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Antibacterial activity of the leaf extract of *Ocimum gratissimum* (Fam. Labiatae)

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Abstract
The antibacterial activity of the leaf extract of *Ocimum gratissimum* was evaluated against isolates of *E. coli, S, aureus. P. aeruginosa and B. subtilis* using agar-diffusion assay. Extraction was achieved using maceration method and the extract further fractionated by column chromatography. The antibacterial properties of both the extract and the column fractions were separately determined against the test isolates. Results of the antibacterial screening revealed that the extract of *O. gratissimum* exhibited some degree of antibacterial activity against isolates of *E. coli* and *B. subtilis* but showed no activity against isolates of *S. aureus and P. aeruginosa.* The column fractions (F_A- F_E), however, showed no antimicrobial activity against the test microorganisms. The probable antimicrobial activity of the plant is likely to be associated with its volatile oil content.

Key words: *Ocimum gratissimum,* antibacterial activity, leaf extract, column fractions.

Introduction

In modern orthodox medicine, plants are also recognised as important sources of drugs. They may be used directly as medicinal agents, or as sources of purified biologically active substances. In the extraction of drugs from plants, the required part of the plant is generally pounded and soaked in cold or warm water, and the infusion may be used either internally or externally. Investigations into the antimicrobial activities of plants have received tremendous attention in recent times.

Ocimum gratissimum is a species of the family – Labiatae – that is native to India (Troupin, 1985). The plant is mainly found around the gardens and is cultivated in Africa for its medicinal uses and as food flavour. It has been found to contain thymol (Papageorgiou, 1979), xanthones (Suzuki, *et al,* 1981) tetraoxygenated xanthones and secoiridoids (Oliver – Bever, 1986) all of which are known to possess biological activity. The leaves of the plant yield aromatic volatile oil which consists mainly of thymol and eugenol. Terpenes and

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lactones have equally been found in the plant (Iwu, 1993). The whole plant is used as an antibacterial agent throughout West Africa (Iwu, 1993) and an infusion of the leaves is used as a remedy for fever and as a diaphoretic while the oil mixed with alcohol is applied as a lotion for skin infections and taken internally for bronchitis. The oil is used externally to keep mosquitoes away although negative results have been reported (Oliver-Bever, 1986). *Ocimum gratissimum* is febrifuge and also used as a stomachic and laxative (Hutchinson, and Dalziel, 1954). The antimicrobial, insect repellent and anthelminthic activity of the essential oil have been reported (Sofowora, 1982). The plant has equally been reported to cure diseases like upper respiratory tract infections (Bouquet, 1969), diarrhoea (Said, *et al*, 1974) headache (Debray, *et al*, 1971) and skin disease (Bouquet, and Debray, 1974). The present study seeks to evaluate the antimicrobial activity of the leaf extract of *O. gratissimum* against isolates of some pathogenic Gram negative and Gram positive bacteria.

Materials and Methods

Plant Materials

The leaves of *O. gratissimum* were collected from Nsukka in Enugu State between March and June, 1999. The identity of the plant was authenticated by Messrs J.M.C. Ekekwe and A.O. Ozioko both of the Botanical Garden, University of Nigeria, Nsukka and voucher specimen has been deposited in the University herbarium. The leaves were dried in an oven at 45 -50 °C for 24 hours. The dried leaves were pulverised and stored at room temperature until used.

Test Microorganisms

A total of eight bacterial samples were used in the experiments. They included two clinical isolates of *E. coli* collected from two different diarrhoea patients at the University of Nigeria Teaching Hospital Enugu (designated as *E.coli* 1 and *E.coli* 2); two clinical isolates of *P. aeruginosa* from urine samples (*P. aeruginosa* 1 and *P aeruginosa* 2); and isolate of *B. subtilis* obtained from stock cultures in the Department of Pharmaceutics, University of Nigeria, Nsukka; Typed culture of *E. coli* (ATCC 11775), *S. aureus* (ATCC 12600) and *P. aeruginosa* (ATCC 10145) collected from Bioresources Development and Conservation Program (BDCP) centre, Nsukka.

Culture Media and Extraction Solvents

Nutrient agar (Oxoid), Mueller- Hinton agar (Oxoid) acetone (BDH), ethanol (BDH), n-hexane (BDH), ethylacetate (BDH) and dimethylsulphoxide (BDH) were used in the experiments as supplied from the manufacturers.

Extraction of the Plant Materials

Extraction of the dried pulverized leaves of *O. gratissimum* was carried out in accordance with the methods used by earlier workers (Said, *et al*, 1969; Timmins, and Court, 1975).

Fractionation of the Crude Extract

Some portion of the extract was separately fractionated in a chromatographic column using silica gel G as the stationary phase and ethyl acetate: n-hexane (2:5) as the mobile phase. Thin layer chromatography was at the same time carried out using silica gel G coated plates with the mobile phase to monitor the column separation of the extract into fractions. The remaining portion of the extract was separated into ethanol-soluble and ethanol- insoluble portions.

