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Antibacterial Activity of *Gongronema latifolium* and *Ocimum gratissimum* Extracts on *E. coli* and *Salmonella typhi*

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The antibacterial activity of ethanolic and aqueous extracts of *Gongronema latifolium* and *Ocimum gratissimum* on *E. coli* and *Salmonella typhi* were determined using disc diffusion method. The antibacterial effect of aqueous extracts of *Gongronema latifolium* was negative on both test organisms and the aqueous extracts of *Ocimum gratissimum* showed very little zone of inhibition on *E. coli* (3.00mm) diameter and on *Salmonella* (2.00mm) diameter. Ethanolic extracts of both *Gongronema latifolium* and *Ocimum gratissimum* showed little zone of inhibition on *E. coli* and *Salmonella typhi*, which was not greater than 3.00 mm diameter. The minimum inhibitory concentration (MIC) evaluated with both ethanolic and aqueous extracts of *Ocimum* and *Gongronema* of various concentrations observed did not show any zone of inhibition. The results suggested that the extracts have low potential for use in the treatment of enteric diseases caused by pathogenic bacteria.

Key words: *Gongronema latifolium*, *Ocimum gratissimum*, antibacterial activity, *Escherichia coli* and *Salmonella typhi*.

Medicinal plants are distributed world wide, but they are most abundant in tropical countries[1]. It is estimated that today, plant materials are presently in or have provided the models for 50% Western drugs[2]. A relatively small percentage of medicinal plants are used as food by both humans and other animal species. It is possible that even more are used for medicinal purposes[3].

In Brazil, around 80,000 species of higher plants were described, which offer enormous prospects for discovery of new compounds with therapeutic properties[4]. Though most of the clinically used antibiotics are produced by soil microorganisms or fungi, higher plants have been a source of antibiotics[5].

Many plant extracts have shown to acquire antibacterial properties active against many microorganisms inside the body or *in vitro* for example, *Garcinia biflavonone* have been found to be active against a wide variety of microorganisms like *Salmonella, E. coli, Staphylococcus*[6]. *Garcinia* is also used in treatment of liver disorder bronchiotitis as a chewing stick and throat infections[7]. The root of *Nauclea latifolia* Smith (Rubiacea) has antibacterial activity against Gram-positive and Gram-negative bacteria and antifungal activity[6]. It is most effective against *Corynebacterium diphteriae, Streptobacillus* spp., *Streptococcus* spp., *Nesseria* spp., *Pseudomonas aeruginosa* and *Salmonella* spp.[7]. Some extracts of green pepper, garlic and onion have been noticed to inhibit the growth of S*higella dysenteria*, *Salmonella typhosa*[8]. Beneficially, they are used to impart colour and flavour and enhance, palatability and preservation on food[9]. In as much as it is, *Gongronema latifolium* (Asclepiadaceae) and *Ocimum gratissimum* (Lamiaceae) are traditionally

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dietary and medicinal herb in Nigeria. They are mainly consumed as spices, flavourants, stimulants and vegetables[10]. *Gongronema latifolium* and *Ocimum gratissimum* are used as vegetable for soup preparations, which exhibit hot and spicy taste and are consumed during cold season. It is claimed that spices and herbs assist in the contraction of the uterus in post-partum women[11].

Ocimum gratissimum is widely distributed in tropical and warm temperature. The plant is commonly used in folk medicine to treat different diseases such as upper respiratory tract infection, diarrhea, headache, fever, ophthalmic, skin disease and pneumonia[12]. The *Ocimum* oil is active against several species of bacteria (*Escherichia coli, Shigella, Salmonella* and *Proteus),* and fungi (*Trichophyton rubrum* and *Trichophyton mentagrophyte*[13]. Various sister species of *Ocimum gratissimum* e.g. *O viridea* Linn, *O. sauva* Linn, *O.basiliaim* Linn. and *O. canumsins* have been reported for their numerous medicinal uses. A screening of crude extracts of plants used in traditional medicine showed that the essential oil of *Ocimum gratissimum* inhibited the growth of *Herpetomonas Sammuel pessoai*[14].

