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Antimicrobial alkaloid fraction from *Commiphora africana* (A. Rich)

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Abstract
An alkaloid fraction was isolated from *Commiphora africana* (Myrrh) and assayed against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes* using the agar diffusion method. The fraction exhibited antimicrobial activities against all the test microorganisms *Bacillus subtilis* was the most susceptible to the alkaloids followed by *Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes* and *Pseudomonas aeruginosa* respectively. The minimum inhibitory concentration ranged between 3.5mg/ml and 4.5mg/ml while the minimum bactericidal concentration ranged between 4.0mg/ml and 5.0mg/ml. The alkaloids fraction from *Commiphora africana* could be a potential source of chemotherapeutic agents.

Keywords: *Commiphora Africana*; Alkaloids; Antimicrobial activity

Introduction

The drugs contained in medicinal plants are known as active principles. The active principles are divided chemically into a number of groups among which are alkaloids, volatile essential oils, phenols and phenolic glycosides, resins, oleoresins, steroids, tannins and terpenes (Mitcher *et al.*, 1988; Habtermariam *et al,* 1993). Some of those chemical compounds of plant origin have been demonstrated to have antimicrobial activities. Miski *et al.* (1983) reported the antibacterial activity of flavonoids from *Salvias palantina.* Dormon and Deans (2000) assessed the volatile oils of black pepper (*Piper nigrum* L.), clove (*Syzygium aromaticum* L.) oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris)* for antibacterial activity against 25 different genera of bacteria. The oils exhibited considerable inhibitory effects against all the organisms tested. Two sesquiterpernoids, mikanolide and dihydromikanolide were isolated by Facey *et al.* (1999) from extracts of *Mikania micrantha.* The compound exhibited inhibitory activities against *S. aureus* and *C. albicans.* Many alkaloids have physiological activities, hence their wide use in medicine (Harbome, 1973).

Commiphora africana commonly called Myrrh is known as "dashi" among the Hausa speaking people of Northern Nigeria and "turari" among the Yoruba speaking people of South Western Nigeria. The gum which exudes from the stem is bitter astringent and expectorant. Extract of the

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plant is used to cure bronchitis, whooping cough and diseases of the genito-urinary organs (Gill, 1992). The objective of this study is to investigate the antimicrobial properties of alkaloid fraction isolated from *Commiphora africana.*

Experimental

Plant material. The root of the plant was collected from Forestry Research Institute, Jos, Nigeria. The plant was duly authenticated at the Institute as *Commiphora africana.*

Sources of microorganisms. Clinical isolates of *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Streptococcus pyogenes* used in this study were obtained from School of Medical Laboratory Technology, National Veterinary Research Institute, Vom. Nigeria.

Standardization of microorganisms. Over night cultures (0.2ml) of each test microorganism was dispensed into 20ml sterile nutrient broth and incubated for 4h to standardize the culture to 10^6cfu/ml. A loopful of the standard cultures was used for the antimicrobial assay (Collins *et al.*, 1995).

Preparation of plant extract. Ethanolic extract of the root of the plant was prepared according to the method described by Okogun (2000) with slight modifications. A 50g sample of the plant root was air-dried under the shade, ground into powder using an electric blender. The blended material was transferred into a beaker and 10ml of 95% ethanol added at room temperature (28 ± 2^0C). The mixture was extracted by agitation on a rotary shaker. Extraction was allowed to proceed for 48h. The extract was decanted and the solvent removed by evaporation at room temperature (28 ± 2^0C) to obtain the extract.