Maintenance, Purification and Standardization of Stock Microbial Cultures.

The stock cultures were maintained on nutrient agar slants at 4°C. In order to purify these stock cultures, subcultures were freshly prepared and incubated at 37° C for 18 – 24 hours before use (Caccamese, and Azzolina, 1979). Standard suspensions of each test microorganism were made by transferring a colony from the subculture into 5 ml of sterile distilled water, and adjusting the volume to obtain a cell population of approximately 1 x 10^6 cfu/ml. A volume of 0.1 ml of such suspensions was used as inoculum in all the tests.

Phytochemical Tests

Phytochemical screening of the dried powdered leaves was carried out using standard procedure (Trease, and Evans, 1989).

Preliminary Antimicrobial Screening of the Extract.

Preliminary antimicrobial screening of the extract was done using the agar-diffusion method (Rios, *et al*, 1988). Sterile cork borer having diameter of 8 mm was used to bore holes into seeded plates containing 20 ml of solidified Mueller-Hinton agar. A 1 ml volume each of the two-fold dilutions (2.0 mg/ml, 4.0 mg/ml, 8.0 mg /ml and 10 mg/ml) was added into each labeled hole using a sterile pipette. The experiment was repeated for all the test microorganisms. Replicate tests were performed and the plates incubated at 37°C for 24 hours. Growth was examined after incubation and the inhibition zone diameter measured.

Antimicrobial Screening of Fractions

Antimicrobial screening of the column factions (F_A- F_E) obtained from *O. gratissimum* was carried out using the agar-diffusion method. Holes of 8 mm in diameter were bored using a sterile cork borer into each of the seeded plates containing 20 ml of solidified Mueller-Hinton agar. A 1 ml volume each of the different dilutions (5 mg/ml, 10 mg/ml, 15 mg/ml, 20 mg/ml) of each stock was added into each labeled hole using a sterile pipette. The experiment was repeated for all the test microorganisms using the extract of *O. gratissimum*. Two replicate tests were performed and the plates were incubated at 37 °C for 24 hours. Growth was examined on the plates and the inhibition zone diameter around each hole measured. A control test was also set up against each test isolate using dimethylsuphoxide (DMSO) as an antimicrobial agent.

Determination of the Minimum Inhibitory Concentrations

The MICs of the extract and the ethanol-soluble portion of *O. gratissimum* were determined separately against the test isolates using the agar-diffusion method. Using DMSO as the diluent, graded concentrations (2.5 mg/ml, 5.0 mg/ml, 10 mg/ml, 20 mg/ml) of the acetone extract were prepared. Mueller- Hinton agar plates, each seeded with the test isolates were also prepared. Five cups were bored on each seeded plate using a sterile cork borer of 8 mm in diameter. Each cup was marked and the different dilutions introduced into them using a sterile pipette. A 1 ml volume of each dilution was further added into each cup. The test was performed for both the acetone extract and ethanol-soluble portion using each test isolate. Two replicate tests were done for each dilution on each test isolate and the plates were incubated at 37 °C for 18 -24 hours. The inhibition zone diameters were recorded by calculating the mean of each set of replicate inhibition zone diameters. The minimum inhibitory concentration was determined by plotting a graph of square of IZD against the logarithm of the concentration.

Results and Discussion

Preliminary phytochemical screening of the dried leaves of *O. gratissimum* revealed the presence of some glycosides especially cardiac glycosides but not the cyanogenic and anthracene glycosides. The presence of saponins, tannins, flavonoids, alkaloids, steroidal aglycones, starch and volatile oil was also indicated. The presence of tannins, alkaloids, flavonoids and saponins suggests possible antimicrobial activity by a plant as proposed by earlier workers (Levan, *et al*, 1979; Ibrahim, *et al*, 1997). More specifically, the presence of saponins and volatile oil is a further indication of the probable antimicrobial activity of the leaf extract of *O. gratissimum* (Chandel and Rastogi, 1980; Ainslie, 1937; Said, *et al*, 1969)

The fractionation of the crude extract of the plant yielded five column fractions (F_A - F_E) which showed no antimicrobial activity from the preliminary antimicrobial tests. The acetone extract, however, showed some degree of antibacterial activity against *E. coli* and *B. subtilis* but exhibited no activity against strains of *P. aeruginosa* and *S. aureus* when assessed in terms of the inhibition zones formed against the test microorganisms. (Table 1). It is evident, therefore, that the antimicrobial effects of the acetone extract from *O.gratissimum* do not appear to justify the ethno-medical uses of the plant as a stomachic, antidiarrhoeal and as a remedy for upper respiratory tract infections (Ainslie, 1937; Said, 1969). It may be reasonable to infer that acetone was either not effective as a solvent for the extraction of the antibacterial principles in *O. gratissimum* or could not extract the antibacterial principles in large enough quantities. A similar observation was made by earlier workers (Njoku, *et al*, 1997) who reported low yield of essential oil (7.5%) from the seed of *Hura crepitan*, using acetone as the extracting solvent.

