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SHORT REPORT

Antidiarrhoeal Activities of Ocimum gratissimum (Lamiaceae)

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ABSTRACT

The antidiarrhoeal activities of leaf extracts of *Ocimum gratissimum* were investigated by disc diffusion and tube dilution methods. The extracts were active against *Aeromonas sobria, Escherichia coli, Plesiomonas shigelloides, Salmonella typhi, and Shigella dysenteriae.* The leaf extracts were most active against *S. dysenteriae* and least active against *S. typhi.* The sensitivity of the organisms measured in terms of zone of inhibition ranged from 8.00 to 19.50 mm. The minimum inhibitory concentrations were from 4.00 to 50.00 mg ml-1, while the minimum bactericidal concentration ranged from 8.00 to 62 mg m1-1. The potentials of the leaf extract for the treatment of diarrhoeal diseases is discussed.

***Keywords*:** Plants, Medicinal; *Ocimum gratissimum;* Antidiarrhoeals; *Aeromonas sobria; Escherichia coli; Plesiomonas shigelloides; Shigella dysenteriae*

INTRODUCTION

Medicinal plants constitute an effective source of both traditional and modern medicine. Herbal medicine has been shown to have genuine utility, and about 80% of rural populations depend on it as their primary health care (1). In Nigeria, various plant parts are used for curing different ailments with remarkable success. Among the enormous number of these medicinal plants are members of the genus *Ocimun L. (Lamiaceae).* The genus is represented by six species in West Africa (2). However, only three species, *O. gratissimum L. O. basilicum L.* and *O. canum Sims* have been reported to have medicinal properties (3). Extracts of leaves of *O. gratissimum* or of whole plants are popular for the treatment of diarrhoea. Cold infusions of the leaves are used for the relief of stomach upset and haemorrhoids(4). The leaf is reported to be rich in thymol, which has antimicrobial properties (5).

The problems of unavailability of pharmaceutical drugs in remote and rural areas, fake drugs and increasing rate of resistance of diarrhoeagenic bacteria to orthodox drugs prompted this investigation. The activities of leaf extracts against some of the bacterial species associated with diarrhoea are reported here.

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MATERIALS AND METHODS

Plant materials

Fresh leaves of *O. gratissimum* were collected from the plants growing on the premises of Lagos State University, Ojo Campus. The plants were authenticated and a voucher sample deposited in the Departmental Herbarium.

Extraction

The dried leaves were milled into a fine powder, using a Waring blender (Mill Mx - 391 N). The ingredients of the powdered leaves (50 g) were then extracted with 200 mL of distilled water in a Soxhlet extractor apparatus. The extract was sterilised, using a membrane filtration unit (Sartorius). The resulting sterile filtrate was aseptically transferred into a labelled sterile bottle.

Microbial cultures

The following bacteria, isolated from stool samples of diarrhoea patients in Lagos University Teaching Hospital, were used for the antimicrobial property determination: *A. sobria, E. coli, P. shigelloides, A. hydrophila, S. typhi, S. dysenteriae, and Pseudomonas aeruginosa.* Three strains of each species were tested. The organisms were maintained on blood agar slopes at 4 ºC and subcultured for 24 hours before use.

Bacterial sensitivity testing

Inocula containing 1x106 cells per ml were introduced onto the surface of sterile nutrient agar plates. They were distributed evenly with a sterile glass spreader. A sterile paper disc previously soaked in the leaf extract was carefully placed at the centre of the labelled plate of each of the bacterial strains. The plates were incubated at 37 ºC and examined for zone of inhibition after 24 hours. Distilled water was used as the control.

Determination of the minimum inhibitory concentration

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the extracts were determined by diluting the extracts to various concentrations (0.0-65.0 mg mL-1), using nutrient broth in test tubes. Each test tube was inoculated with a bacterial suspension containing $1x10^6$ cells per mL and incubated at 37 ºC for 24 hours. The MIC was regarded as the lowest concentration of the extract that did not permit any visible growth when compared with drug free broths inoculated with each of the bacterial suspensions. The MBC was determined using the method of Rotimi et al (6). Tubes that showed no visible growth were streaked on fresh nutrient agar plates, incubated at 37oC for 24h and examined for growth. The MBC was regarded as the lowest concentration of the extracts that prevents the growth of any bacterial colony on solid medium.

RESULTS

The sensitivity of the aqueous extract of *0 gratissimum* measured by the zone of inhibition varies from (means + 1 SEM) 8.0±1.8 mm in *S. typhi* to 19.5±0.6 mm in *S. dysenteriae. P. aeruginosa and A. hydrophila* were not inhibited by the extract (Table I). The antibacterial activity of the leaf extracts was measured in terms of MIC and MBC. The lowest minimum inhibitory concentration of 4.0 mg mL^{-1} was against *S. dysenteriae,* while the highest value of 50.0 mg mL^{-1} was against *S. typhi* (Table II). Also the MBC ranged from 8.0 mg mL^{-1} in *S. dysenteriae* to 62.0 mg mL^{-1} in *S. typhi* (Table II).

Table I. Sensitivity of bacteria to leaf extracts of *O. gratissimum*

Test organism	Zone of inhibition*(mm)
S. dysenteriae	19.5±0.6
P. shigelloides	15.5±0.3
A. sobria	13.6±1.3
E. coli	11.5±1.5
S. typhi	8.0±1.8
A. hydrophila	0.0
P. aeruginosa	0.0
Sterile distilled water (control)	0.0

* means ± 1 SEM of 3 values.

Table II. Antibacterial activities of leaf extracts of *O. gratissimum* measured in terms of MIC and MBC

Organisms tested	MIC (mg mL^{-1})	MBC (mg mL^{-1})
S. dysenteriae	4.0	8.0
P. shigelloides	8.0	16.0
A. sobria	16.0	31.0
E. coli	32.0	45.0
S. typhi	50.0	62.0

DISCUSSION

The aqueous extracts of the leaves of *0 gratissimum* contain substances with antibacterial properties. This agrees with the works of Olowokudejo and Pereira-Sheteolu (3) and Sofowora (5). The extracts were active against the following bacteria of medical importance. *A. sobria, P. shigelloides, E. coli, S. dysenteriae, and S. typhi.* Some of the infections by these bacteria include diarrhoea, gastrointestinal disorders, and typhoid fever. Resistant strains of these organisms to many pharmaceutical drugs have been widely reported. *Aeromonas* spp, and *Plesiomonas* spp. which have been implicated in diarrhoeal diseases, are mostly resistant to penicillin, ampicillin, and carbenicillin (7).

The extract was most active against *S. dysenteriae* and least active against *S. typhi*. Previous chemical analyses of the plant showed the presence of thymol and eugenol that might be responsible for the antibacterial properties. The MIC values of the extract were lower than the MBC values, suggesting that the plant extract is bacteriostatic at lower concentrations but bactericidal at higher concentrations. These results offer a scientific rationale for the traditional use of the aqueous extract for the treatment of diarrhoeal diseases. In Nigeria, where the plant grows wild, the leaves are plucked, squeezed in water, sieved and the aqueous extract is drunk to treat diarrhoea with remarkable successes.

Recently, there has been an increase in the number of people in Nigeria depending on herbal drugs because of decline in purchasing power and the increasing fear of purchasing fake orthodox drugs. Herbal drugs are cheap, readily available and unadulterated. Their antibacterial activity could be increased by partial purification and subsequent concentration of the active ingredients.

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