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ANTIBACTERIAL SPECTRUM OF EXTRACTS OF *OCIMUM GRATISSIMUM* L. (BASIL) AND *XYLOPIA AETIOPICA* A. RICH. (DUNAL).

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ABSTRACT

Preliminary phytochemical analysis showed that fruits of X. aetiopica contain phenolic compounds (including tannins) and cardiac glycosides while leaves of O. gratissimum possess these components and also saponins. Antibacterial activities of aqueous, ethanol and acetone extracts of the leaves of O. gratissimum and fruits of X. aetiopica were studied by the agar diffusion method. In case of extracts of O. gratissimum, the most effected organisms were S. aureus and K.pneumoniae whereas in case of X. aetiopica , the most effected ones were K. pneumoniae, S. aureus and S.typhi at prob. of F 0.01. The effect of extracts of O. gratissimum was most effective on tested organism (mean value 3.69) compared to the extracts of X. aetiopica (mean value 3.44) at prob. of F 0.01. In case of both the plants, the ethanol extract (mean value 3.83) was most effective compared to acetone extract (mean value 3.56) and aqueous extract (mean value 3.30) at prob. of F 0.01 against the tested organisms. Some of the tested organisms were treated with ethanol extract containing medium (McConkey broth for S. typhi and S. paratyphi and Nutrient broth for other bacterial isolates) for 48 hours. Gram staining revealed that these treated organisms were gram-variable. The minimum inhibitory concentration, MIC (15-20 mg/ml) and the minimum bactericidal concentration, MBC (20-25 mg/ml) of both the extracts were comparable to those of chloramphenicol, ampicillin, penicillin and flagyl (MIC is 1.5-2.0 mg/ml and MBC is 2.0-10.0 mg/ml) considering the crude nature of the antibacterial substances present in the extracts.

INTRODUCTION

Ocimum gratissimum L. is a perennial shrub which belongs to a family Labiate. Various species of Ocimum plant have been reported to contain eugenol while *O. gratissimum* was reported to contain also thymol (Oliver, 1960; Sofowora, 1984). The leaves of *O. gratissimum* are used in the treatment of diarrhea, cough, fever, vomiting and nausea in African countries like Nigeria, Ghana and French Guinea (Kafaru, 1994). According to Sofowora(1984) thymol is the active ingredient of *O. gratissimum* which accounts for its antimicrobial activity on the treatment of diarrhea. *Xylopia aetiopica* (dunal) A. Rich. belongs to the family Annonaceae. The fruits have the odor and taste of pepper and are used as spice, stimulant, flavor and as a carminative. The fruit is a common ingredient of many indigenous medications which are employed in the control of many ailments. The diterpenes of the fruits of *X. aetiopica* include xylopic acid and their derivatives which are known to possess antimicrobial activity (Boakye- Yiadom et al., 1987).

Because of the wide uses of these traditional herbs in Nigeria, an attempt has been made in the present investigation to determine the effect of organic and aqueous extracts of the plants on some bacterial isolates responsible for gastro enteric infections and some respiratory infections of bacterial origin.

MATERIALS AND METHODS

Materials

The leaves of *O. gratissimum* were collected from the outskirts of Minna Town, Nigeria and the dried fruits of *X. aetiopica* were purchased from Minna Main Market, Minna. The plant parts were identified and authenticated by Dr. M.I.S. Ezenewa, School of Agriculture and Agricultural Technology, FUT, Minna (Keay and Onochie, 1960 and Ivens et al., 1976). Leaves of *O. gratissimum* were sundried to a constant weight and then crushed into powder using a blender (National MX391M, Matushita Electric). The dried fruits of *X. aetiopica* were also crushed into powder using a blender. The powdered samples were used for extraction purpose.

Extraction Procedure

For extraction purpose, distilled water, ethanol and acetone were employed as extracting agents for both the samples. Each of the samples (50 g) was treated separately with 200 ml of water, ethanol and acetone for 24 hours and then each of the extracts was filtered and a rotary evaporator was used in vacuo at 40°C to concentrate the extracts. The dried extracts were used for diffusion test and also for MIC and MBC test.

