

Antibacterial Effect of *Gongronema latifolium* Leaf Extract on *Staphylococcus aureus* Isolates from Skin of Human Subjects in Lafia Metropolis

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Abstract

Antibiotics have been critical in the fight against infectious diseases. However, plants have also been used to treat diseases due to the presence of some chemical compounds (active ingredients) that possess medicinal properties. This study investigated the antibacterial effect of Gongronema latifolium (amaranth globe) leaf extract on Staphylococcus aureus isolates from the skin of human subjects in Lafia metropolis, Nasarawa State, Nigeria. Aqueous and ethanol leaf extracts of G. latifolium were screened for antibacterial activity against S. aureus isolates using the agar disk diffusion method. A total of forty (40) specimens were collected and S. aureus was isolated using standard biochemical methods. Hospital isolates had the highest lowest percentage sensitivities of 85.71 and 0.00% from 300 mg/mL ethanol and 200 mg/mL aqueous extracts, respectively. Also, restaurant isolates had the highest percentage and lowest sensitivities of 83.33 and 0.00% from 300 mg/mL ethanol and 200 mg/mL aqueous extracts, respectively. Both extracts showed significant differences observed in their concentration effects against S. aureus isolates from restaurant subjects (p<0.05). MIC was 75 mg/mL for the aqueous extract against isolates obtained from both sources and that for the concentration ranges of 300-9.375 and 200-6.25 mg/mL ethanol extract was observed to be 37.5 and 50 mg/mL, respectively. The result of the study showed that both extracts of G. latifolium have an inhibitory effect proportional to concentration on the test organism S. aureus isolates obtained from both subjects. Hence, ethanol extract of G. latifolium may potentially control skin respiratory and enteric infections caused by S. aureus.

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Introduction

Antibiotics have been critical in the fight against infectious diseases such as boils (abscesses), impetigo, cellulitis caused by bacteria and and other microorganisms particularly methicillin-resistant S. aureus (MRSA) has long been observed among hospitalized patients since 1960 [1]. These microbial infections, if not properly treated as a result of overuse or misuse of drugs or their components, could result in the development of resistant strains by the microorganisms that could pose a threat to society and are predicted to cause about 10 million deaths by 2050 [2, 3]. Plants can fight against diseases due to the presence of some chemical compounds (active ingredients) that give plants their medicinal properties and thus, maintain one's state of good health since they nourish and supply the body with important nutrients in cases of nutrient deficiencies or by attacking the causative organisms themselves [4]. Medicinal plants otherwise known as traditional medicine (TM) have been applied traditionally in the

treatment of certain health problems in every community and have been handed down from generation to generation its inception can be traced as far back as the origin of humankind [5, 6]. In developing countries including Nigeria, infectious diseases described as a product of host-parasite interaction from the ecological point of view, account for a high rate of health-related issues induced by pathogenic microorganisms [7].

Staphylococcus aureus, a gram-positive bacterium of clinical significance, constitutes a major public health threat, being one of the most common causes of hospital and community-acquired infections implicated with skin, respiratory tract, and gastrointestinal infections [8, 9]. The organism is frequently resistant to a wide variety of antibiotics. Infections caused by methicillin-resistant *S. aureus* (MRSA) and Vancomycin-resistant *S. aureus* (VRSA), leading strains of hospital-associated and communityassociated microbial pathogens are associated with

