Original Research Papers

In-vitro screening of antimicrobial activities of *Ocimum gratissimum* on clinical isolates

Abstract

This study was undertaken to evaluate the antimicrobial activities of crude ethanol and methanol extracts of the leaves of *Ocimum gratissimum* L. (scent leaf) on *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus auerus* and *Candida albicans*.The antimicrobial activities were carried out using agar well diffusion method. The Minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) of the plant extracts on the test isolates were determined by the agar dilution method. Ciprofloxacin and fluconazole (positive controls) were used in comparison with crude extract of ***Ocimum*** *O. gratissimum* leaves and also, Dimethyl sulfoxide (DMSO) was used as the negative control. The ethanolic extract of *O. gratissimum* showed antibacterial activity with the mean inhibitory zone diameter of 3 -7mm against *S. auerus*, 2 mm against *E. coli*, 2 – 12 mm against *K.pneumoniae*, 2 mm against *P.aeruginosa.* Ethanol and methanol crude extracts of *O.gratissimum* leaves showed no effect on *C.albicans*. ***Ocimum*** *O. gratissimum* extracts showed the lower antimicrobial activity than the commercially available antibiotics (ciprofloxacin and fluconazole). The minimum Inhibitory Concentration and Minimum Bactericidal Concentration of the extracts on the test organisms also increased in the following order; methanol < ethanol. Hence, this extract could only serve as antibacterial agent in the management of bacterial infection because it has no antifungal activities on *Candida* isolates used in this study.

Keywords: Antibacterial agent, Clinical isolates, *Ocimum gratissimum,* Agar well assay

INTRODUCTION

Surgical site infection has been a major public health concerns and some of these clinical isolates may either cause endogenous infection or auto-infection as in the case of wound infection [1; 2]. A wound is a lesion on the skin which accompanied by the exposure of subcutaneous tissue following the loss of skin integrity that allow microorganisms to strive and cause infection [3]. This open lesions are susceptible to cause infection with proliferation of microorganism on human host or environment [4].

Infection occurs when one or more invaders or foreign bodies penetrate the host and start to multiply in large number, attack and harm the host [5].One of the most serious cases of wound infection is known Surgical site infection which constitute a global health problem both health and human term [6]. Some of Organisms commonly found in infected wound regions include Gram positive cocci such as *S.aureus, Streptococcus spp,*

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Gram negative bacilli mostly *Enterobacter, E. coli, Proteus spp, P. aeruginosa, Klebsiella spp* and *candida species* are also isolated in immunocompromised individuals [7].

There is increasing number of studies on multiple antibiotics resistance, making the need for exploring possible alternatives a necessity[8]. Herbs have been very important therapeutic agent in the past for the treatment of infectious diseases [9]. For instance, traditional treatment of circumcision wounds in those days locally prepared with herbs [11;12].

Ocimum *O. gratissimum* is an aromatic medicinal plant which belongs to the family *Lamiaceae* with genus *Ocimum* and species *gratissimum* [15]. It is a natural inhabitant of the tropical and warm regions such as India including sub-Sahara Africa especially in Kenya and Nigeria [13; 14]. In Nigeria, they called it different names, like "Efinrin" in Yoruba; "Nchoanwu" or "Ahuji" in Igbo; "Aramogbo" in Edo and"Daidoya" in Hausa[14].

MATERIALS AND METHOD

Source and maintenance of test organisms

The clinical isolates of *E. coli, K. pneumoniae, P. aeruginosa, S. aureus, and C. albicans* were obtained from Medical microbiology department at NAUTH while Pure cultures of standard strains of *S. aureus* (NCTC 6571) and *E. coli* (NCTC 10418), (control organisms), were obtained from Department of Pharmaceutical Microbiology and Biotechnology, Nnamdi Azikiwe University, Awka, Nigeria.

Collection and identification of plant sample

O. gratissimum leaf was bought from local market in Awka, Anambra State, Nigeria and was identified by Mr Paulinus Ugwuoke, in the Department of Botany, Nnamdi Azikiwe University, Awka.

Preparation of Extraction

O. gratissimum leaf was allowed to air-dried after washing with distilled water at room temperature, it was grounded into fine powder with a mechanical grinder. 200 g of *O. gratissimum* grounded powder was weighed and dissolved into each 95% ethanol and methanol respectively for three days. After it's dissolution, the solution of the plant extracts were filtered through No. 1 What- man filter paper and the

resulting solutions dried in a rotary evaporator at 60^{o}C. The dried extracts recovered were placed in sterilized screw-capped bottles and kept in refrigerator at 4^{o}C.

Phytochemical Analysis

The phytochemical analysis of methanol and ethanol extract of *O. gratissimum* (scent leaf) was carried out using standard methods as described by [15].