Organisms

The bacterial isolates used in this study were *S. typhi, S. paratyphi* B, *K. pneumoniae*, *E. coli* , *S. aureus, S. faecalis, S. pyogenes* and *P. aeruginosa*. All the isolates were collected from National Veterinary Research Institute, Vom, Jos. *S. typhi, S. paratyphi* and *K. pneumoniae* were maintained on McConkey agar slants while the other isolates were maintained on Nutrient Agar slants.

Preliminary phytochemical analysis

This was performed according to Cuilei (1982) and Sofowora (1984).

Determination of antibacterial activity using diffusion method

The acetone and ethanol extracts of the samples obtained after extraction were first reconstituted in minimum amount of ethanol (0.1-0.2 ml) to dissolve the extracts and then each of the extracts was diluted with glycerol (20.0 ml) to get a final concentration of 100 mg/ml for this test. For agar diffusion test, *S. typhi* and *S. paratyphi* B were inoculated on McConkey broth and after 24 hours growth the cultures were diluted to obtain the final concentration of 10^7 cells/ml and in case of other bacteria, the same procedure was followed except Nutrient broth was used.

The agar diffusion method was employed for antimicrobial assay of the extracts. For *S. typhi* and *S. paratyphi* B, four cups were bored into each dried McConkey plate previously seeded with 10^7 cells/ml of test bacteria and 0.5 ml of each of the extracts at 100 mg/ml concentration was aseptically

introduced into the cups. Glycerol as control was introduced into fourth cup. Two agar plates were used for each organism. The plates were then

incubated at 37°C for 24 hours. The same procedure was repeated for other isolates except Nutrient agar was used.

Effects of extracts on growth and cell morphology of bacterial isolates

For this purpose, the ethanol extracts of both plants were chosen at 20 mg/ml and 50 mg/ml concentration. The solutions were made from the stock solution 100 mg/ml using glycerol as the neutral solvent. To each of the test tubes containing 2.0 ml of the broth (McCockney broth for *S. typhi* and *S. paratyphi* B and Nutrient broth for other bacterial isolates), a loopful of the test organism at 10^7 cells/ml concentration was introduced and was allowed to grow for 24 hours. Then 0.5 ml of each of the two concentrations (50 mg/ml and 20 mg/ml) of ethanol extract of *O. gratissimum* and *X.aetiopica* was added and after 24 hours the growth and cell morphology was observed.

Determination of MIC and MBC of the extracts

MIC test:

For MIC test, the concentrations used were 80.0 mg/ml, 40.0 mg/ml, 20.0 mg/ml, 10.0 mg/ml, 5.0 mg/ml, 2.5 mg/ml and 1.25 mg/ml. All the concentrations were prepared from the 100 mg/ml stock solution. To each test tube containing 2.0 ml of the broth (McConkey for *S. typhi* and *S. paratyphi* B and Nutrient broth for other isolates.

0.5 ml of varying concentrations of the reconstituted samples was added and inoculated with the test organism. For each of the selected bacterial species, the procedure was repeated using chloramphenicol, ampicillin, penicillin and flagyl which were diluted to required concentrations using distilled water. After proper incubation, the highest dilution of the extract and antibiotics that prevented visible growth of the test strains was taken as MIC (Rotimi et al., 1987).

MBC test

From MIC test, a loopful of broth was collected from those which did not show any growth and was inoculated on agar plates (McConkey for *S. typhi* and *S.*

paratyphi B and Nutrient agar for other isolates). MBC was determined by the highest dilution at which there is no visible growth on the solid media.