Furthermore, *Gongronema latifolium* a secondary plant in humans may have reduced the risk of chronic diseases. An edible rainforest plant native of the South Eastern part of Nigeria had been widely used in folk medicine as a spice and vegetable for maintaining healthy blood glucose level[15]. G*ongronema* posses desirable hopping characteristics[16].

Ocimum gratissimum is rich in alkaloids, tannins, phylates flavonoids and oligosaccharides and it has tolerable cyanogenic glycoside content[17], which are the chemical compounds active against microorganisms. In other words, *Gongronema latifolium* is rich in alkaloids, tannins, phenol, flavonoid and saponin.

Although, since ages, herbs have served humans in many ways, such as drugs, foods and flavours. It was consequent upon these arbitrary use of spices and herbs like *Ocimum gratissimum* and *Gongronema latifolium* in folk medicine that necessitated this research of verifying *in vitro*, of the antibacterial activity of extracts of *O. gratissimum* and *G. latifolium* on some enteric organisms such as *E. coli* and *Salmonella typhi.* Also, to provide a guide or direction on the concentration of these herbs to the populace who use them for treatment of some enteric diseases and to prevent the side effects associated with high doses, by ascertaining whether these medicinal herb extracts could effect clear zones of inhibition on the test organisms *in vitro*.

MATERIAL AND METHODS

Collection and identification of plant material

The major plant materials used were freshly harvested leaves of *Gongronema latifolium* and *Ocimum gratissimum*. The plant materials were collected from Omoba Rural Community in Isiala Ngwa South Local Government Area, Abia State, Nigeria.

The plants were identified and classified by Prof. H.O. Edeoga (taxonomist) of the Department of Biological Sceicnes, Michael Okpara University of Agriculture, Umudike. *Ocimum gratissimum* belongs to the family Lamiaceae and commonly known as "Nchuanwu" and whereas *Gongronema latifolium* locally known as "Utazi" belongs to the family Asclepiadaceae[18]. The plant samples were deposited in the herbarium.

Preparation of extracts

The plant materials were thoroughly washed with clean water and were oven dried in the laboratory using moisture extraction oven at the temperature of 65°C and the drying lasted for 48 hours. The dried leaves were pulverized to powder using Thomas Wiley mill model ED5[19] from Soil Science Laboratory, National Root Crops Research Institute, Umudike (NRCRI).

The powder was weighed using Satorium AG Gottingen Electronics weighing balance. Weight of the total powder is as follows:

Gongronema latifolium 180.00g
Ocimum gratissimum 160.00g.

Ethanolic extract preparation

Twenty grams of each of the pulverized powder of the leaves of the plant materials were weighed using electronic weighing balance, and the weighed samples were soaked separately in a clean 250ml conical flask containing 200ml of ethanol. The mixtures were vigorously stirred with a stirrer. After 24hours elapsed with interval stirring, the mixture were filtered using

Whatmann No. 1 filter paper[20], into a clean beaker, and the filtrate concentrated to dryness by evaporation using steam bath at 100°C.

Aqueous extract preparation

Twenty grams of each of the pulverized powder of the leaves of the plant materials were also weighed and soaked separately into a clean 250ml conical flask containing 200mls of distilled water. The mixtures were stirred vigorously with a stirrer. After 24 hours elapsed with interval stirring, the mixtures were filtered using Whatmann No. 1 filter paper (2), into a clean beaker, and the filtrate were concentrated to dryness by evaporation using steam bath at 100°C. After evaporation the extracts were recovered and weighed:

Gongronema latifolium aqueous extract	12.0g
G. latifolium ethanol extract	17.0g
Ocimum gratissimum aqueous extract	10.0g
O. gratissimum ethanol extract	15.0g

