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Research Article

Antibacterial Activity of Leaves Extracts of *Gongronema latifolium* against Some Urinary Bacterial Isolates from Students of Federal University Dutsin-Ma

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ABSTRACT

Gongronema latifolium are known for various antimicrobial properties. Hence, this study assayed the *in-vitro* effects of methanolic, aqueous and ethanolic leaves extract of *Gongronema latifolium* on bacterial species isolated from urine samples of students from Federal University Dutsin-Ma. Thirty (30) urine samples were aseptically collected from the university students. Standard laboratory techniques were used for the isolation and identification of the organisms. The results showed the presence of *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.* Results of the phytochemical screening revealed the presence of tannins, steroids, alkaloids, glycosides and saponins in the leaves extract. Six (6) millimeter sterile discs were impregnated with methanolic, aqueous and ethanolic extracts at different concentrations and the test organisms were spread evenly on Mŭller -Hinton plate. All the test organisms showed sensitivity to both methanolic and ethanolic leaves extracts of *G. latifolium.* The aqueous extracts showed less activity against the isolates. The zone of inhibition ranges from 10mm to 15mm for methanolic extract, 8mm to 14mm for ethanolic extract and 0mm to 3mm for aqueous extract. The findings of MIC and MBC revealed that *S. aureus* appears more susceptible to the methanolic extract (lower MIC and MBC). Both *E. coli* and *P. aeruginosa* show similar patterns of susceptibility to methanolic and ethanolic extracts while aqueous extracts show no measurable or weaker effects. The study has therefore established the antibacterial activities of extracts of *G. latifolium* against the isolates and hence, there is need for further research to ascertain the various medicinal properties of *G. latifolium.*

Keywords: *Gongronema latifolium*; Minimum bactericidal concentration; Minimum inhibitory concentration; Phytochemical screening; Sensitivity

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INTRODUCTION

Gongronema latifolium (*G. latifolium*), commonly called 'Utazi' and 'Arokeke' in the South Western and South Eastern parts of Nigeria, and Bush Buck in English is a tropical rainforest plant primarily used as a spice and vegetable in traditional folk medicine (Ugochukwu and Babady, 2002; Ugochukwu *et al.,* 2003). It is a leafy green plant characterized by its large, glossy, dark green leaves. The leaves are heart-shaped with serrated edges and have a slightly bitter taste and aromatic flavour and are the most commonly used part of the plant. The plant can grow as a vine or shrub and can reach heights of about 5 meters when matured. During the last century, the practice of herbalism became mainstream throughout the world. Despite great advances observed

in modern medicine, plants still make an important contribution to health care. This is due to the recognition of the value of traditional medical systems, particularly of Asian origin, and the identification of medicinal plants from indigenous pharmacopeias, which have significant healing power. Medicinal plants are distributed worldwide, but they are most abundant in tropical countries In Brazil alone, about 80,000 species of higher plants were described which offer enormous prospects for discovering new compounds with therapeutic properties (Calixto, 2000). Various studies had reported that *G. latifolium* contains essential oils, saponins, and pregnanes among others (Morebise and Fafunso, 1998; Morebise *et al.,* 2002). Ugochukwu and Babady (2002), Ugochukwu *et al*. (2003), and Ogundipe *et al.* (2003) reported that aqueous and ethanolic extracts of *G. latifolium* had hypoglycaemic hypolipidemic, and antioxidative properties; while Morebise *et al.,*(2002) showed that it has anti-inflammatory properties. The leaves of Utazi are used in various culinary dishes in African cuisine, particularly in Nigeria. They are added to soups, stews, and sauces to impart a distinctive bitter taste and flavour to the dishes. Morebise and Fafunso (1998) stated that in traditional medicine, the leaves of *Gongronema latifolium* are believed to have various health benefits. They also have anti-diabetic, and digestive properties, among others. However, these uses require further scientific research and validation.