RESULTS

For aqueous extraction, a brown semisolid substance was obtained weighing about 6.0-8.0 g per 50 g of the leaves of *O. gratissimum* and fruits of *X. aetiopica* whereas for ethanolic and acetone extracts the yield was in the range of 3.0-5.2 g per 50 g of plant parts. The results in Table 1 showed that leaves of *O. gratissimum* contained cardiac saponins, glycosides, phlobatanins, polyphenols and tannins whereas frulits of *X. aetiopica* contain . saponins, cardiac glycostdes and tannins.The results in Table 2 showed that there was no significant difference regarding the activity of different extract of *O. gratissimum*(3.93-4.53) and *X. aetiopica* (3.33-4.23) against *K. pneumoniae* and *S. aureus* at prob. of F 0.01.Table 3 showed that the effect of extract of *O. gratissimum* was significantly different for different organisms at prob. of F 0.01. The most effected ones are *K. pneumoniae* and *S. aureus*. In case of *X. aetiopica*, the most effected ones were *S. paratyphi* and *S. typhi*. Table 4 showed that when *S. faecalis* was grown in medium containing 20 mg/ml extract of *O. gratissimum* and *X.aetiopica*, there was reduction in branching, the cells were swollen and some cells were gram positive whereas some were gram negative. In case of *K. pneumoniae*, there was no branching and part of the cells were gram positive and some were gram negative. In case of *E. coli* and *S. paratyphi*, the cells were also gram variable. At 50 mg/ml there was no cell growth observed. Table 5 showed that the MIC and MBC of the extracts were greater than those of chloramphenicol, ampicillin and flagyl.

DISCUSSION

Preliminary phytochemical analysis showed that leaves of *O. gratissimum* possess phenolic compounds, tannins, cardiac glycosides and saponin while fruits of *X. aetiopica* also possess these components except saponin. Ampofo and Bado (1979) found cardiac glycosides and saponin in all organs of the plant *Canthium subcordaum* by thin layer chromatography. Van Sumere et al, 1975, has shown that tannins or phenolic compounds and saponin are present in the bark and pulp of dicotyledonous plants. According to Gonzalel and Mather (1982), saponin, tannins and phenolic compounds have antibacterial properties. Table 2 indicated that ethanol and acetone extracts of both plant

parts possess significant antibacterial activity against *K. pneumoniae*, *S. typhi*, *S. aureus*, *E. coli* and *S. paratyphi* B. Previous studies by Wolinsky and Sote (1984) have shown that tannins and poyphenols are soluble in ethanol. Acetone has the specific property of extracting tannins and must be the factor responsible for the significant antibacterial activity of the acetone extracts. The aqueous extracts did not show significant antibacterial activity compared to the organic extracts, although the traditional healers use the extract of *O. gratissimum* raw by crushing the fresh leaves in a given quantity of water after which it is allowed to settle and the filtrate used for medicinal purposes. The dried fruits of *X. aetiopica* is soaked in equal volume of water overnight before use. This type of treatment may be necessary for the herbalists to get the proper dosage of the herbs. When plant materials are ground in water, plant cells are otherwise damaged and a number of phenolases and hydrolases are released (Akpata et al., 1977). This enzyme might overwhelm the active principles in the extracts and so prevent them from expressing their antimicrobial activities. Table 4 showed that there was a significant distortion in the morphology of all the organisms tested after treatment with the extract at 20 mg/ml. From this observation it may be suggested that the site of action of the antibacterial substance was at the cell wall while the gram reaction after treatment was variable for all the isolates tested. This is in agreement with the work of Emeruwa (1982) on the extracts of *C. papaya*. The MIC and MBC values for the plants against the tested organisms were generally higher than those of some standard antibiotics. The crude form of the extracts may have been responsible for their high MIC and MBC values compared to the antibiotics. The plant extracts were in crude form and have not been refined to exclude other ingredients that may affect the active substances. *E. coli* is known to be responsible for various infections like enteritis, cystitis and urethritis, while cases of a typical pneumonia and septicemia are as a result of infection by *K. pneumoniae*, *S. typhi* and *S. paratyphi* are known to be the causative agents for typhoid and paratyphoid fever respectively. *S. faecalis* is known to be associated with peritonitis and possible septicemia among others. The extracts of the two plants may be used to cure the above diseases preceded by further experiments. The antibacterial activities of the extracts against the test organisms suggest that there is a scientific basis for their utilization in traditional medicine for the treatment of some enteric and respiratory infections.

