chemical composition, age and time of harvest of the plant, extraction method as well as method used for antimicrobial study; it is therefore important that scientific protocols be clearly identified, adequately followed and reported.

## Competing Interests

Authors have declared that no competing interests exist.

## References

[1] Giacometti, A., Cirioni, O., Schimizzi, A. M., Del Prete, M. S., Barchiesi, F., Giacometti, N., Drapeau, C. M. J., Nicastri, E., Martini, L., Ippolito, G., Moro, M. L. Surgical Site Infections in Italian Hospitals: A Prospective Multicenter Study. *Biomedical Complementary and Infectious Diseases,* 2000; 8 (34): 1471-1480.

[2] Batzing, B. L. Wound infections *In*: Horne, N. (Ed.). *Microbiology- An Introduction* Thomson Learning, Inc. Pacific Grove, USA". 2002; pp. 631-642.

[3] Kingsley, A. A proactive approach to wound infection. *Nurs. Stand.,* 2001; 15: 1-54.

[4] WUWHS. Principles of best practice: Wound infection in clinical practice. An International Consensus. A World Union of Wound Healing Societies' Initiative. London: MEP Ltd. http://www.*woundsinternational.com/clinicalguidelines/wound- infection-in-clinical-practice-an-international-consensus.* 2014; pp. 1-12.

[5] Emmerson, T. Presence of pathogens in wound and the delicate balance between a colonized wound and an infected wound; *Journal of Science,* 1998; 73 (1): 5-28.

[6] Bowler, H. D. Signs of Clinical Infection by Stable Poly-Microbial Communities*. Clinical Wound Management,* 2001; 84 (3): 179.

[7] Mordi, R. M., Momoh, M. I. Incidence of Proteus species in Wound Infections and Their Sensitivity Pattern in the University of Benin Teaching Hospital. *African Journal of Biotechnology,* 2009; 8 (5): 725-730.

[8] Sani, R. A., Garba, S. A., Oyewole, O. A. Antibiotic Resistance Profile of Gram Negative Bacteria Isolated from Surgical Wounds in Minna, Bida, Kontagora and Suleja Areas of Niger State. *American Journal of Medicine and Medical Sciences,* 2012; 2 (1): 20-24.

[9] Castello, M. C., Phatak, A., Chandra, N., Sharon, M. Antimicrobial activity of crude extracts from plant parts and corresponding calli of *Bixa Orellana L. Indian J. Exp. Biol.,* 2002; 1378-1381.

[10] Sofowora, A. "Traditional medicine and medicinal plants of Africa. Spectrum books Ltd. Ibadan, Nigeria. 1989, pp. 150-153.

[11] Balick, M. J., Paul, A. C. Plants that heals. In their plants, people and culture; the science of ethanobotany. New York, Scientific American Library. 1996; pp. 25-61.

[12] Ugochukwu, N. H., Babady, N. E., Cobourne, M., Gasset, S. R. The effect of *Gongronema latifolium* extracts on serum lipid profile and oxidative stress in hepatocytes of diabetic rats. *J. Biosci.,* 2003; 20 (1): 1-5.

[13] Okafor, J. C., Ejiofor, M. A. U. Strategies for enhancement of utilization of potential edible woody forest species of southeastern Nigeria. *The biodiversity of African plant*. Kluwe. The Netherlands, 1996; pp. 684-695.

[14] Hutchinson, J. The families of flowering plants. Oxford at the Clarendon Press. 1973; pp. 408-409.

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

[16] Nwanjo, H. U., Okafor, M. C., Oze, G. O. Anti-lipd peroxidative activity of *Gongronema latifolium* in Streptozotocin-induced diabetic rats. *Nigerian Journal of Physiological Sciences,* 2006; 21 (1-2): 61-65.

[17] Egbung, G. E., Atangwho, I. J., Iwara, I. A., Eyong, U. E. Micronutrient and phyto-chemical composition of root bark and twig extracts of *Gongronema latifolium*. *Journal of Medicine and Medical Sciences*, 2011; 2 (11): 1185-1188.

