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Full Length Research Paper

A study of aqueous and ethanolic extracts of *Ocimum gratissimum* and *xylopia aethiopica* and their antimicrobial activities

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Aqueous and ethanolic extracts of *Ocimum gratissimum* and *xylopia aethiopica* were analyzed for their antimicrobial activities against five pathogenic organisms; *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus fecalis*, *Pseudomonas aeruginosa* and *Lactobacilli*. Ethanolic extracts of *O. gratissimum* had a minimum inhibitory concentration (MIC) of 30 μg/ml against *S. aureus*, *E. coli, P. aeruginosa* and *lactobacilli* while for *S. fecalis* the MIC was 15 μg/ml. Aqueous extracts of *O. gratissimum* had an MIC of 12.5 μg/ml against *S. aureus, E. coli* and *S. fecalis*, while for *P. aeruginosa* and *lactobacilli* the MIC was 6.25 and 25 μg/ml, respectively. Ethanolic extracts of *X. aethiopica* showed an MIC of 10 μg/ml in the five organisms tested. While its aqueous extract gave an MIC of 30 μg/ml for *S. aureus* and *Lactobacilli*, and 15 μg/ml for *E. coli, P. aeruginosa* and *S. fecalis*. Our findings suggest that the anti-microbial activity of these spices reside in their aqueous fractions and also indicate that very low concentrations are required to achieve antimicrobial effects.

Key words: Antimicrobial effect, *Xylopia aethiopica, Ocimum gratissimum,* spice.

INTRODUCTION

Ocimum gratissimum and *Xylopia aethiopica* are used as spices in the preparation of foods and medicines. They are particularly used locally in preparation of potions and teas for women during peuperium. *O. gratissimum* is used by the Ibos of Southeastern Nigeria in the management of the baby's cord. It is believed to keep the baby's cord and wound surfaces sterile. It is also used in the treatment of fungal infections, fever, cold and catarrh (Iwu, 1986). Clinical trials in creams formulated against dermatological diseases have yielded favourable results (Edeoga and Eriata, 2001). Phytochemical evaluation of the plant has shown that it is rich in alkaloids, tannins, phytates flavonoids and oligosaccharides. It has tolerable cyanogenic glycoside content (Ijeh et al., 2004). Characterization of its ethanolic extracts revealed the presence of non-cyclic sesquiterpenes, phenols (Esvanzhuga, 1986), eugenol, alpha-pinen, camphor and terpinene (Bever, 1986).

X. aethiopica is one of the most pungent spices available. It has an attractive aroma and has been applied in ethnomedicine in the treatment of cough, bronchitis, dysentery and female sterilization (Iwu, 1986). It is believed to aid uterine contraction and is applied as an abortifacient (Iwu, 1986). Crushed powdered fruits are used in mixture with shea butter fat and coconut oil in creams and cosmetic products (Murray, 1995). Some of its investigated uses include termite anti-feedant activity (Murray, 1995) and antiseptic properties (Iwu, 1995). Its oils have been characterized and shown to contain a number monoterpenes and sesquiterpenes (Sofowora, 1984). Phytochemichal evaluation shows that *X. aethiopica* is rich in alkaloids, tannins, flavonoids, steroids,

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Table 1. Anti-microbial effect of aqueous extracts of *O. gratissimum*.

Organism	Zone of inhibition (mm)				MIC (μg/ml)
	50 μg/ml of extract	25 μg/ml of extract	12.5 μg/ml of extract	6.5 μg/ml of extract	
S. aureus	14.0	7.0	3.0	0.0	12.5
E. Coli	15.0	10.0	5.0	0.0	12.5
P. aeruginosa	12.0	10.0	6.0	3.0	6.25
S. fecalis	16.0	10.0	3.0	0.0	12.5
Lactobacilli	8.0	2.0	0.0	0.0	25.0

Table 2. Anti-microbial effect of ethanolic extract of *O. gratissimum*.

Organism	Zone of inhibition (mm)			MIC (μg/ml)
	60 μg/ml of extract	30 μg/ml of extract	15 μg/ml of extract	
S. aureus	5.0	2.0	0.0	30
E. Coli	10.0	6.0	0.0	30
P. aeruginosa	8.0	3.5	0.0	30
S. fecalis	11.0	7.0	2.0	15
Lactobacilli	5.0	0.8	0.0	30

oligosaccharides and has tolerable levels of cyanogenic glycosides (Ijeh et al., 2004).

A major limitation to the use of these plants is the side effects associated with their use in high doses. This present study is aimed at providing guide concentrations to users of this plant in the treatment of number of common infections.

MATERIAL AND METHODS

Collection and preparation of plant materials

Fresh leaves of *O. gratissimum* and seed of *X. aethiopica* were bought from Umuahia main market in Abia State, Nigeria. They were botanically identified at the taxonomy unit of the Department of Forestry of the Michael Okpara University of Agriculture, Umudike, Nigeria. The leaves and seeds were sun-dried to a constant weight over a ten-day period. They were milled to fine powder with the aid of a clean electric blender. 50 g milled plant material was soaked in 200 ml of distilled water to prepare the aqueous extract, and in 200 ml of ethanol to prepare the ethanolic extract. It was allowed to stand for 24 h after which it was filtered using a whatman No. 1 filter paper and the filtrate was evaporated to dryness over a steam bath. The residue after filtration was redissolved in de-ionized water to obtain varying concentrations which were used for the anti-microbial tests.