high morbidity and mortality rates, high treatment costs, and long stays in hospitals [10, 11]. Certain plants serve as sources of antimicrobial agents. The drugs utilized in contemporary society are products of research and development produced and approved by major pharmaceutical firms, among which the most important raw materials studied and developed are plant materials, particularly those with ethno pharmacological properties that occur in nature, currently being utilized by developing countries including Nigeria and about 80% of the world population [12, 13, 14]. There is a growing interest in plants, a promising alternative to ineffective antibiotics with antimicrobial activity for therapeutic usage. Scientists are actively involved in the screening of such plants to establish their potential antimicrobial effects and identify the compounds responsible for the antimicrobial properties [15, 16]. A wide range of herbal plant parts possess high medicinal properties termed "bioactive compounds" and are used as extracts for drug production. The different plant parts used include leaf, stem, barks, root, flower, fruits, seeds, and twig exudates [17, 18]. Scientists have shown a great deal of interest in plants with antimicrobial activity and increasingly becoming involved in the screening of such plants to establish their potential antimicrobial effects and identify the bioactive agents responsible for the antimicrobial properties [14, 19]. One plant that often possesses therapeutic properties is G. latifolium, a highly valued plant in the Western part of Africa particularly Nigeria, known as 'Utazi' in the southeastern and 'Arokeke' in the southwestern part of Nigeria, a tropical rainforest plant that belongs to the family Asclepiadaceae [20, 21, 22, 23]. G. latifolium plant possesses effective nutritional benefits and pharmacologic activities such as analgesic, antimicrobial, anti-bacterial, anti-plasmodial, antioxidant, anti-ulcer, anti-sickling, anti-asthmatic, antipyretic, hepatoprotective, hypoglycemic and antiproperties inflammatory [21, 24, 25]. The development of resistance in microorganisms and economic incentives although challenging, has led to research and development in the search for new antibiotics to maintain a pool of effective drugs at all times [26, 27]. While developing resistant strains is inevitable, the careless ways we administer and use antibiotics have greatly worsened the process, which result in the evolution of resistant strains [28]. Due to the rise of microbial-resistant strains to antibiotics, the active ingredient or component in plants is expected to be inimical to the growth of some microorganisms, especially the pathogenic ones, e.g. Escherichia coli, Staphylococcus aureus, Klebsiella sp., etc. [29, 30]. The study is aimed at determining the antibacterial potentials of leaf extract of G. latifolium on S. aureus isolates obtained from the skin of human subjects and it would be a useful source of scientific information to medical/health practitioners and the local populace on the benefit of G. latifolium for the treatment of Staphylococcal infections.

Materials and Methods

Collection of specimens

Specimens were obtained from individuals' skin (upper arm) using sterile swab sticks dipped in normal saline and kept intact inside sterile screwcapped tubes. These were obtained in two locations namely; Hospitals (Dalhatu Araaf Specialist Hospital, Kowa Hospital, and Agu Hospital) and three Restaurants within the Lafia metropolis, Nasarawa State. The swabs collected were transported to the microbiology laboratory within six hours of collection.

Isolation of *Staphylococcus aureus*

The specimens were streaked on Mannitol salt agar and incubated at 37° C for 24 h. Yellow colonies were picked and sub-cultured on nutrient agar plates to produce pure cultures. The cultured isolates were maintained on the plates at 4°C from where colonies were obtained for microscopic staining technique and biochemical tests to ascertain the organisms' identity. Microscopic technique and standard biochemical tests including gram staining, catalase, coagulase, and motility tests were performed on the pure cultures to identify the organisms [31]. The isolates were sub-cultured in nutrient broth at 37° C for 6 h before antibacterial testing [32].

Collection of plant material

Fresh leaves of *G. latifolium* were purchased from traditional dealers at a local market in Lafia, Nasarawa State. The plants were identified and authenticated at the Department of Botany, Federal University Lafia, Nasarawa State. A voucher specimen was deposited at the Herbarium for reference purposes.

Preparation of extracts

Fresh leaves of *G. latifolium* were properly washed and air dried at a temperature below 45° C until they became crispy and then pulverized into powder form using a sterile blender. Two solvents were used for the preparation of the extracts, namely distilled water and 60% (v/v) ethanol. 50 g per dried powdered leaves were soaked in 300 mL of distilled water and 60% (v/v) ethanol for 72 h. The extracts were filtered using Whatman No. 1 filter paper and placed in a water bath to obtain aqueous and ethanolic crude extracts. Extracts were kept at 4°C in a refrigerator for at least 24 h [33].