Preparation of stock solutions

stock solutions were prepared by dissolving 400mg of the extracts in 2mL of DMSO (to make 200mg/mL). Also, in the determination of the minimum inhibitory concentrations of the plant extracts, stock solutions were prepared by dissolving 2000 mg/mL in 4mL of DMSO (to make 500mg/mL). These were stored in screw capped tubes at 4°C for further use.

In-vitro screening of antimicrobial activities of the plant leaf extracts.

The agar well diffusion assay method described by [16], was used to evaluate the antibacterial and antifungal activities of the crude extracts of *O. gratissimum* against the test microorganisms. Dilutions of 100, 50, 25, 12.5, and 6.25mg/mL were prepared from the 200mg/mL stock solution of the plant extracts in a 2-fold dilution process. Twenty (20) mL of molten Mueller Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) (for bacterial and fungal isolates respectively) were poured into sterile Petri dishes (90 mm) and allowed to set. Standardized concentrations (McFarland 0.5) of overnight cultures of test isolates were swabbed aseptically on the agar plates and holes (6mm) were made in the agar plates using a sterile metal cork-borer. Twenty (20µl) of the various dilutions of the plant extract and control were put in each hole under aseptic condition, kept at room temperature for one hour to allow the agents to diffuse into the agar medium and incubated accordingly. Ciprofloxacin (5µg/mL) and fluconazole (50µg/mL) were used as positive controls in the antibacterial and antifungal evaluations respectively; while DMSO was used as the negative control. The MHA plates were then incubated at 37^{o}C for 24 hours, and the SDA plates were incubated at room temperature (25-27^{o}C) for 2-3days. The inhibition zones diameters (IZDs) were measured and recorded. The size of the cork borer (6mm) was deducted from the values recorded for the IZDs to get the actual diameter.

This procedure was conducted in triplicate and the mean IZDs calculated and recorded.

Determination of Minimum Inhibitory Concentration (MIC) of the plant leaf extracts on test isolates

The Minimum inhibitory concentration (MIC) of the plant extracts on the test isolates were determined by the agar dilution method as described by [17].

The stock solutions (500mg/mL) were further diluted in a 2-fold serial dilution to obtain the following concentrations: 250, 125, 62.5, 31.25, 15.625, 7.8125, 3.91, 1.95, and0.98 mg/mL. Agar plates were prepared by pouring 4 mL of molten double strength MHA and SDA (for bacterial and fungal isolates respectively) into sterile Petri plates containing 1mL of the various dilutions of the extract making the final plate concentrations to become 100, 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78, 0.39, and 0.19 mg/mL

The test isolates which were grown overnight in broth were adjusted to McFarland 0.5 standard and streaked onto the surface of the agar plates containing dilutions of the extract.

The MHA plates were then incubated at 37^{o}C for 24 hours and the SDA plates were incubated at room temperature (25-27^{o}C) for 2-3days, after which all plates were observed for growth.

The minimum dilution (concentration) of the extracts completely inhibiting the growth of each organism was taken as the MIC. This procedure was conducted in triplicate.

Determination of Minimum Bactericidal/Fungicidal Concentrations (MBCs/MFCs) of the plant leaf extracts on test isolates

The MBC/MFC of the plant extracts was derived by sub culturing portions of the agar from plates that showed no growth in the tests for determination of MICs. These agar portions were transferred respectively into plates containing freshly prepared MHA and SDA .

These plates were incubated at 25-27^{o}C for 2-3 days and were observed daily for growth. The absence of growth at the end of incubation period signifies total cell death. The minimum concentration of the plant extracts that produces total cell death is taken as the MFC.

The antibacterial effectiveness of the leaf extracts at concentrations of 200mg/mL, 100mg/mL, 25mg/mL and 6.25mg/mL as compared with the activity of ciprofloxacin was shown in Table1. *K. pneumonia* was only found to be susceptible to all the concentrations of crude ethanol extract of *O. gratissimum* leaves with mean zone of inhibition ranging between 2-12mm . Typed isolate of *E. coli* and clinical isolate of *C. alblicans* were found to be resistant to all the concentrations of crude ethanol extract of *O.gratissimum* while typed and clinical isolates of *E. coli, K. pneumoniae , P. aeruginosa and C. alblicans* were found to be resistant to all the concentrations of crude methanol extract of ***Ocimum*** *O. gratissimum* leaves (Table 2). However, the commerical antibiotics (Ciprofloxacin) showed greater antibacterial activity compared to its corresponding extract of ethanol and methanol.