Extraction of alkaloids. Sample of ethanolic extract (10g) was taken into a beaker and strong ammonium solution was added just to moisten it. The mixture was allowed to stand for 10min and chloroform: ethanol (1:1) mixture was added in sufficient amount to soak and suspend the powder. The mixture was allowed to stand for 2min with occasional stirring with a glass rod, the mixture was filtered through a plug of cotton wool in a filter funnel. The filtrate was evaporated to dryness on a water bath. The residue was allowed to cool and it was dissolved in 5ml chloroform in a separating funnel. It was shaken with 3ml of sulphuric acid. The layers were allowed to separate and the chloroform layer drawn off and discarded. The upper layer which contained the alkaloid fraction was retained. This procedure was repeated until the upper layer become colourless and was made alkaline with strong ammonia and extracted with 3ml of chloroform. The extract was retained and evaporated to dryness (Trease and Evans, 1983).

Sensitivity test. The antimicrobial test was performed using the agar diffusion method of Nair and Chanta (2005). The test organisms were inoculated on nutrient agar plates and spread uniformly using a sterile glass spreader. Wells of 5mm diameter were made on the nutrient agar using a sterile cork borer. The cut agar disks were carefully removed by the use of forceps sterilised by flaming. To each well was introduced different concentrations (0.5 – 3.0mg/ml) of alkaloid fraction isolated from the plant extract. The various concentrations were obtained by dissolving the appropriate amount of alkaloid fraction in appropriate volume of sterile distilled water. Control experiments comprising inoculums without alkaloid fraction were set up. The plates were allowed to stand for 1h at room temperature (25±2°C) for diffusion of the substances to proceed before the growth of organisms commenced. The plates were incubated at 37^0C for 24h. The zones of inhibition were then recorded. zone sizes ranging from 4.0mm – 18.0mm were considered to be resistant.

Determination of Minimum Inhibitory Concentration (MIC) of alkaloids. Various concentrations of alkaloid fraction from *Commiphora africana* ranging between 0.5mg/ml and 8.0mg/ml were prepared by dissolving dry alkaloid fraction in appropriate volume of sterile distilled water, they were introduced into different test tubes, each tube was inoculated with an overnight culture of *E. coli, B. subtilis, S. aureus, Ps. aeruginosa* or *S. pyogenes* diluted to give a final concentration of 10^6cfu/ml. The tubes were incubated at 37^0C for 24h. The least concentration of alkaloid fraction that did not permit any visible growth of the inoculated test organism in broth culture was regarded as the MIC in each case (Collins *et al.*, 1995).

Determination of Minimum Bactericidal Concentration (MBC) of alkaloids. After culturing the test organisms separately in nutrient broth containing various concentrations of the active ingredients, the broth that showed no microbial growth was inoculated onto freshly prepared alkaloid free agar plates to assay for the bactericidal effect. The culture was incubated at 37^0C for 24h. The lowest concentration of alkaloid fraction that does not yield any colony growth on the solid medium after the incubation period was regarded as MBC (Alade and Irobi, 1993).

Table 1: Antimicrobial effect of alkaloid faction isolated from *Commiphora africana*

Concentration (mg/ml)	Mean diameter of zone inhibition (mm) ±SD				
	Ec	Bs	Sa	Pa	Sp
0.5	0	0	0	0	0
1.0	0	10.0±0.1	8.0±0.01	0	0
1.5	6.5±0.1	14.0±0.01	10.5±0.05	4.0±0.1	0
2.0	11.5±0.1	16.0±0.5	11.5±0.2	9.5±0.1	8.5±0.01
2.5	11.5±0.1	16.5±0.01	13.5±0.1	10.5±0.01	11.5±0.1
3.0	13.0±0.1	18.0±0.01	15.0±0.02	10.5±0.1	11.5±0.1

Ec = *E. coli*, Bs = *B. subtilis*, Sa = *S. aureus*, Pa = *P. aeriginosa*, Sp = *S. pyogenes*

Table 2: Minimum inhibitory concentration (MIC) of alkaloid fraction isolated from *Commiphora Africana*

Organism	Concentration of alkaloid fraction (mg/ml)												
	8.0	5.0	4.5	4.3	4.2	4.0	3.8	3.5	3.0	2.0	1.0	0.5	MIC
E. coli	-	-	-	-	-	-	+	+	+	+	+	+	4.0
B. subtilis	-	-	-	-	-	-	-	-	+	+	+	+	3.5
S. aureus	-	-	-	-	-	-	-	+	+	+	+	+	3.8
P. aeruginosa	-	-	-	-	+	+	+	+	+	+	+	+	4.3
S. pyrogenes	-	-	-	+	+	+	+	+	+	+	+	+	4.5

+ = Presence of growth - = Absence of growth MICs were regarded as the lowest concentration that did not permit any visible growth of test organism in broth culture.