Preparation of disks impregnated with the extracts Disks of diameter 6 mm were perforated from sterile Whatman No. 1 filter papers using a perforator. These disks were placed in sterile aluminum foil paper and then sterilized using the autoclave at a temperature of 121°C and pressure of 15 psi (pounds per square inch). The sterilized discs wrapped in foil paper were immediately placed in the hot air oven and allowed to dry at 100°C for one hour. The disks were carefully removed and aseptically placed in labeled sterile Petri dishes using sterile forceps. 0.1 mL of each of two different extracts of two different the concentrations of 200 and 300 mg/mL respectively were impregnated on each disk contained in the labeled petri dishes owing to the given concentrations.

Ethanol (60% v/v) and distilled water which served as controls were impregnated on disks contained in separate petri dishes. The impregnated discs were left for 20 min for proper adsorption and then dried in the hot air oven at 25° C.

Antimicrobial susceptibility testing Disk agar diffusion method

The disk agar diffusion method as originally described by Bauer et al. [34] was adopted. Mueller-Hinton agar plates were prepared. Test organisms from growth on 24 h overnight nutrient broth cultures incubated at 37°C were suspended in normal saline solution (0.85% NaC1) and adjusted to match a turbidity of 0.5 McFarland Standard. 0.1 m1 each of the standardized suspensions was inoculated on the surfaces of Mueller-Hinton agar plates using sterile cotton swab sticks. These were further spread to obtain an unbiased even distribution of bacterial cells via the confluent streak method. The plates were left for about 30 min for proper adsorption of the suspension; the impregnated discs were aseptically transferred directly into the sensitivity plates with the aid of sterile forceps. Discs impregnated with ethanol (60% v/v) and distilled water which served as controls were also placed on the surfaces of the media. The extracts on the discs were allowed to adsorb into the media. After 30 minutes of application, the plates were inverted, and incubated at 37°C for 24 h, and the diameter of inhibition around the discs was measured in millimeters (mm) [34, 35].

Determination of minimal inhibitory concentration

The minimum inhibitory concentration of the various extracts was determined by the broth dilution method as described by the NCCLS [36, 37]. Two-fold serial dilutions of the extracts were made and subjected to broth cultures of isolates that were sensitive to any of the extracts. Different dilutions of both the aqueous and ethanolic 60% (v/v) extracts were prepared in Mueller-Hinton broth to obtain a concentration range of 200.0, 100.0, 50.0, 25.0, 12.5, and 6.25 mg/mL for the ethanolic extract and 300.0, 150.0, 75.0, 37.5, 18.75 and 9.375 mg/mL for both the aqueous and ethanolic extracts. Standard densities of the test organisms were inoculated into varying concentrations of the extracts and incubated at 37°C for 24 h. The minimum concentration that completely inhibited macroscopic growth was regarded as the minimum inhibitory concentration of the respective extracts [35].

Statistical analysis

The results were statistically analyzed using a paired Ttest. The Data are expressed as mean \pm standard deviation (mean \pm SD) using Statistical Package for Social Science (SPSS).

Results and Discussion

Antibacterial sensitivity of *G. latifolium* leaf extracts in two concentrations on *S. aureus* isolates from hospital specimens

The highest percentage sensitivity of 85.71% was obtained from 300 mg/mL ethanolic extract of *G. latifolium* while the lowest percentage sensitivity of 0.00% was obtained from 200 mg/mL aqueous

extract. Statistically, no significant difference was observed among the different concentrations of each extract (p>0.05). Also, in comparison of 300 mg/mL of both extracts statistically, there was no significant difference observed (p>0.05). The antibacterial sensitivity of the extracts on the various isolates is shown in Table 1.