Global prevalence of infectious diseases caused by bacteria is a major public health problem (Tabil and Mahasneh, 2010). The bacterial agents including: *Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli* cause several human infections (Peirano, 2008). Recent emergence of antibiotic resistance and related toxicity issues limit the use of antimicrobial agents and is prompting a revival in research of the antimicrobial role of plants against resistance strains due to comparable safety and efficacy (Alviano and Alviano, 2009). It has been reported that *G. latifolium* has an antimicrobial activity against some species of microorganisms (Enyi-Idoh *et al.,* 2012). It is already known and documented that some of the aforementioned bacterial species are resistant to many antibiotics traditionally used to treat bacterial infections, such as penicillin and ampicillin. Therefore, this study was aimed at assaying the *in-vitro* effects of aqueous, ethanolic and methanolic leaves extract of *G. latifolium* on bacterial isolates from urine samples of students from Federal University, Dutsinma.

Materials and Method

Study Area

The study was carried out in Dutsinma Local Government Area of Katsina State.

Collection of Samples

The major raw material used in this work were freshly purchased *G. latifolium* leaves obtained from a central market in Kaduna State and put into a sterile polythene bag. The samples were air-dried and then made into a fine powder using a sterile mortar and pestle and transported aseptically to chemistry laboratory for phytochemical screening at Federal University Dutsinma. Urine samples were collected from students of Federal University Dutsinma into sterile plastic bottles and then taken to the microbiology laboratory for further analysis in the same University.

Analysis of Samples

Preparation of the media

Eosin methylene blue (EMB) agar, Cetrimide agar and Mannitol salt agar (MSA) were used for the cultivation of bacteria. All media were prepared according to the manufacturers' instructions.

Isolation and Identification of Bacteria

Inoculation

The container of each urine sample was gently swirled to mix the sample. Loopfuls of each sample were spread-inoculated on EMB, Cetrimide agar and MSA. All the plates were incubated aerobically at 35–37 °C for 18–24 hr.

Gram's staining

Smears of bacterial pure cultures were made on grease-free glass slides and placed on a staining rack in a sink. The smears were flooded with crystal violet, (a primary stain) and allowed for one minute and then rinsed with water. Gram's iodine was added as a mordant for around 60 seconds to allow the crystal violet to permeate the organisms and it was then rinsed with water. To remove the primary dye, ethanol was added for fifteen seconds, after which it was rinsed with water. Finally, the counter stain, (safranin) was added for 60 seconds, and rinsed with water and allowed to air dry. A drop of oil immersion was applied to the stained portion of the slide and viewed using oil immersion lens (100×) of the light microscope for various shapes of the bacteria. (Chessbrough, 2018).

Biochemical tests

Citrate utilization test

A citrate utilization test is used to determine whether or not a bacterium can use sodium citrate as a sole carbon source. The agar contains citrate, ammonium ions which serve as nitrogen sources, and bromothymol blue which acts as an indicator. The test organism was then inoculated into the citrate agar and incubated for 48 hours at 37°C. A positive result showed growth with a

color change from the initial green to a blue indicating that citrate has been utilized

Indole test

Bacterial colonies were inoculated unto the test tube containing Tryptophan soy broth and then incubated at 37^{o}C or 48 hours. Then 0.5 ml of Kovacs reagent was added to the test tubes and then gently. The production of indole is confirmed by the formation of red ring coloration on the surface of the medium, which indicated a positive reaction, while the negative results showed no colour change (Bachoon *et al.,* 2008).

Methyl red test

Glucose Phosphate broth was prepared and distributed into test tubes and further sterilized by autoclaving. The sterilized medium was inoculated with a pure culture of *Escherichia coli* and incubated at 37°C for 24 hours. After incubation, five drops of methyl red indicator were added to the medium and observed for the development of red colour (Koneman, 2016).

Voges Proskauer (VP) test

For the VP test, after incubation, 0.6ml of alpha naphthol and 0.2M of KOH solution per ml of culture broth media were added continuously and shaken properly and then kept in a slant position for about 1 hour and the result was noted down (Bachoon *et al*., 2008).

Motility test

The motility test is used to determine whether an organism is motile or non-motile. A sterile hanging drop slide was picked and Vaseline was applied around the cavity of the slide. A drop of the fresh broth culture of the test organism was placed on the center of Vaseline on the slide. The hanging drop slide was then used to cover the drop of the culture. The preparation was quickly turned so that a drop of the fresh broth was suspended. It was examined under both low and high power magnification for the presence or absence of motility.