[18] Dalziel, J. M. The useful plants of Tropical West Africa, Crown agents for the colonies, London. 1937; pp 18-22.

[19] Morebise, O., Fafunso, M. A., Makinde, J. M., Olajide, O. A., Awe, E. O. Anti-inflammatory property of the leaves of *Gongronema latifolium*. *Phytotherapy Research*, 2002; 16: 75-77.

[20] Ugochukwu, N. H., Babady, N. E. Antioxidant effects of *Gongronema latifolium* in hepatocytes of rat models of noninsulin dependent diabetes mellitus. *Fitoterapia*, 2002; 73: 612-618.

[21] Chinedu, I., Uhegbu, F. O., Imo, C. K., Ifeanacho, N. G. Ameliorating effect and haematological activities of methanolic leaf extract of *Gongronema latifolium* in acetaminophen-induced hepatic toxicity in wistar albino rats. *International Journal of Biosciences*, 2013; 3 (11): 183-188.

[22] Onuoha, S. C., Chinaka, N. C. Carbon tetrachloride induced renal toxicity and the effect of aqueous extract of *Gongronema latifolium* in Wistar rats. *Drug Discov.,* 2013; 4 (11): 15-16.

[23] Imo, C., Uhegbu, F. O., Ifeanacho, N. G., Azubuike, N. C. Histological and hepatoprotective effect of ethanolic way extract of *Gongronema latifolium* berthin Acetaminophen induced hepatic toxicity in male albino rats. *Intern. J. Prev. Med. Res.,* 2015; 1 (4): 217-226.

[24] Omodiale, P. E., Enitan, S. S., Adejumo, E. N., Akinleye, W. A., Bella, A. S. Protective effect of aqueous root extract of *Gongronema latifolium* against paracetamol induced hepatotoxicity and chloroquine induced nephrotoxicity in rats. *International Journal of Herbal Medicine*; 2017; 5 (3): 115-120.

[25] Egba, S. I., Omeoga, H. C., Njoku, O. U. Oral administration of methanol extract of *Gongronema latifolium* (utazi) Up-Regulates cytokine expression and influences the immune system in wistar albino rats. *World Appl. Sci. J.,* 2014; 31 (5): 745-750.

[26] Akinnuga, A. M., Bamidele, O., Ekechi, P., Adeniyi, O. S. Effects of an ethanolic leaf extract of *Gongronema latifolium* on some Haematological parameters in rats. *Afr. J. Biomed. Res.,* 2011; 14: 153-156.

[27] Robert, A. E., Luke, U. O., Udosen, E. O., Ufot, S. U., Effiong, A. E., Ekam, V. S. Anti-diabetic and anti-hyperlipidemic properties of ethanol root extract of *Gongronema latifolium* (Utazi) on Streptozotocin (STZ) induced diabetic rats. *ARPN J. Sci. Technol.,* 2013; 3 (10): 995-998.

[28] Owu, D. U., Nwokocha, C., R., Obembe, A. O., Essien, A. D., Ikpi, D. E., Osim, E. E. Effect of *Gongronema latifolium* ethanol leaf extract on gastric acid secretion and cytoprotection in Streptozotocin-induced diabetic rats. *West Indian Med. J*., 2012; 9.

[29] Aka, P. A., Uzodinma, S. U., Okolo, C. E. Antidiabetic activity of aqueous and methanol extract and fraction of *Gongronema latifolium* (Aselepidaceae) leaves in Alloxan diabetic rats. *J. Appl. Pharmaceut. Sci.,* 2011; 1 (9): 99-102.

[30] Bagavan, A., Rahuman, A. Evaluation of larvicidal activity of medicinal plant extracts against three mosquito vectors. *Asian Pacific J. Trop. Med.,* 4: 29-34.

[31] Cheesbrough, M. "Biochemical tests to identify bacteria" *In*: Cheesbrough, M. (ed.). District Laboratory Practice in Topical Countries, Part 2. Cambridge University Press, Cape Town, South Africa. 2006; pp. 62-70, 180.

[32] Buchanan, R. E., Gribbons, N. E. Bergey's Manual of Determinative Bacteriology (8th edition). Williams and Wilkins Co. Baltimore USA. 1974.