Table1: Preliminary phytochemical analysis of *O. gratissimum* and *X. aetiopica.*

Components	Leaves of *O.gratissimum*	Fruits of *X. aetiopica*
Alkaliods	-	-
Anthraquinones	-	-
Saponins	-	+
Cardiac glycosides	+	+
Phlobatanins	+	+
Polyphenols	+	+
Tannins	+	+
Sesquiterpenes	-	+

'+' indicates active component present; '-' indicates active component absent.

Table 2: Zones of inhibition produced by aqueous(AQ), ethanol(ET) and acetone (AC)extracts of *O. gratissimum* and *X. aetiopica.*

Organisms	Zones of inhibition(cm)					
	O.gratissimum			*X.aetiopica*		
	AQ	ET	AC	AQ	ET	AC
P.aeruginosa	3.03	3.40	3.06	2.76	3.17	2.93
K. pneumoniae	3.93	4.53	4.23	3.06	3.60	3.33
S. aureus	3.93	4.47	4.20	3.03	3.87	3.43
S. faecalis	3.00	3.47	3.13	2.97	3.83	3.43
S. paratyphi B	3.47	3.90	3.50	3.43	3.77	4.13
S. typhi	3.57	3.93	3.73	3.43	4.07	3.63
Mean	3.49	3.95	3.64	3.12	3.72	3.48
Prob. Of F	0.01					
LSD	0.131	0.236	0.193	0.157	0.170	0.163
SE	0.029	0.052	0..043	0.035	0.038	0.037

Table 3: Statistical analysis showing the effect of extracts of *O. gratissimum* and *X. aetiopica* on some organisms

Organism	Extract	
	O. gratissimum	*X.aetiopica*
K. pneumoniae	4.23	3.33
P. aeruginosa	3.17	2.96
S. aureus	4.20	3.44
S. faecalis	3.20	3.41
S. paratyphi B	3.62	3.78
S. typhi	3.74	3.71
Mean	3.69	3.44
Prob. of F	0.01	
SE	0.024	0.021
LSD	0.092	0.080

Table 4: Effects of extracts of *O. gratissimum* and *X. aetiopica* on growth and cell morphology of some organisms

Organisms	Cell morphology and Grams Reaction		
	Before treatment	Treatment with OG	Treatment with XA
A	Abundant growth, Short chained Diplococcic, Gram Positive	Reduced growth reduced branching of swollen cells, gram variables	Scanty growth, gram variable, swollen cells appear singly
B	Abundant growth Short rods with Branching, Gram negative	Swollen cells with changes in length and direction; no branching, appear singly	scanty growth, swollen single rods gram variable
C	Medium growth, Rod shaped and Gram negative	Scanty growth, gram variable and cell appears singly	"
D	Moderate growth, Rod shaped and appears singly	Scanty growth round and swollen cell, gram variable	Swollen short rods, gram variable
E	"	"	"

OG- *Ocimum gratissimum*; XA- *Xylopia aetiopica*; A- *S. faecalis*, B- *K.pneumoniae*, C-*E. coli*, D- *S. typhi*, E- *S. paratyphi*.

Table 5: Determination of MIC and MBC of ethanol extracts of *X. aetiopica* (XA) and *O.gratissimum* (OG) and some standard antibiotics.

Extracts & Antibiotics	MIC (mg/ml)					MBC(mg/ml)				
	A	B	C	D	E	A	B	C	D	E
XA	15.0	20.0	15.0	15.0	20.0	20.0	25.0	20.0	20.0	20.0
OG	20.0	15.0	15.0	15.0	-	20.0	20.0	20.0	20.0	-
PE	-	2.0	-	-	-	-	5.0	-	-	-
CH	-	-	1.5	1.5	-	-	-	2.0	2.0	-
FL	5.0	-	-	-	-	10.0	-	-	-	-
AM	-	-	-	-	2.0	-	-	-	-	-

CH- chloramphenicol, PE- penicillin, FL- flagyl, AM- ampicillin; A- *S. faecalis*, B- *K. pneumoniae*, C- *S. typhi*, D- *S. paratyphi* B, E- *E. coli*. '-' indicates not carried out.

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