Coagulase test

The test is used to identify *Staphylococcus aureus* by detecting coagulase enzyme production. A drop of plasma was placed on a sterile slide. A bacterial sample was collected with a sterile wireloop and mixed with the blood plasma. It is then observed for clumping within 10-15 seconds. It was positive when clumping occurred and negative when there was no clumping.

Catalase test

The test is used to determine if a bacterium produces the enzyme catalase. A drop of hydrogen peroxide was placed on a sterile glass slide and then a sterile loop was used to collect a bacterial sample and mixed with drop of hydrogen peroxide. It was catalase positive when bubbles erupted and catalase negative when no bubble was formed.

Preparation of McFarland Standard

Barium Sulphate (1% v/v solution of sulfuric acid) was prepared by adding 1 ml of concentrated H_2SO_4 in 99mLof distilled water. The solution was combined with 99.4ml of sulphuric acid solution to yield 1.096/v barium sulfide suspension. 0.1ml of each broth culture of *Escherichia coli* was then dispensed into the separate test tubes containing the sterile suspension. This served as the inoculums standard used for the antibacterial testing.

Antibacterial Susceptibility Test

Muller Hinton Agar (MHA) and Nutrient Broth (NB) were made following the manufacturers' instructions. Under aseptic conditions, the products were autoclaved at 121°C, cooled to room temperature, and then dispensed unto the sterile disposable Petri dishes. Before usage, the plates were kept at 4°C. The antibacterial activity of the *G. latifolium* leaves extracts was assessed using agar well diffusion method. Four wells were made on each agar plate using a sterile cork borer: 3 of the wells were for different extract concentrations and 1 for ciprofloxacin (which served as positive control). The extract was diluted in 3 sterile test tubes containing 2ml of Dimethyl Sulfoxide (DMSO) each labeled with concentrations of 300mg/ml, 200mg/ml, and 100mg/ml respectively and then a syringe was used to add 0.1ml drop in each of the well accordingly. The plates were incubated at the appropriate temperature (37°C) for a defined period of 24 hours. The diameter of the inhibition zones around the wells was measured and recorded as an indicator of antibacterial activity.

Phytochemical screening of the crude extract

Methanolic, ethanolic and aqueous leaves extracts of *G. latifolium* were subjected to qualitative tests for the identification of various active constituents like alkaloids, cardiac glycosides, steroids, saponins, and tannins.

Test for Alkaloids

Two drops of Mayes's reagent were added to the side of the test tube containing aliquots of the extracts. A green-coloured precipitate confirms the test as positive.

Test for Saponins

A drop of sodium bicarbonate was added in the test tube containing 50 ml extract of the sample. The mixture was vigorously shaken and kept for two minutes. If there is formation of a honey comb like froth indicates the presence of saponins (Ameya *et al*., 2018).

Test for Tannins

0.5g of extract was stirred with about 10mls of distilled water and then filtered. Few drops of 1% ferric chloride

solution were added to 2ml of the filtrate. The occurrence of a blue black, green or blue green precipitate indicates the presence of tannins

Test for Glycosides

Extract was mixed with 2mL of glacial acetic acid containing 2 drops of 20% $FeCl_3$. The mixture was poured into another tube containing 2mL of concentrated sulfuric acid. A brown ring at the interface indicates the presences of glycosides.

Test for Steroids

About 0.2 g of the powdered sample was dissolved in 2 ml of chloroform.0.2 ml of concentrated H2SO4 was carefully added to form a layer. A reddish-brown color at the interface between the layers indicates the deoxy-sugar characteristics of cadenolides which indicates the presence of steroid.

Determination of minimum inhibitory concentration (MIC)

A total of twelve (12) sterile test tubes were placed in a test tube rack for each organism. Two-fold dilutions were made using the different extracts, and mixed thoroughly. Each tube was inoculated with the test organism and incubated at 37°C for 24hours. Then MIC was taken to be the lowest dilution of the extract that had no visible bacterial growth (or turbidity).