Table 1: Antibacterial sensitivity of G. latifolium leaf
extracts on <i>S. aureus</i> isolates from Hospital specimens

	Diameter of zone of inhibition (mm)							
Isolate		Aqueous	Ethanolic extract					
	Control	300	200	Control	300	200		
		mg/mL	mg/mL		mg/mL	mg/mL		
HSP1	0 (R)	0 (R)	0 (R)	0 (R)	9 (S)	9 (S)		
HSP2	0 (R)	0 (R)	0 (R)	0 (R)	8 (S)	7 (S)		
HSP3	0 (R)	7 (S)	0 (R)	0 (R)	0 (R)	0 (R)		
HSP6	0 (R)	0 (R)	0 (R)	0 (R)	8 (S)	7 (S)		
HSP9	0 (R)	0 (R)	0 (R)	0 (R)	8 (S)	0 (R)		
HSP13	0 (R)	8 (S)	0 (R)	0 (R)	9 (S)	0 (R)		
HSP18	0 (R)	0 (R)	0 (R)	0 (R)	8 (S)	0 (R)		
%Sen.	0(0.00)	2(28.57)*	0(0.00)	0(0.00)	6(85.71)*	3(42.86)*		

HSP = Hospital isolate;%Sen. = Percentage sensitivity;R= Resistance; S= Sensitive; *= no significant difference

Table 2:	Anti	ibac	cterial se	nsitivity o	of G. la	<i>tifolium</i> leaf
extracts	on	<i>S</i> .	aureus	isolates	from	Restaurant
specimer	ıs					

	Diameter of zone of inhibition (mm)							
Isolate		Aqueous	extract		Ethanolic extract			
	Control	300 mg/mL	200 mg/mL	Control	300 mg/mL	200 mg/mL		
Rest3	0 (R)	7 (S)	0 (R)	0 (R)	0 (R)	0(R)		
Rest11	0(R)	7 (S)	0(R)	0(R)	8 (S)	0(R)		
Rest13	0 (R)	7 (S)	0 (R)	0 (R)	9 (S)	7 (S)		
Rest14	0 (R)	0 (R)	0 (R)	0 (R)	8 (S)	0 (R)		
Rest18	0 (R)	0 (R)	0 (R)	0 (R)	8 (S)	0 (R)		
Rest20	0 (R)	8 (S)	0 (R)	0 (R)	9 (S)	0 (R)		
%Sen.	0(0.00)	4(66.67)**	0(0.00)**	0(0.00)	5(83.33)**	1(16.67)*		

Rest= Restaurant isolate; **%Sen**. = Percentage sensitivity; **R**= Resistance; **S**= Sensitive; **= significant difference

Antibacterial sensitivity of *G. latifolium* leaf extracts in two concentrations on *S. aureus* isolates from restaurant specimens

The highest percentage sensitivity of 83.33 was obtained from the ethanolic extract of *G. latifolium*, while the lowest percentage sensitivity of 0.00 was obtained from 200 mg/mL aqueous extract. Statistically, a significant difference was observed among the different concentrations of each extract (p<0.05). In comparing 300 mg/mL of both extracts statistically, there was no significant difference between the extracts (p>0.05). The antibacterial sensitivity of the extracts on the various isolates is shown in Table 2.

Inhibitory effect of concentration ranges 300–9.375 mg/mL aqueous extract, 300–9.375 mg/mL and 200–6.25 mg/mL ethanolic extract of *G. latifolium* against *S. aureus* isolates from Hospital and Restaurant specimens

The highest inhibitory effect of the aqueous extract of *G. laitfolium* of concentration ranges from 300-9.375 mg/mL was observed at a concentration of 300 mg/mL of the extract against isolates obtained from hospital and restaurant subjects, while the lowest



inhibitory effect was observed at 75 mg/mL of the extract. The minimum inhibitory concentration (MIC) is therefore found to be 75 mg/mL of the aqueous extract. The MICs of the extracts against isolates obtained from both hospital and restaurant subjects are shown in Table 3, while clear zones of inhibition were observed in different dilutions of the ethanolic extract contained in test tubes.