Table 3: Minimum bactericidal concentration (MBC) of alkaloid fraction isolated from *Commiphora Africana*

Organism	Concentration of alkaloid fraction (mg/ml)												
	8.0	5.0	4.5	4.3	4.2	4.0	3.8	3.5	3.0	2.0	1.0	0.5	MIC
E. coli	-	-	-	-	-	-	+	+	+	+	+	+	4.0
B. subtilis	-	-	-	+	+	+	+	+	+	+	+	+	4.5
S. aureus	-	-	+	+	+	+	+	+	+	+	+	+	5.0
P. aeruginosa	-	-	-	+	+	+	+	+	+	+	+	+	4.5
S. pyrogenes	-	-	+	+	+	+	+	+	+	+	+	+	5.0

+ = Presence of growth - = Absence of growth

MBCs were regarded as the lowest concentrations that prevented growth of test organisms in nutrient agar

Results and Discussion

The alkaloid fraction isolated in this study exhibited antimicrobial activity against *E. coli, B. subtilis, S. aureus, P. aeruginosa* and *S. pyogenes* (Table 1).*B. subtilis* was the most susceptible to alkaloid fraction isolated from the plant material assayed in this study. The alkaloid fraction may interfere with folic acid metabolism and hence inhibit *B. subtilis* DNA synthesis. The inhibitory activities exhibited by the alkaloids tends to agree with the reports of Leven *et al.* (1979) and Scherbanvaskil (1971) both of which linked the antibacterial properties of plants to the presence of tannins, alkaloids, flavonoids and saponins. Several plants which are rich in alkaloids have been shown to possess antimicrobial activity against a number of microorganisms. For example Adebajo *et al.* (1983) investigated the antimicrobial activity of leaf extract of *Eugenia uniflora* and reported that alkaloids, tannins and glycosides were detected and that the ethyl acetate and methanolic leaf extracts of the plant were active against *E. coli, P. vulgaris, K. pneumoniae* and *A. niger. P. aeruginosa* was resistant to the alkaloid fraction (1.0mg/ml) assayed in this study. The bacterium may produce enzymes that destroy or inactivate alkaloids.

The large size of the zones of inhibition produced by the alkaloids isolated from the plant material used in this work against the test organisms indicated the potency of the active ingredients. *B. subtilis* appeared to be the most susceptible to the alkaloid fraction from *Commiphora africana*. The bacterium may lack an alternative biochemical pathway which cannot be affected by the alkaloid fraction. The resistance of *Ps. aeruginosa* to the alkaloid fraction may be as a result of the ability of the alkaloid fraction to penetrate the cell wall and get to the susceptible sites in *P. aeruginosa*. The MIC of the alkaloids ranged between 3.5mg/ml and 4.5mg/ml. The effect of the alkaloids on the MIC for the test microorganisms correlate with the report that microorganisms varied widely in the degree of their susceptibility (Emeruwa, 1982). Antimicrobial agents with low activity against an organism have a high MIC while a highly active antimicrobial agent gives a low MIC. The MIC and the MBC which is normally used to evaluate the efficacy of the agents such as antiseptics, disinfectants and indeed chemotherapeutic agents (Croshow, 1983) under standard conditions also support the sensitivity tests results. This study showed that the alkaloids isolated from *Commiphora africana* could be useful as a source of antimicrobial agent.

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