Determination of minimum bactericidal concentration (MBC)

The minimum bactericidal concentration was determined all tubes in minimum inhibitory concentration that showed no visible growth were sub-cultured on freshly prepared Nutrient agar plates and incubated at 37°C for 24hours. The minimum bactericidal concentration (MBC) was regarded as the lowest concentration of the extract that killed the bacteria.

RESULTS

Morphological and Biochemical Characterization

Results of the colonial morphologies, gram reaction and biochemical tests of the bacteria are shown in Table 1. Findings of the investigations revealed the presence of both gram positive and negative bacteria

Phytochemical Screening Profile of the Extract

The methanolic, ethanolic and aqueous phytochemical screening conducted on *G. latifolium* extracts showed that it contained phytochemicals such as alkaloids, steroids, glycoside, tannins, and saponins. The results are presented in Table 2.

Antibacterial Activity of *Gongronema latifolium*

The antibacterial activities of leaves extracts *G. latifolium* were assessed against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* are shown in Table 3. The methanolic extract against *Staphylococcus species* (15mm) exhibited the largest zone of inhibition for indicating the highest antibacterial activity among the three extracts. The ethanolic extract showed a zone of 14mm, while the aqueous extract had the smallest zone of inhibition 10mm. For *E. coli* and *P. aeruginosa* their zones of inhibitions for both methanolic, ethanolic and aqueous extracts are shown in Table 3.

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

The minimum inhibitory concentration (MIC) of *Gongronema latifolium* leaves extract (methanolic, ethanolic and aqueous) was determined against the selected isolates. *Staphylococcus aureus* appears more susceptible to the methanolic extract (lower MIC and MBC) while the aqueous extracts show weaker or no measurable effects. The results are presented in Table 4.

Table 1. Morphological and Biochemical profile of some urinary bacteria pathogens

Cell Shape	Gr Rx	Ci	In	MR	VP	Mo	Co	Ca	Suspected Organism
Rod	-	-	+	+	-	+	-	-	*E. coli*
Rod	-	-	+	+	-	+	-	-	*E. coli*
Rod-	-	+	-	-	-	+	-	-	*P. aeruginosa*
Rod	-	-	+	+	-	+	-	-	*E. coli*
Rod	-	+	-	-	-	+	_	-	*P. aeruginosa*
Spherical	+	-	-	-	-	-	+	+	*S. aureus*
Rod	-	-	+	+	-	+	+	-	*E. coli*

Keys: += positive; -=negative

Table 2: Phytochemical Screening of Methanolic, Ethanolic and Aqueous Leaves Extract of *Gongronema latifolium*

Phytochemical Compound	Methanolic Extract	Ethanolic Extract	Aqueous Extract
Alkaloids	+	+	+
Tannins	+	+	+
Saponins	+	+	+
Glycosides	+	+	+
Steroids	+	+	+

KEY: + indicates the presence of phytochemicals

Table 3: Antibacterial Activity of the *Gongronema latifolium* leaves extract against the selected isolates

Bacterial Strain	Extract Type	Zone of Inhibition (mm)
Staphylococcus aureus	Methanolic	15
	Ethanolic	14
	Aqueous	3
Escherichia coli	Methanolic	13
	Ethanolic	11
	Aqueous	2
Pseudomonas aeruginosa	Methanolic	10
	Ethanolic	8
	Aqueous	0
Control	Ciprofloxacin	R

KEY: R=Resistance

Table 4. Minimum inhibitory concentration and Minimum bactericidal concentration (mg/mL)

Isolates	Methanolic		Ethanolic		Aqueous	
	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	75	150	150	300	300	-
E. coli	150	300	300	300	300	-
P. aeruginosa	150	300	150	300	-	-

