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Short Communication

Antimicrob activity of leaf extracts of *Ocimum gratissimum* on diarrhoea causing in southwestern Nigeria

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The antibacterial activity of different extracts from the leaves of *Ocimum gratissimum* was tested against S*taphylococcus aureus, Escherichia coli, Salmonella typhi and Salmonella typhimurium*, pathogenic bacteria that cause diarrhea. These extracts evaluated include cold water extract (CWE), hot water extract (HWE) and steam distillation extract (SDE). Only SDE has inhibitory effects on the selected bacteria and the minimum inhibitory concentration (MIC) ranged from 0.1% for *S. aureus* to 0.01% for *E. coli* and *S. typhimurium*, and 0.001% for *S. typhi*.

Key words: Antibacterial activity, *Ocimum gratissimum,* diarrhea.

INTRODUCTION

During the last century, the practice of herbalism became mainstream throughout the world. In spite of great advances observed in modern medicine, plants still make an important contribution to health care. This is due to the recognition of the value of traditional medical systems, particularly of Asian origin, and the identification of medicinal plants from indigenous pharmacopoeias, which have significant healing power. Medicinal plants are distributed worldwide, but they are most abundant in tropical countries (Calixto, 2000; Lewis, 2001). In Brazil alone, about 80,000 species of higher plants were described which offer enormous prospects for discovering new compounds with therapeutic property (Nakaruma et al., 1999).

Ocimum gratissimum (labiatae) is widely distributed in tropical and warm temperate regions. The plant is commonly used in folk medicine to treat different diseases such as upper respiratory tract infections, diarrhoea, headache, ophthalmic, skin diseases, pneumonia, cough fever and conjunctivitis (Onajobi, 1986). Moreover, a lot of work has been done to show the antimicrobial properties of this plant to some selected pathogens. For example, *O. gratissimum* has been reported to be active against several species of bacteria and fungi (Nwosu and Okafor, 1995; Nakaruma et al., 1999).

Although, much has been documented on the antimicrobial properties of this plant, this work however is designed to evaluate the antidiarrhoea property of different extracts of this plant in order to know the best extract to use in the treatment of diarrhoea in folk medicine.

MATERIALS AND METHODS

Extraction methods

Leaves of *O. gratissimum* were collected from surrounding gardens and bushes. For hot water extraction, the plucked leaves were placed into a pot and boiled for 30 min. This was then allowed to cool and the leaves were then squeezed to obtain the extract. To

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Table 1. Antimicrobial activity of the different extracts of the leaves of *O. gratissimum* on selected bacteria that cause diarrhoea.

Microorganisms	Zones of inhibition (mm)			
	Hot	Cold	Steam	Control
S. aureus	0.0	0.0	37.0	0.0
E. coli	0.0	0.0	34.0	0.0
S. typhi	0.0	0.0	30.0	0.0
S. typhimurium	0.0	0.0	39.0	0.0

Table 2. MIC of the oil from the leaves of *O. gratissimum* against the test organisms.

Microorganisms	Zones of inhibition (mm)		
	0.1%	0.01%	0.001%
S. aureus	30.0	0.0	0.0
E. coli	29.0	11.0	0.0
S. typhi	26.0	18.0	8.0
S. typhimurium	28.0	7.0	0.0

make the cold water extract, the leaves were macerated in a mortar with a pestle, and the extract was obtained by sieving with sterile cheesecloth.

In the steam distillation extraction, 400 g of fresh leaves of *O. gratissimum* were cut into pieces and subjected to steam distillation using the Clavenger apparatus. The leaves were placed into the distillation flask containing 300 ml of sterile distilled water. The flask was then connected to the still head, the stopper was removed, and water run in to the orifice until it overflows at the junction tube. The flask was then heated with frequent agitation until ebullition begins and distillation continues at a rate that leaves the lower part of the condenser cold. The flask was rotated occasionally to wash down any material adhering to the upper part of the walls. At the end of 1 h, heating was discontinued, and after 5 min, the volume of the oil collected was read in the graduated portion of the tube. The distillation was further continued for another 1 h, and again the volume of the oil was measured. This continued until successive readings of the volume of the oil was constant. The measured yield of oil was taken to be the content of volatile oil in the leaves. The oil was then stored in the freezer until use. This constitutes the stock solution (100%) of the oil of *O. gratissimum* used for this work. From the stock solution, different dilutions of the oil were prepared using dimethyl sulphoxide (DMSO) to give 0.1, 0.01, and 0.001% (v/v).

Antimicrobial assay

The following bacteria were used in this study; *Salmonella typhi, Salmonella typhimurium, Staphylococcus aureus and Escherichia coli.* These organisms were collected from the microbiology laboratory of Obafemi Awolowo University Teaching Hospital Complex, Ile- Ife, Nigeria. Cultures of these bacteria were grown in nutrient agar at 37°C and maintained on slopes of nutrient agar. Each of the organisms was transferred into a separate test-tube containing nutrient broth to reactivate them by culturing overnight at 37°C.The different prepared extracts of the leaves of *O. gratissimum* was tested for antimicrobial activity against the test organism using the agar diffusion method of Navarro et al. (1966). The organisms were used to seed different nutrient agar plates; one organism per plate, wells were made on the plates with a sterile cork borer of 6 mm diameter to contain the different extracts and the plates were incubated at 37°C for 24 h. The zones of inhibition were measured at the end of the incubation period.

Determination of the minimum inhibitory concentration (MIC)

To the prepared nutrient agar plates containing specific bacteria on which different wells have been made, different dilutions of the extracts of *O. gratissimum* oil was introduced. The plates were incubated at 37°C for 24 h. Zones of inhibition were measured at the end of the incubation period. The MIC was taken to be the lowest dilution inhibiting the growth of the organism.

RESULTS AND DISCUSSION

Following the extraction of the leaves of *O. gratissimum* using cold water, hot water and steam distillation methods, the antimicrobial activities of the different extractions were determined. Table 1 shows the antimicrobial activity of the different extracts on the selected pathogenic bacteria that cause diarrhoea. Of all the different extracts tested, only the steam distillation extract inhibited the growth of the organisms with diameter of zones of inhibition ranging from 30.0 mm for *S. typhi* to 39.0 mm for *S. typhimurium*. Table 2 shows the minimum inhibitory concentration of the steam-distilled oil. It ranged from 0.001% (v/v) for *S. typhi* to 0.1% (v/v) for S*. aureus*.

From this investigation, only the oil from the leaves had antibacterial activity. The resistance of the organism to the other extracts may be due to the high volatibility of the oil, leading to the escape or evaporation of the oil during the boiling and for the cold-water extract, it may be due to insufficient release of the oil during extraction. The antimicrobial property present in the oil that is probably responsible for this observation is likely to be eugenol. This component has been demonstrated to have both antibacterial (Nakaruma et al., 1999) and antihelmintic activities (Pessoa et al., 2002). The result of this work however agrees with the findings of Orafidiya et al. (2000) who showed that the oil extract of *O. gratissimum* was active against enteroaggregative *E. coli*. It is therefore conceivable that this extract can be used to treat cases of diarrhea caused by these organisms in infected individuals.

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