Also, the highest inhibitory effect of the ethanolic extract of G. latifolium for the higher concentration range, 300-9.375 mg/mL was observed at 300 mg/mL of the extract against isolates obtained from hospital and restaurant subjects, while the highest inhibitory effect for the lower concentration range. 200-6.25 mg/mL was observed at 200 mg/mL of the extract. The lowest inhibitory effect for the higher concentration range, 300-9.375 mg/mL was observed at 37.5 mg/mL against the isolates obtained from hospital and restaurant subjects, while that for the lower concentration range, 200-6.25 mg/mL was observed at 50 mg/mL of the extract against the isolates. The MICs of the extracts against isolates obtained from hospital and restaurant subjects are shown in Table 3. In contrast, clear zones of inhibition were observed in different dilutions of the aqueous extract contained in test tubes.

Table 3: MICs of concentration range 300-9.375mg/mL aqueous extract, 300-9.375mg/mL aqueous extract, 300-9.375mg/mL ethanolic extract of G. latifoliumon S. aureus isolates from Hospital andRestaurant specimens

Isolate	MIC (300-9.375	MIC (300-9.375 mg/mL) ethanolic	· ·
Isolate	extract	extract	extract
HSP1	-	37.5	50
HSP2	-	37.5	50
HSP3	75	-	-
HSP6	-	37.5	50
HSP9	-	37.5	-
HSP13	75	37.5	_
HSP18	-	37.5	-
Rest3	75	-	-
Rest11	75	37.5	-
Rest13	75	37.5	50
Rest14	-	37.5	-
Rest18	-	37.5	-
Rest20	75	37.5	-

HSP = Hospital subject; **Rest**= Restaurant subject; - = no clear zone of inhibition

The study is focused on the antibacterial activity of aqueous and ethanolic extracts of *Gongronema latifolium* on *Staphylococcus aureus* isolates from hospital and restaurant subjects. The result of the study showed that aqueous and ethanolic extracts of *G. latifolium* have an inhibitory effect proportional to concentration on the test organism *S. aureus* isolates obtained from both hospital and restaurant subjects. This conforms with a study conducted by Ndubueze *et al.* [38], who showed that ethanolic and aqueous extract of *G. latifolium* on both *Pseudomonas aeruginosa, S. aureus, Klebsiella pneumoniae,* and *Escherichia coli* are concentration-dependent and effective techniques of extraction [38].

In Table 1, the activity of the extracts on isolates from hospital subjects was observed that 300 mg/mL ethanolic extract of G. latifolium had the highest percentage sensitivity of 85.71%, while 200 mg/mL of aqueous extract had the least percentage sensitivity of 0.00%. It was also observed that 200 mg/mL ethanolic extract had a percentage sensitivity of 42.86% while 300 mg/mL aqueous extract had a percentage sensitivity of 28.57%. This result conforms to a study conducted by Ilodibia et al. [39] which showed that a higher sensitivity/inhibitory effect was obtained from ethanolic extracts [39]. Statistically, no significant difference was observed between the different concentrations of the aqueous and ethanolic extracts on the isolates obtained from hospital subjects, and also between 300 mg/mL of both the aqueous and ethanolic extracts (p>0.05). This is because resistant strains of S. aureus isolates may have been developed from specimens obtained from the hospital subjects. These strains may have device mechanisms that resist the effects of phytochemical compounds such as saponins, tannins, glycosides, etc., which may inhibit the bacteria.