The yields were recovered as percentage of the quantity of initial plant material 20.0g used[21].

$$\text{Yield}(\%)=\frac{\text{Yield in 'g'}}{20.0\text{g}}\times\frac{100}{1}$$

Test of purity and sterilization of materials

The dried extracts were exposed to ultraviolet rays for 24 hours and checked for sterility[22], by streaking on freshly prepared nutrient agar plates and was incubated at 37°C for 24 hours. It was observed that there was no organism or artifact to contaminate the sensitivity testing. All the glassware were thoroughly washed with detergent and rinsed properly with water drain dried, foiled and carefully packaged into the autoclave for sterilization at 121°C, 115atm. for 15 minutes.

Bacterial species confirmation

Clinical strains of microorganism used are *Escherichia coli* and *Salmonella typhi*. The bacteria stock of *Escherichia coli* coded E.COR 2002 obtained from the Microbiology Laboratory of Federal Medical Centre (FMC) Umuahia, Abia State, Nigeria. The isolate of *Salmonella typhi* were obtained from the Department of Biological Science Laboratory, Michael Okpara University of Agriculture, Umudike.

The identity of the two test organisms biochemical and morphological characteristics were further confirmed by standard method. The bacteria species were re-isolated in nutrient agar and were subcultured into nutrient and MacConkey agar slant[22].

Preparation of different concentration of extracts

The aqueous crude extracts were reconstituted by weighing 0.4g quantity of each extract into sterile test tubes and 4mls of distilled water were added to give concentration of 100mg/ml, also the ethanolic crude extracts were also reconstituted by dissolving 0.4g of each crude ethanolic extract weighed into a sterile test tube with 4mls of water and di-methyl sulphoxide (DMSO) to a concentration of 100mg/ml[23]. Also, dilutions of 160mg/ml, 80mg/ml, 40mg/ml and 20mg/ml concentrations were made.

Disc diffusion assays

The disc diffusion method as reported by[22] was adopted by the determination of the antibacterial activity of extracts. Whatmann No. 1 filter paper was used with slight modification. The filter papers were cut into circular discs 6mm in diameter using a perforator. The discs were treated by boiling for 30 minutes, to kill the chemical used in preserving the filter paper and also to avoid the inhibition of the antimicrobial actions of the extracts on test organism. After boiling, the disc were evaporated to dryness on steam bath for 3 hours until the discs were completely dried and were stored in a sterile vial bottle for use.

Determination of the absorptive capacity of the disc

The absorptive capacity of the disc was determined by placing it in a sterile glass petridish of predetermined weight of water. After 30 minutes, the disc was removed and the glass petridish with its content weighed again using electronic balance. The reduction in the weight of a glass petridish was recorded as the volume of water received or absorbed by the disc. The disc was found to absorb a maximum volume of 0.02ml solution. In other words, a total of 100mg/2ml solution of each of the reconstituted extracts were used to soak about 400 disc for 30 minutes and each absorbed 0.02ml of the extracts and the discs were allowed to dry and it was used immediately

and the remaining was kept in a sterile vial for further use.

Screening the extracts for antibacterial activity

Nutrient agars and MacConkey media were prepared by weighing 7g of the agar into 250ml distilled water in a clean flask; this was stirred and autoclaved at 121°C, 115atm for 15 minutes. The medium was cooled to 50°C and 20ml of the medium was poured into a sterile glass petridish and allowed to solidify[19]. The sterility of the medium was tested by allowing it to stay for 8 hours observing for contamination. The serial dilution of the bacterial culture was made using normal saline. A flamed sterile wire loop was used to take a loopful of the organisms from the pure culture plate into the test tube containing 10mls of normal saline from the tube, serial dilution of 10^{-1} to 10^{-5} was made, from the dilution of 10^{-3} and 10^{-4} a sterile swab stick was used to seed the nutrient media culture plates. In an inoculating room or chamber having set especially a sterile working bench, the already prepared discs were carefully transferred into an inoculated culture plates with the use of sterile forceps. Seven discs of the 100mg/ml concentration were used. As well as the disc from serial dilution of 160mg/ml, 80mg/ml, 40mg/ml and 20mg/ml concentration after appropriate labeling and dating, the plates were incubated for 24 hours at a temperature of 37°C.