Table 1: Preliminary Antimicrobial Activity of the Acetone Extract of *O. gratissimum* measured as mean IZD (mm)

Concentration µg/ml	*P. aeruginosa* 1	*P. aeruginosa* 2	*P. aeruginosa* ATCC10145	*E. coli* 1	*E. coli* 2	*B. subtilis*	*E. coli* ATCC 11775	*S. aureus* ATCC 12600
10.0	0	0	0	0	3.0	10.0	0	0
8.0	0	0	0	0	2.0	6.0	0	0
4.0	0	0	0	0	1.0	3.0	0	0
2.0	0	0	0	0	0	0	0	0

Table 2: MIC values of *O. gratissimum* extracts and tetracycline hydrochloride

Test Isolate	*O. gratissimum* (MIC mg /ml)		Tetracycline hydrochloride MIC mg/ml
	Acetone extract	Ethanol – soluble extract	
P. aerug. 1	-	-	16.0
P. aerug. 2	-	-	31.0
P. aerug. (ATCC10145)	-	-	29.0
E. coli 1	-	-	22.0
E. coli 2	9.44	4.26	12.0
E. coli (ATCC 11775)	-	-	50.0
S. aureus (ATCC 12600)	-	-	19.0
B. subtilis 2	2.52	3.50	19.0

Key: – = no activity.

At the concentration of 10 mg/ml, the ethanol – soluble portion of *O. gratissimum* showed some activity against *B. subtilis* and *E. coli* while the column fractions (F_A- F_E) produced no effects against any of the test isolates (Figs. 1 and 2). It is discernible, from Figures 1 and 2, that the column fractions showed complete absence of activity against the test isolates when compared to the acetone extract. These results may be attributed to the fact that a number of plant constituents, which on their own appear biologically inactive, may potentiate one another in a combination (Trease and Evans, 1989). The separation of the acetone extract of *O. gratissimum* into its different column fractions may have resulted in the isolation of individual constituents that are less potent when tested singly.

The MIC values of the ethanol-soluble portion and the acetone extract of the plant along with those of tetracycline hydrochloride which was used as a standard are presented in Table 2. It is equally evident from Table 2 that the extract of *O. gratissimum* exhibited no activity against *P. aeruginosa* and *S. aureus* as also shown in Table 1. The acetone extract inhibited the growth of *E. coli* and *B. subtilis* at minimal concentrations of 9.44 and 2.52 mg / ml respectively while the ethanol-soluble portion achieved the same effects at minimal concentrations of 4.26 and 3.50 mg/ ml respectively (see Table 2). The probable antimicrobial activity of the plant has been associated with its essential oil content. The weak

antibacterial activity of the acetone extract of *O. gratissimum* had earlier been attributed to the inability of acetone to extract the antibacterial principles (essential oil) of the plant in large enough qualities. It would be pertinent, in a separate study, to confirm this proposition and to establish the full antibacterial spectrum of the extracts of *O. gratissimum* using a more effective extraction solvent or technique.

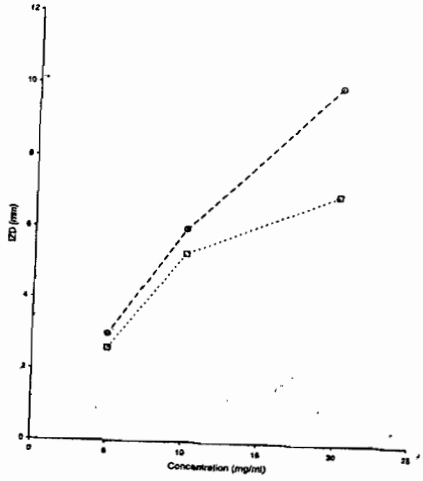

Fig. 1: Antimicrobial activity of the acetone extract, ethanol-soluble fraction of *O. gratissimum* against *B. subtilis*

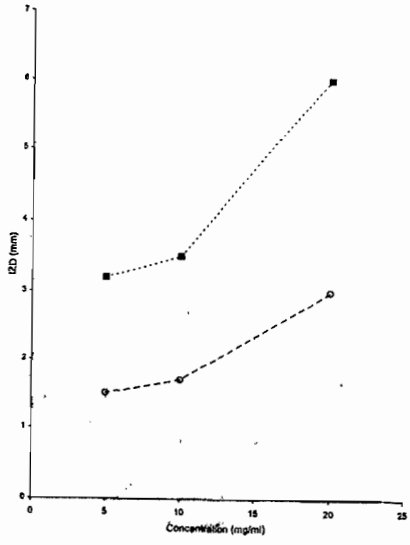

Fig. 2: Antimicrobial activity of the acetone extract and ethanol-soluble portion of *O. gratissimum* against *E. coli* 2

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