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Evaluation of the Antibacterial Activity of *Gongronema latifolium* and *Costus afer* Leaf Extracts on *E. coli* (ATCC 29455) and *S. aureus* (ATCC 25923)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors played considerable roles in the research. Author NPA designed the study, performed the statistical analysis and supervised laboratory proceedings. Author CN carried out laboratory proceedings and literature searches while author IOH wrote the first draft of the manuscript as well as some literature searches and analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Man's use of medicinal plants in treating illnesses is as old as human existence and many plants have been used for this purpose because of their phytochemical constituents that prove many times to be antimicrobial. The antibacterial activity of the leaf extract of *Gongronema latifolium* and *Costus afer* on *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25923) was investigated using standard microbiological procedures of sub-culturing, identity confirmation, water and ethanol extraction of leaves and sensitivity testing via agar well diffusion method. Results revealed that *S. aureus* and *E. coli* were both inhibited by the aqueous extract of *C. afer* with zone diameter of 16 mm and 15 mm respectively as well as the ethanolic extract of *C. afer* with diameter of 18mm and 15 mm respectively. However, aqueous and ethanolic extracts of *G. latifolium* proved ineffective against the strains of *E. coli* and *S. aureus* used in this study. Results of minimum inhibitory concentration revealed MIC of the aqueous extract of *C. afer* on *E. coli* and *S. aureus* to be 50 $mgml^{-1}$ and 25 $mgml^{-1}$ respectively while that of the ethanolic extracts of *C. afer*

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was 12.5 $mgml^{-1}$ and 6.25 $mgml^{-1}$ for *E. coli* and *S. aureus* respectively. Comparatively *E. coli* showed high sensitivity to Ciprofloxacin, Gentamycin and Septrin with zones of inhibition of 37, 32 and 24 respectively and resistant to Ampicillin, Erythromycin and Tetracycline with zones of inhibition of 6, 0 and 0 respectively. *S. aureus* on the other hand proved sensitive to Ciprofloxacin, Erythromycin, Gentamycin and Tetracycline with zones of inhibition of 35, 28, 29 and 34 respectively and resistant to Ampicillin and Septrin with zones of inhibition of o respectively. This study has revealed that some positive effect can be achieved against *S. aureus* and *E. coli* infections using *C. afer* at good concentrations. Better results could also be achieved using ethanol as extracting medium with instead of water as is common practice.

Keywords: Antibacterial; E. coli; S. aureus; Gongronema latifolium; Costus afer.

1. INTRODUCTION

In today's world, plants used for therapeutic purposes constitute an effective source of both orthodox and traditional medicine; herbal medicine has been shown to have genuine use with over 80% of rural dwellers depending primarily on it for primary health care [1].

There is a growing interest in plants with antimicrobial activity and medicinal plants have been exploited for their phytochemical constituents which have been shown to be antimicrobial [2]. Scientists are increasingly becoming involved in the screening of such plants with the aim of establishing their potential antimicrobial effects and identifying the compounds responsible for the antimicrobial properties [3,4]. The synergistic effects of herbs such as ginger, garlic, turmeric and bitter cola on *Pseudomonas* spp. isolates have been recently confirmed [5].

Africa is a Continent endowed with a great diversity of plants. African medicinal plants rank highest among plants used for the investigations of antimicrobial properties. Africans and other humans have long used plants for the local treatment of infections such as cough, intestinal disorders, respiratory problems, sore throat, gonorrhoea, syphilis and rheumatic pains [3].

Green plants possess the broadest spectrum of synthetic activity and have been the source of many useful compounds. Nigeria is blessed with most of these green plants which have shown considerable pharmacological activities such as antioxidant, antimicrobial, anti-inflammatory, anticancer, antiviral, anti-allergic and vasodilatory properties [4]. In Nigeria, over 300 plants are used for treating various diseases including HIV/AIDS opportunistic infections such as pneumonia, diarrhea, typhoid fever, candidiasis, tuberculosis and other ailments [6,7,8].

Gongronema latifolium (Amaranth globe) is popularly known in Nigeria by the Igbos as 'utazi', the Efik/Ibibio people as 'Utasi' and the Yorubas as 'arokeke' or 'madumaro' [9]. Its parts particularly the leaves are used in various delicacies. On the other hand, Costus *afer* is a perennial rhizomatous herb, commonly called "spiral ginger", 'ginger lily' or 'bush cane' [10] 'eti' by the Isokosand Urhobos and 'bush sugar cane' or 'monkey sugarcane' in Warri and most parts of Delta State, Irekeomode in Yoruba, opete or okpete in Igbo, Kakii-zuwaa in Hausa and Mbritem in Efik. Most rural dwellers use this medicinal plant to treat upper respiratory tract and gastro-intestinal infections [11], gonorrhea [12]; and syphilis.