Preparation of the innocula

Microorganism (*Staphylococcus aureus*, *Escherichia coli*, *Streptococcus fecalis*, *Pseudomonas aeruginosa* and *Lactobacilli*) used in the work were obtained from the stock culture of the microbiology laboratory of the Federal Medical Center, Umuahia, Abia State, Nigeria. Viability test of each isolate was carried out by resuscitating the organisms in buffered peptone broth and thereafter subcultured into nutrient agar medium and incubated at 37°C for 24 h.

Antimicrobial screening tests

The sensitivity of selected organisms to the aqueous and ethanolic extracts of *O. gratissimum* and *X. aethiopica* was evaluated by the cup-plate agar diffusion method (Ebi and Ofoefule, 1997). 20 ml of molten nutrient agar was seeded with 0.2 ml of broth cultures of the test organisms in sterile petri-dishes. The petri-dishes were rotated slowly to ensure a uniform distribution of microorganisms. They were then left to solidify and in the dish. Cups of 8.0 mm diameter were made in the agar using a sterile cork borer. Different concentrations of the test extracts were inoculated into the cups using a sterile Pasteur pipette. The dishes were allowed to stand for about 30 min at room temperature to allow for proper diffusion of the extracts to take place. The plates were then incubated at 37°C for 24 h. This procedure was repeated for each extract concentration and the mean diameter/zone of inhibitions were measured and recorded. The minimum inhibitory concentration (MIC) was determined by comparing the different concentrations of a particular extract having different zones of inhibition and the selecting the lowest concentration for each extract.

RESULTS AND DISCUSSION

The tested organisms showed a higher sensitivity to aqueous extracts of *O. gratissimum* and *X. aethiopica*. *Lactobacilli* appear to be the least sensitive to all the extracts (Tables 1 and 2 show that it had the highest MIC for both aqueous extracts). *P. aeruginosa* was found to

Table 3. Antimicrobial effect of aqueous extracts of *X. aethiopica*.

Organism	Zone of inhibition (mm)			MIC (μg/ml)
	60 μg/ml of extract	30 μg/ml of extract	15 μg/ml of extract	
S. aureus	11.0	7.0	0.0	30
E. Coli	12.0	9.0	4.0	15
P. aeruginosa	10.0	9.0	3.0	15
S. fecalis	14.0	8.0	6.0	15
Lactobacilli	9.0	4.0	0.0	30

Table 4. Antimicrobial effect of ethanolic extracts of *X. aethiopica*.

Organism	Zone of inhibition (mm)				MIC
	500 μg/ml of extract	100 μg/ml of extract	50 μg/ml of extract	25 μg/ml of extract	(μg/ml)
S. aureus	8.0	5.0	0.0	0.0	100
E. Coli	12.0	8.0	0.0	0.0	100
P. aeruginosa	9.0	6.5	0.0	0.0	100
S. fecalis	10.0	6.0	0.0	0.0	100
Lactobacilli	8.0	6.8	0.0	0.0	100

be more sensitive to aqueous extracts of *O. gratissimum* than all the other organisms tested. This finding justifies the ethnomedical use of *O. gratissimum* leaves as a plaster to cover wound surfaces and baby cord. *P. aeruginosa*, *E. coli, S. aureus* are common human commensals and have been incriminated in the infection of wounds (Duguid et al., 1985). This finding also justifies the application of *O. gratissimum* in dermatological creams and indicates that effective doses could be achieved at very low concentrations. We have previously reported an ED_{50} of 23.95 μg/ml for aqueous extracts of *O. gratissimum* (Ijeh et al., 2004) . This compares well with our present finding of a minimum inhibitory concentration of 6.25 to 25.0 μg/ml for aqueous extracts of *O. gratissimum*. Also the present finding of an MIC 15 to 30 μg/ml with the ethanolic extract compares well with an ED_{50} of 26.30 μg/ml previously reported (Ijeh et al., 2004) for ethanolic extracts of *O. gratissimum*.

A higher MIC was recorded for both ethanolic fractions of *X. aethiopica* (100 μg/ml) and *O. gratissimum* (15 – 30 μg/ml) (Tables 2 and 4). Our present findings show that the aqueous fractions of both plants have more potential as an antimicrobial agent than its ethanolic fractions. The use of hot decoctions of these plants in the treatment of cough, dysentery and female sterilization is often limited by the disagreeable peppery and bitter taste associated with its use in high concentrations. This limitation can be considerably reduced if the finding of a low MIC in this work supported by our earlier reported low ED_{50} (Ijeh et al., 2004) is applied as a guide concentration in the preparation of these decoctions. *S. aureus* and *E. coli* have been frequently incriminated in food poisoning incidence which is often associated with gastroenteritis (Entani et al., 1998). Our findings support the ethnomedicinal use of both *O. gratissimum* and *X. aethiopica* in the management of stomach aches and gastroenteritis.

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