After the incubation period, the zones of inhibitions were measured and recorded. The same procedure was repeated for four different extracts on the test organisms (*E.coli* and *Salmonella typhi*) up to three times[21].

Determination of minimum inhibitory concentration (MIC)

The Minimum Inhibitory Concentration (MIC) is the concentration giving the least inhibitory activity and below which there is no further inhibition. It was regarded as the concentration giving the lowest possible zones of inhibition. Using a sterile forcep, the paper discs of different concentration from (160mg/ml, 80mg/ml, 40mg/ml and 20mg/ml) and the 100mg/ml were placed at different portions of the inoculated plates labeled 10^{-1}-10^{-4} respectively. The plates were incubated at 37°C for 24 hours.

Control experiment using antibiotics

In order to compare the diameter of zone of clearing from the extracts and already standardized antibiotics, control experiment is necessary, and it was carried out aseptically[19]. This would encourage the prescription of either antibiotics or herbs with antimicrobial activities. The antibiotics used included ciprofloxacin (500mg), gentamycin (280mg) and chloramphenicol (250mg) (24). The working solution concentration was made 5mg/ml and 0.5 ml was added to 100 discs. These discs were placed on plates containing *E.coli* and *S.typhi* inocula, using sterile forceps. Then the plates were incubated at 37°C for 24 hours. The zones of inhibition were measured and recorded[21].

RESULTS AND DISSCUSSION

The yields of the plants extracts (ethanol and aqueous) were calculated and recovered as percentage of the quantity of initial powdered sample of plant materials shown in Table 1. The ethanolic extract of *Gongronema latifolium* gave the highest yield (8.5g) representing 117.0%. The next was the ethanolic extract of *Ocimum gratissimum*, which yielded 7.5g representing 15.0%. The percentage yield of aqueous extract of *Gongronema* and *Ocimum* were 12.0% and 10.0% respectively.

Antibacterial activity of different plant extracts showed the result of the sensitivity test of different organisms with the concentrated extracts using the paper disc diffusion method (Table 2). Aqueous extracts of *Gongronema latifolium* was completely resistant to both *E.coli* and *Salmonella typhi* whereas, aqueous extract of *Ocimum gratissimum* showed very little zone of inhibition on both *E.coli* and *Salmonella typhi*

Similarly, both ethanolic extracts of *G.latifolium* and *O.gratissimum* showed also little zone of inhibition not greater than 3.00mm diameter on both *S.typhi* and *E.coli*. On the other hand, 2mg/ml of gentamycin showed wider zones of inhibition on both *E.coli* and *S.typhi* which could not be compared to 100mg/ml concentration of the extract of plant materials. Also 5mg/ml of ciprofloxacin inhibited *E.coli* more than *S.typhi* and 5mg/ml of chloramphenicol was resistant to both *S.typhi* and *E.coli.*

The inhibitory activities of ethanolic extracts were found to be a little bit greater than

Table 1. Percentage yield of the crude extracts of *Ocimum gratissimum* and *Gongronema latifolium*

Plant species	Extract type	Weight of powered Sample used (g)	Weight of extract (g)	Percentage yield of extract (%)
G. latifolium	Aqueous	20.0g	6.0	12.0
	Ethanol	20.0g	8.5	17.0
O. gratissimum	Aqueous	20.0g	5.0	10.0
	Ethanol	20.0g	7.5	15.0

Table 2. Antibacterial activities of various extract

Bacteria species	*Gongronema latifolium*		*Ocimum gratissimum*	
	Ethanol	Aqueous	Ethanol	Aqueous
S. typhi	+	-	±	±
E. coli	±	-	+	+