This study was carried out to evaluate the antibacterial activity of the leaf extract of *Gongronema latifolium* and *Costus afer* on *Staphylococcus aureus* and *Escherichia coli*.

2. MATERIALS AND METHODS

2.1 Collection of Leaf Samples and Preparation of Extracts

Fresh leave samples of *Gongronema latifolium* were bought from Mile 3 Market at Port Harcourt, Rivers State while the fresh leaves of *Costus afer* were harvested from matured trees in Umuode Osisioma Ngwa LGA of Abia State. The leaves sample were washed and air-dried. They were further dried in vacuum oven at 50°C for 10-15 h. The leaves were milled completely into powder by grinding. Two solvents were used for the preparation of the extracts, namely distilled water (Aqueous extract) and 70% ethanol [13]. For Aqueous extract and ethanolic extracts, one hundred and eighty gram (180 g) of dried milled leaves powder was soaked in 300 ml of sterile distilled water and 70% ethanol respectively for 5 days at 4°C. The two solutions were filtered

separately with Whatman filter paper into two 250 ml conical bottle flask and both centrifuged at 10,000 rpm for 5 min. The filtrates were dried at 50°C for 2 weeks until a constant dry weight of the extracts were obtained in a vacuum oven [13,14].

2.2 Collection and Confirmation of Test Organisms

The test organisms employed for the antibacterial activity screening include: *Escherichia coli* (ATCC 29455) and *Staphylococcus aureus* (ATCC 25923) which were obtained from Larhol Research laboratory, Benin City in Edo State, Nigeria.

The pure cultures were sub cultured on sterile nutrient broth test tubes and were incubated for 24 h at 37°C for further confirmation. The confirmation of bacterial isolates were carried out using morphological examination, and biochemical characterization which include; Gram staining, motility, protease, catalase, citrate, oxidase, coagulase, citrate, indole, methyl red, starch hydrolysis, sugar fermentation (sucrose, glucose and lactose) and pathogenicity tests which include; capsule staining and haemolysis.

2.3 Antibacterial Screening

2.3.1 Preparation of inoculums

Active cultures for screening were prepared by transferring a loopful of cells from the stock cultures to test tube of nutrient broth and were incubated without agitation for 24 h at 37°C [15].

2.3.2 Antibiotic sensitivity

The cultures were standardized by serially diluting with fresh nutrient broth to achieve a McFarland standard of 0.5 corresponding to a cell density of $1.5x10^8$ cfu ml^{-1}. These were used to inoculate the Mueller-Hinton plates by using 0.1 ml inoculum suspension to swab uniformly using sterile cotton wool.

2.3.3 Sensitivity using extracts

Sterile cork borer of 6.0 mm diameter were used to bore holes into the organisms seeded Mueller-Hinton agar plates and 0.3 ml of reconstituted extract of water and ethanol extract were aseptically dropped into each, appropriately labelled wells on the plates. Incubation was done at 37°C for 24 h before zones of inhibition were measured.

3. RESULTS AND DISCUSSION

The results shown on Table 1 reveal the zones of inhibition encountered when selected antibiotics were used on the two test organisms which in this case served as controls for comparative analysis.

The zones of inhibition show that *S. aureus* was non-sensitive or resistant to Ampicillin and Septrin with zones of inhibition of zero (0) respectively. *E. coli* on the other hand was resistant to Ampicillin, Erythromycin and Tetracyclin with zones of inhibition of 6, 0 and 0 respectively. Ciprofloxacin had the highest zone of inhibition on *E .coli* and *S. aureus* in conformation with [14] with diameters 37 mm and 35 mm respectively showing that ciprofloxacin has very high antibacterial effect on the two test organisms. Gentamycin also had a considerable effect on the two organisms tested with zones of 32 and 28 mm for *E. coli* and *S. aureus* respectively. Ampicillin proved to be the poorest antibiotic for both organisms.

The results for the antibacterial activity of the aqueous extract of *C. afer* are presented on Table 2. The results indicated that at concentration of 100 $mgml^{-1}$, *S. aureus* and *E. coli* were both inhibited by the aqueous extract of *C. afer* with zone diameter of 16 mm and 15 mm respectively while no zone of inhibition was shown on the extracts of *G. latifolium* for the two isolates. On the other hand, The results of the effect of the ethanolic extracts on Table 3 show that at concentration of 100 $mgml^{-1}$, *S. aureus* and *E .coli* were both inhibited by the ethanolic extract of *C. afer* with diameter of 18 mm and 15 mm respectively while no zone of inhibition was also shown on the extracts of *G. latifolium* for the two isolates.