Table 3 shows the MIC and MBC of the ethanol and methanol extract of plant and ethanol extract exhibited the highest activity against clinical isolate of *K.pneumoniae,* then, *S.auerus* followed by *P.aeruginosa* and *E.coli.*

Table 1: Susceptibility Testing of Ethanol Extract of *O. gratissimum* leaves showing the Inhibition Zone Diameters (IZDs)(mm) produced by clinical bacterial and yeast isolates

Concentrations of plant extract	200mg/mL	100mg/mL	50mg/mL	25mg/mL	12.5mg/mL	6.25mg/mL	Cipro floxacin	DMSO
Tested organisms	IZD(mm)	IZD(mm)	IZD(mm)	IZD(mm)	IZD(mm)	IZD(mm)	5ug/mL	
S.aureus (NCTC6571)	4	2	1	0	0	0	9	0
E.coli (NCTC10418)	0	0	0	0	0	0	12	0
S.auerus	7	5	3	0	0	0	12	0
E.coli	2	0	0	0	0	0	22	0
K.pneumoniae	12	10	8	6	4	2	10	0
P.aeruginosa	2	0	0	0	0	0	12	0
							Fluco nazole 50ug/mL	
C.albicans	0	0	0	0	0	0	24	0

0: Resistant

Table 2: Susceptibility Testing of Methanol Extract of *O. gratissimum* leaves showing the Inhibition Zone Diameters (IZDs)(mm) produced by Clinical bacterial and yeast isolates

Concentrations of plant extract	200mg/mL	100mg/mL	50mg/mL	25mg/mL	12.5mg/mL	6.25mg/mL	Cipro floxacin 5ug/mL	DMSO
Tested organisms	IZD(mm)	IZD(mm)	IZD(mm)	IZD(mm)	IZD(mm)	IZD(mm)		
S.aureus (NCTC6571)	4	2	1	0	0	0	9	0
E.coli (NCTC10418)	0	0	0	0	0	0	12	0
S.auerus	8	6	4	2	1	0	12	0
E.coli	0	0	0	0	0	0	22	0
K.pneumoniae	0	0	0	0	0	0	10	0
P.aeruginosa	0	0	0	0	0	0	12	0
							Fluco nazole 50ug/mL	
C.albicans	0	0	0	0	0	0	24	0

0 : Resistant

Table 3 : MIC and MBC of the extracts against tested organisms.

Isolates	Ethanol		Methanol	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
S.auerus (NCTC6571)	50	50	50	50
E.coli (NCTC10418)	-	-	-	-
S.auerus	25	25	12.5	25
E.coli	200	200	-	25
K.pneumoniae	3.125	3.125	-	200
P.aeruginosa	200	200	-	-
C.albicans	-	-	-	-

- No Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

DISCUSSION

The antibacterial effectiveness of the leaf extracts at concentrations of 200mg/mL, 100mg/mL, 25mg/mL and 6.25mg/mL as compared with the activity of ciprofloxacin. *K. pneumoniae* was only found to be susceptible to all the concentrations of crude ethanol extract of *O. gratissimum* leaves with mean zone of inhibition ranging between 2- 12mm . Typed isolate of *E. coli* and clinical isolate of *C.alblicans* were found to be resistant to all the concentrations of crude ethanol extract of *O. gratissimum* while typed and clinical isolates of *E. coli, K. pneumoniae , P. aeruginosa* and *C. alblicans* were found to be resistant to all the concentrations of crude methanol extract of ***Ocimum*** *O. gratissimum* leaves. This present study was not in line with the work of [16],who shown the various activities of *O. gratissimum* extract tested *in vitro* against some bacterial and fungal isolate.

However, the commerical antibiotics (Ciprofloxacin) showed greater antibacterial activity compared to its corresponding extract of ethanol and methanol. This is possibly due to the failure of the active ingredient to

dissolve in it and all the sensitive extracts were more at higher concentrations than lower concentration. Also, the comparsion of the activity of the plant extract with conventional antibiotics, such as ciprofloxacin and fluconazole confirmed reports by other workers [18], that constitutional antibiotics are more active than plant extracts. The ethanol extract showed the highest activity against clinical isolate of *K.pneumoniae,* then, *S.auerus* followed by *P.aeruginosa* and *E.coli.* The ethanolic extract was both bacteriostatic and bacteriocidal at a concentration of 3.125mg/ml, 25mg/ml, 50mg/ml and 200mg/ml on the clinical isolate of *K.pneumoniae,* clincal isolate of *S.auerus ,* typed isolate of *S.auerus,* clinical isolates of *P.aeruginosa* and *E.coli* respectively while the methanolic extract was both bacteriostatic and bacteriocidal at a concentration of 50mg/ml on the typed isolate of *S. auerus*.

Conclusion

This study reveals that ethanolic extract of *O.gratissimum* was observed to be more susceptible to *K.pneumoniae* at all concentrations, thus showing higher antibacterial activity than the methanolic extract. *C. alblicans* was found to be resistant at any concentrations of crude extract of *O. gratissimum* leaves.

Consequently, failure of some of the extract to exert antimicrobial effect on the test organism is not enough to conclude that the leaves do not contain substances that can exert antimicrobial activity against the test organism because the potency of extract depends on method used to obtain the extract.

Further attention and research to identify the active components responsible for the plant antifungal activity should also be carried out.

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