+= Inihibition < (3.00min) diameter; ± = Trace; - = Resistant

Table 3. Antibacterial activities of various extract diameter of inhibition of Table 2

Bacterial species	*Gongronema latifolium*		*Ocimum gratissimum*	
	Ethanol	Aqueous	Ethanol	Aqueous
S. typhi	3.00	0.00	2.00	2.00
E. coli	2.00	0.00	3.00	3.00

Table 4. Minimum inhibitory concentration of ethanolic and aqueous extract of *Ocimum gratissimum* and *Gongronema latifolium*

G. latifolium										
	Ethanol					Aqueous				
	160	80	40	20	MIC	160	80	40	20	MIC
E. coli	0.00	0.00	0.00	0.00	Nil	0.00	0.00	0.00	0.00	Nil
S. typhi	0.00	0.00	0.00	0.00	Nil	0.00	0.00	0.00	0.00	Nil
O. gratissimum										
	Ethanol					Aqueous				
	160	80	40	20	MIC	160	80	40	20	MIC
E. coli	0.00	0.00	0.00	0.00	Nil	0.00	0.00	0.00	0.00	Nil
S. typhi	0.00	0.00	0.00	0.00	Nil	0.00	0.00	0.00	0.00	Nil

zone of inhibition of the different concentrations (Table 4). The non-inhibition of the aqueous extract of *G.latifolium* agreed on the work done by[25], on antimicrobial properties of some medicinal plants, which confers the absence of the activity against the *Salmonella* and *E.coli*. Also, a low level of activity at low extract concentrations may suggest that the concentrations of active constituent in the extracts are too low for many appreciable antibacterial activity[26].

In addition, low concentration of diffusible water soluble active constituents or excessive heating which often affects biologically active substances such as flavonoids, essential oils and other heterogeneous phytoconstituents present in the extract[25], might also influence their respective activity.

The result of this work corresponded to the findings of[27] who in their work found weak antibacterial activity of both ethanolic and aqueous extracts on the test organisms like *Salmonella* and *E.coli*. The effectiveness of an antimicrobial agent varies with the nature of the organism being treated since microorganisms differ markedly in their susceptibility. So, bacterial endospores are much more resistant to most antibacterial agents than the vegetative forms.

The work done by[28] showed that *O.gratissimum* and *G.latifolium* possess anti diarrheal activities which they investigated using disc diffusion method[29] supports the ethanomedicinal use of both *O.gratissimum* and *Xylopia aethipica* in the management of gastroenteritis. However, the presence of active principles in plants is influenced by several factors such as age of plant, method of extraction, extracting solvent and time of harvesting plant materials[30].

Furthermore, for the control experiment (Table 5), gentamycin had the widest zones of inhibition in both *S.typhi* and *E.coli* showing 12.00mm and 10.00mm diameter as shown in Plates 1,2,3 and 4 whereas ciprofloxacin against *E.coli* had diameter zone of inhibition of 8.00mm and 4.00mm on *S. typhi*. Chloramphenicol did not show any zone of inhibition on both test organisms.

Table 5. Diameter zone of inhibition (MM) of the control experiment using strandard antibiotics

Antibiotics	Bacterial Species	
	E. coli	*S. typhi*
Ciprofloxacin	8.00	4.00
Gentamycin	10.00	12.00
Chloramphenicol	0.00	0.00

CONCLUSION

The result obtained with the extracts opens perspectives to find more effective drugs of plant origin, which are less toxic and available for low socio-economic population in the treatment of enteric diseases caused by pathogenic bacteria. In this study, *G.latifolium* and *O.gratissimum* produced narrow inhibitory effect on *E.coli* and *S.typhi* in vitro. In other words, in vitro data may be helpful in determining the potential usefulness of the plant material. Further studies will be needed to purify the bioactive compounds of the ethanolic extract and characterize the aqueous fraction of these plants. Also their phytochemical mode of action should further be investigated.

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