[33] Miles, A. A., Misra, S. S. Bacterial counts. *Journal of Hygiene* (London), 1938; 38: 732.

[34] Slack, M. P. E. "Practical aspects of antimicrobial chemotherapy". *In*: Weatherall, D. J., Warrel, D. A. and Ledingham, J. G. G. (eds.). Oxford Textbook of Medicine. 1985; 1: 530-543.

[35] Shott, S. "Statistics for health professionals". Saunders, W. B., Co. Philadelphia, 1990; pp. 313-336.

[36] Morebise, O., Fafunso, M. A. Antimicrobial and phytotoxic activities of saponin extracts from two Nigerian edible medicinal plants. *Biokemistri*., 1998; 18 (2): 69-77.

[37] Nwinyi, O. C., Chinedu, N. S., Ajani, O. O. Evaluation of antibacterial activity of *Pisidium guajava* and *Gongronema Latifolium. Journal of Medicinal Plants Research*; 2008; 2 (8): 189-192.

[38] Adeleye, I. A., Omadime, M. E., Daniels, F. V. Antimicrobial activity of essential oil and extracts of *Gongronema latifolium* on bacterial isolates from bloodstream of HIV infected patients. *J. Pharmacol. Toxicol.,* 2011; 6 (3): 312-320.

[39] Edim, E. H., Egomi, U. G., Ekpo, U. F., Archibong, E. U. A review on *Gongronema latifolium* (Utasi): A novel antibiotic against *Staphylococcus aureus* related infections. *International Journal of Biochemistry and Biotechnology*, 2011; 1 (8): 204-208.

[40] Enyi-Idoh, K., Utsalo, S., Arikpo, G., Eja, M. Time-dependent evaluation of the antibacterial and phytochemical properties of *Vernonia amygdalina* and *Gongronema latifolium*. *The Internet Journal of Herbal and Plant Medicine*, 2012; 1 (2): doi: 10.5580/2cb2.

[41] Omodamiro, O. D., Ekeleme, C. M. Comparative study of in vitro antioxidant and antimicrobial activities of *Piper guineense*, *Curmuma longa, Gongronema latifolium, Allium sativum,* and *Ocimum gratissimum. World Journal of Medicine and Medical Science*, 2013; 1 (4): 51-69.

[42] Singh, K. V., Shukla, N. P. Activity on multiple resistant bacteria of garlic (*Allium sativum*) Extract. *Fitoterapia,* 1984; 55: 313-315.

[43] Jezowa, L., Rafinski, T., Wrocinski, T. Investigations on the antibiotic activity of *Allium sativum L. Herba pol*., 1996; 12: 3-13.

[44] Enitan, S. S., Olley, M., Uhunmwangho, S. E., Akele, Y. R., Ehiaghe, A. F., Enitan, C. B., Antibacterial activity of methanolic leaf extract of *Plukenetia conophora Mull. arg.* against selected bacteria isolated from urinary tract infection. *International Journal of Microbiology and Application*, 2014; 1 (1): 1-10.

[45] Rao, Y. R., Rout, P. K. "Geographical location and harvest time dependent variation in the composition of essential oils of *Jasminum sambac. (L.)* Aiton". *Journal of Essential Oil Research, JEOR*. 2013; http://findarticles.com/p/articles/mi_qa4091/is_200311/ai_n9325900.

[46] Evans, W. C. Trease and Evans Pharmacognosy. 15th edn. Elsevier. 2005; pp. 20.

[47] Ilodibia, C. V., Ezeja, I. J., Akachukwu, E. E., Chukwuma, M. U., Egboka, T. P., Emeka, A. N. Phytochemical Screening and Antimicrobial Effects of Aqueous and Ethanol Leaf and Stem Extracts of *Gongronema latifolium* Benth. *Research Journal of Botany*, 2015; 10 (2): 50-60.

[48] Amagase, H., Milner, J. Impact of various sources of garlic and their constituents on 7, 12-dimethylbenz (a) anthracene binding to mammary cell DNA. *Carcinogenesis,* 1993; 14: 1627-1631.