DISCUSSION

Findings of this research carried out had revealed the presence of both gram-positive bacteria (*Staphylococcus aureus*) and gram-negative bacteria (*E. coli, and Pseudomonas aeruginosa)* from urine samples that were examined. The result for phytochemical analysis of *Gongronema latifolium* leaves extracts revealed the presence of several phytochemical compounds to include tannins, alkaloids, saponins, cardiac glycoside and steroids. The positive presence of tannins and other phytochemicals in the extracts aligns with previous study by Ugochukwu and Babady (2003) who reported the presence of saponins, tannins and other constituents in *Gongronema latifolium.* The presence of alkaloids in the extract is noteworthy, as alkaloids are known for their diverse biological activities. Some alkaloids have demonstrated antibacterial properties, and their presence in the extract could contribute to the observed antibacterial effects. Alkaloids have been associated with medicinal uses for centuries. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms (Banso, 2009). The results of investigations on the antibacterial effects of methanolic and ethanolic extracts of the plants revealed that extracts has antimicrobial on the test isolates. The aqueous extracts has weaker activity. Many studies have shown that methanolic extracts often exhibit strong antimicrobial activity due to the solvent's ability to extract a wide range of bioactive compounds (Ramos *et al*., 2008). The result of the study showed that aqueous, methanolic and ethanolic extracts of *Gongronema latifolium* have concentrations dependent inhibitory effect on the test organisms, *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa.* This is in agreement with the work of Ilodibia *et al.* (2015) who reported that both aqueous, methanolic and ethanolic extracts of *G. latifolim* possess antibacterial activities. Although, much research work has not been carried out on the antimicrobial activity of *G. latifolium* which has been used for ages by people of West Africa particularly Nigerians for dietary and medicinal purposes (Akubuo *et al.,* 2011), the results of this investigations has established both the presence of phytochemicals in the plants extracts and their antimicrobial activities on both gram positive (*S. aureus)*

and gram negative (*E.coli* and *P.aeruginosa*) bacteria. The antimicrobial activity revealed in this study also aligns with the work reported by Nwinyi *et al.,* (2009) in which they reported that *G. latifolium* has antimicrobial activities against *S. aureus* and *E. coli.* The aqueous extracts proved not to be effective against *P. aeruginosa* and does agree with the report of Oshodi *et al.,* (2004) which showed that the aqueous extract doesn't possess antimicrobial activity but those not conformed to the report of Hannah *et al.,* 2019 which showed that the aqueous extract possess inhibitory action against the test isolates. The ethanolic and methanolic extract of the leaves inhibited activities all the test organisms. This corroborates with the report of Adeleye *et al.* (2011).

The minimum inhibitory concentration and minimum bactericidal concentration results showed that both ethanolic and methanolic extracts had activity on the isolates. The aqueous extracts however, portrayed a little or no measurable effect. The concentrations were prepared from 300mg/ml, 150mg/ml, 75mg/ml and 37.5mg/ml. The inhibition and bactericidal effect of methanolic and ethanolic extracts against the test isolates suggest that the plant possesses broad spectrum antibacterial properties which could be used in the treatment of some infections due to these organisms. In the present study, the Gram-positive bacterium (*Staphylococcus aureus)* was more susceptible than the Gram-negative bacteria (*Escherichia coli and Pseudomonas aeruginosa*).

Conclusion

The study have demonstrated the antibacterial potential of *Gongronema latifolium* leaves extracts against *Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa*. The methanolic and ethanolic extracts exhibited significant inhibitory and bactericidal effects on the growth of these bacteria, suggesting their potential as natural antimicrobial agents. These findings support the traditional use of these plants in medicine and highlight their potential for developing new antibacterial therapies. Antibacterial effect of *G. latifolium* which is evident from this study explains the long history of the use of these plants in traditional medicine for the treatment of different bacterial infections such as the use in the treatment of stomach pains and infections. However, the full potential of these plants is dependent on the characterization of the biologically active components.

The work suggests further research in the use of the leaves extract of *Gongronema latifolium* and other herbs in the treatment of infections caused by different of bacterial organisms (both Gram-positive and Gram-negative).

Pharmaceutical industries are encouraged to consider extracting and purifying the active ingredients of *Gongronema latifolium* in the production of novel antibiotics which could be of help in curbing the menace of antibiotic resistance.

The traditional medicinal uses of the leaf extract are encouraged but traditional health practitioners must adhere to a dosage to avoid toxicity.

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