Both results on Tables 1 and 2 imply that aqueous and ethanol extract of *G. latifolium* has no inhibitory activity against *S. aureus* and *E. coli*. This result of *G. latifolum* against the two test organisms was contradictory to the results from several other findings on the antibacterial activity of *G.latifolum* leaf such as those of [16] and [17].

The inhibition of both test organisms by aqueous and ethanolic extracts of *C. afer* conforms to the findings of [17]. The ethanolic extract showing higher inhibitory zones than the aqueous extract

as seen for *S aureus* may be due to the fact that the active ingredients are more soluble in ethanol than in water. As also seen on Tables 1 and 2, the extract also has higher zone of clearance against *S. aureus* (Gram positive bacteria) than *E. coli* (Gram negative bacteria) which conforms to [18].

The lack of inhibitory activity by extracts of *G. latifolium* used in this present study as against the works of [16] and [17] may be due to factors such as genetic differences between the microbial strains and the plants used, 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, the clinical isolates used in the studies could be drug-resistant strains and as a result may not be sensitive to the extracts.

Other reasons may include the, differences in topographical factors, and nutrient concentrations of the soil, drying and extraction method as well as method used for antimicrobial study.

The results of the minimum inhibitory concentration (MIC) of the extracts which proved effective on the test organisms (aqueous and ethanol extract of *C. afer*) as shown on Table 4 and Table 5 for concentrations of 50 $mgml^{-1}$, 25 $mgml^{-1}$, 12.5 $mgml^{-1}$, 6,25 $mgml^{-1}$, 3.135 $mgml^{-1}$, 1.56 $mgml^{-1}$, 0.78 $mgml^{-1}$ are quite unique.

The minimum inhibitory concentration (MIC) test showed that the MIC of the aqueous extract of *C. afer* on *E. coli* was 50$mgml^{-1}$ and 25$mgml^{-1}$ on *S. aureus* (Table 4). On the other hand, ethanol extract of *C. afer* on *E. coli* was 12.5$mgml^{-1}$ and 6.25 $mgml^{-1}$ for *S. aureus* (Table 5).

Table 1. Antibiotics susceptibility pattern of different antibiotics on agar well

Antibiotics	Concentrations(µg)	Organisms and their zone of inhibition (mm)	
		E. coli	***S. aureus***
Ampicillin	10	6	0
Ciprofloxacin	10	37	35
Gentamicin	10	32	28
Septrin	25	24	0
Erythromycin	10	0	29
Tetracyclin	25	0	34

Table 2. Effect of aqueous extract of *Costus afer* and *Gongronema latifolium on Staphylococcus aureus* and *Escherichia coli*

Plant extract	Organisms and their zones of inhibition (mm)	
	E. coli	***S. aureus***
C. afer	15±0.58	16±0.58
G. latifolium	0±0.00	0±0.00

Table 3. Effect of ethanolic extracts of *Costus afer and Gongronema latifolium* on *Staphylococcus aureus* and *Escherichia coli*

Plant extract	Organisms and their zones of inhibition (mm)	
	E. coli	***S. aureus***
C. afer	15±0.58	18±0.58
G. latifolium	0±0.00	0±0.00

Table 4. Minimum inhibitory concentration (MIC) of aqueous extract of *costus afer*

Test organism	Concentration (mg/ml)						
	50	**25**	**12.5**	**6.25**	**3.135**	**1.56**	**0.78**
E. coli	-	+	+	+	+	+	+
S. aureus	-	-	+	+	+	+	+

Table 5. Minimum inhibitory concentration (MIC) of ethanolic extract of *costus afer* on *E. coli* and *S. aureus*

Test organism	Concentration (mg/ml)						
	50	25	12.5	6.25	3.135	1.56	0.78
E. coli	-	-	-	+	+	+	+
S. aureus	-	-	-	-	+	+	+

Aqueous extract had lower zones of inhibition hence less active so the isolates are more susceptible to the ethanol extract than aqueous extract [19].

The results also imply that at a lower concentration, ethanol extract of *C. afer* inhibited *E. coli* and *S. aureus*.

4. CONCLUSION AND RECOMMENDATIONS

Despite the existing findings supporting the antimicrobial potential of extracts of *G. latifolium* against various clinical isolates, the findings in this current study do not support claims made by some researchers in previous studies. The result of this present work shows that leaf extract of *C. afer* has moderate effect against *S. aureus and E. coli* while *G. latifolium* has no inhibition against the strains of the organisms used in this study. This study has provided the basis for the use of *Costus afer* in the treatment of infections caused by *E. coli and S. aureus*. The antibacterial effects of the plants could be enhanced by extracting with ethanol instead of water as applied in the traditional practice.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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