In Table 2, the activity of the extracts on isolates from restaurant subjects was observed that 300 mg/mL ethanolic extract had the highest percentage sensitivity of 83.33%, while 200 mg/mL of aqueous extract had the least percentage sensitivity. It was also observed that 200 mg/mL ethanolic extract had a percentage sensitivity of 16.67% while 300 mg/mL aqueous extract had a percentage sensitivity of 66.67%. This result conforms to a study conducted by Nwinyi et al. [40], who showed that higher sensitivity/inhibitory effect occurred in ethanolic extracts of Psidium guagava and G. latifolium showed in comparison with the aqueous extract. Statistically, a significant difference was observed between the different concentrations of aqueous and ethanolic extracts on the isolates from restaurant subjects (p<0.05) but no significant difference was observed between 300 mg/mL of the aqueous and ethanolic extracts (p>0.05) [40]. This inhibitory effect observed may be due to the phytochemicals such as tannins, glycosides, saponins, alkaloids, phytates, and other useful secondary metabolites present in the plant. These phytochemicals are known to have medicinal properties. This correlates with phytochemical studies conducted by Morebise et al. [41], who showed that the antibacterial properties of these plants depend on certain active ingredients, especially oils such as saponins, tannins, and flavonoids. G. latifolium contains saponins and these have been known to be responsible for its antioxidant and antimicrobial properties [41]. Commercially produced ethanol popularly referred to as alcohol and largely consumed by local communities has been shown to have been to possess potentials (i.e. dissolves organic substances) due to its ability to extract phytochemical compounds such as tannins, glycosides, saponins, phytates etc, that have inhibitory

effects against *S. aureus isolates* and other bacteria. According to Cheremisinoff [42], the reason for the potential abilities of ethanol is that ethanol and water are classified as polar solvents, although ethanol is less polarized than water [42].

In Table 3, the inhibitory activity of the aqueous extract on isolates from hospital subjects was observed that clear zones of inhibition occurred at concentrations of 300, 150, and 75 mg/mL respectively against all the isolates' sensitivity to the extract. The Minimum Inhibitory Concentration (MIC) was observed at 75 mg/mL. At this concentration, the S. aureus strains isolated from the hospital subjects were observed to be susceptible to the extract, even at much higher concentrations, while below this concentration level, the organisms developed resistance against the inhibitory activity of the extract. This does not correlate with research work conducted by Akani et al. [43], who showed that no MIC of aqueous extract of G. latifolium was observed against S. aureus but that MIC of Costusafer was observed at concentrations of 25 mg/mL against S. aureus and 50 mg/mL against E. coli. The inhibitory effect could be a result of the geographical location and environmental conditions (i.e. temperature, humidity) of the plant which can influence the potency constituents of the plants, as well as the isolates used for the study [43].

Also, in Table 3, the inhibitory activity of ethanolic extract on isolates from restaurant subjects was observed that clear zones of inhibition occurred at concentrations of 300, 150, 75, and 37.5 mg/mL, respectively against all the isolates sensitive to the extract for the higher concentration range, while for the lower concentration range, clear zones of inhibition occurred at 200, 100 and 50 mg/mL respectively for isolate sensitive to the extract. The MICs were found at 37.5 and 50 mg/mL for both lower and higher concentration ranges respectively. The MIC for the higher concentration obtained implies that the strains of S. aureus isolated are susceptible to the extract at that concentration and much higher concentrations. Below such concentration, the extract is ineffective against the isolates. The MIC obtained for the lower concentration range implies that the extract is active against the isolated strains, even at much higher concentrations. However, below the MIC obtained, the extract is inactive against the isolates due to resistant strains that may have been developed. This does not correlate with much lower values obtained in a study conducted by Obroh et al. [44] which showed that the MIC of Gongronema latifolium and Moringa oleifera was found between 12.5-25.0 mg/mL for S. aureus [44].

Conclusion

In conclusion, this study has shown that ethanolic extracts of the plants exhibited greater inhibitory effects on the organisms. The potential antibacterial effects of the plants could be enhanced by extracting with ethanol instead of water as applicable in the traditional practice.

Recommendations

From the findings of this research, we recommend the following:

- The populace particularly the local communities should be sensitized on the benefit of *G. latifolium* in the treatment of diseases.
- Leaves of *G. latifolium* should be highly encouraged as spices in our local delicacies due to their nutritional benefits.
- Solvents with potential abilities such as alcohol and others not harmful to the body should be used to produce liquors of *G. latifolium*.
- Researchers should conduct tests using different extract methods against *S. aureus* and other microbial pathogens such as protozoa, fungi, and other helminthic parasites.

Conflict of interests: The authors declare that they have no conflict of interests.

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