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In vitro antimicrobial activity of some medicinal plants from Cameroon.

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Abstract

The antimicrobial properties of crude methanolic extracts derived from ten Cameroonian medicinal plants were carried out. The screening of the antimicrobial activity of extracts was conducted by a disc diffusion test against Gram-positive, -negative bacteria and yeast. The active extracts (inhibition diameter ≥9 mm) were assayed for the minimum inhibitory concentration. All extracts were submitted to phytochemical screening by chemical tests. The results obtained indicated that the activity was more pronounced against Gram-positive bacteria and yeast than Gram-negative bacteria, which no activity was observed. The most active antimicrobial plant was *Plagiostyles Africana* (Euphorbiaceae).

Keywords: antimicrobial, medicinal plants, Cameroon, bacteria, Yeast.

1. Indroduction

Medicinal plants have long been the subject of human curiosity and need. In many parts of Cameroon, there is a rich tradition in the use of herbal medicine for the treatment of various infectious diseases, inflammations, injuries and other diseases (Adjanohoun et al., 1996). Plant derived products are present in 14 of the 15 therapeutic categories of pharmaceutical preparations that are currently recommended by medicinal practionners and, they form an important part of the health-care system in the werstern world (Phillipson and Anderson, 1989). Among the more than 250 000 species of higher plants, only about 5-10% are chemically investigated (Nahrstedt, 1996). Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, much recent attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine (Kokoska et al., 2002; Nostro et al., 2000). Plant based antimicrobials represent a vast untapped source for medicines and, further exploration of plant antimicrobials needs to occur. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Iwu et al., 1999; Cowan, 1999). The present study was conducted to investigate antimicrobial properties of methanol extracts of some medicinal plants used in folk medicine in Cameroon.

2. Materials and Methods

2.1. Plant material

The plants were collected from various places in Cameroon (table 1) during a trimester (from September to November 2003). Samples were identified by reference to the National Herbarium of Cameroon. Voucher specimens have been deposited at National Herbarium of Cameroon.

2.2. Preparation of extracts

The plant material (leaves, seeds and stem) were air dried and powdered. An amount of 15t0 60 g of the powder were extracted with 500 ml of Methanol. The extraction was done at room temperature under constant shaking for 24 hours. The different extracts obtained were filtered and concentrated under reduced pressure to dryness.

2.3. Microorganisms

The methanol extracts were individually tested against a panel of microorganisms, including Gram-positive bacteria [*Enterococcus hirae* ATCC 9790, *Staphylococcus aureus* ATCC 25923, 4 clinical strains of *Staphylococcus aureus* (1, 2, 3, 4), *Staphylococcus epidermidis*], Gram-negative bacilli.[*Escherichia coli* ATCC 25922, *E. coli* 35218, 10 clinical strains of *E. coli*, *Pseudomonas aeruginosa* ATCC 27823, 3 clinical strains of *P. aeruginosa*, 4 *Klebsiella pneumoniae*] and yeast species (7 clinical strains of *Candida albicans*).

2.4. Antimicrobial assay

The determination of antimicrobial activities of extracts were done by disc diffusion test according to the methods of the National Committee for Clinical Laboratory Standards (NCCLS) (1999). The minimum inhibitory concentrations of strains which exhibit inhibition diameter more than 8 mm were determined by the agar dilution method (NCCLS, 1999). The MICs of penicillin and econazole were also determined in parallel experiments in order to control the sensitivity of the test microorganisms. All tests were performed in triplicate.

2.4.1. Disc diffusion method

The dried plant extract were dissolved in DMSO (10%)/Tween 20 (0.5%) (v/v) to a final concentration of 100mg/ml and sterilized by filtration using 0.22 µm Millipore filters. Antimicrobial test were then carried out by disc diffusion method (NCCLS, 1999) using 100 µl of saline suspension containing 10^8 CFU/ml of bacteria, 10^6 CFU/ml of yeast on Mueller Hinton agar (MH) and Sabouraud dextrose agar medium respectively. The discs (6 mm in diameter) were impregnated with 15 µl of extracts and placed on the inoculated agar plate. Negative controls were prepared using the same solvents employed to dissolve the plants extracts. The inoculated plates were incubated at 37 °C for 24 h for bacteria strains and 48 h for yeast. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms.

2.4.2. MIC' agar dilution assay

MIC values of the Gram-positive and yeast isolates were studied based on the agar dilution method according to NCCLS (1999). The extract was added aseptically to sterile melted MH agar medium at the appropriate volume to produce the concentrations range of 2-1024 µg/ml. The resulting MH agar solutions were immediately poured into Petri plates after vortexing. The plates were spot inoculated with 2µl of each Gram positive bacteria and yeast isolates. Penicillin G was used as a reference antibiotic drug and econazole as a reference antifungal drug. The inoculated plates were incubated at 37 °C for bacteria and yeast for 24 h and 48 h respectively. At the end of incubation period, the plates were evaluated for the presence or absence for growth. MIC values were determined as the lowest concentration of the extract where absence of growth was recorded.

2.5. Phtochemical screening

Tests for alkaloids, coumarins, flavonoids, sterols and triterpenes were carried out according to the methods of Harborne (1973).

3. Results

3.1 Disc diffusion test

The results of disc diffusion testing of plant extracts are listed in table 2.

The DMSO/Tween 20 10%/0.1% (v/v) negative control showed no inhibiting effects.

All the plant extracts were not active against Gram-negative bacilli (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*).

The extracts from *Mammea africana*, *Ouratea sulcata* and *Plagiostylse africana* showed a good activity against *Staphylococcus* spp. whereas the extract from *M. africana* was also active against *Entercoccus hirae*.

Yeasts were found to be sensitive to extracts from *Crepis cameroonica*, *Crotalaria retusa*, *Lophira lanceolata*, *Ochna afzelii*, *Ouratea flava* and *Plagiostyles aficana*. The extract from *P. africana* showed a high activity (inhibition diameters ranged from 10 to 20 mm) against these microbes.

3.2 Minimum inhibitory concentration

The MIC values of plant extracts are given in table 3.

The DMSO/Tween 20 negative control showed no toxic effect at 10%/0.1% (V/V).

The positive controls showed MIC value ranged from <0.25 to 4 µg/ml (Penicillin G) against Gram-positive bacteria and from 32- >1024 µg/ml (econazole) against yeasts.

The MIC values of plant extracts for Gram-positive bacteria were >1024 µg/ml except *P. africana* which is the most active extract with MIC ranged from 64-1024 µg/ml against these bacteria.

Against yeasts, the MIC values of all plant extracts ranged from 512->1024 µg/ml.

3.3 Phytochemical screening

The results of the phytochemical screening of all plant extracts are listed in Table 3. The chemical tests showed the presence of flavonoids in all the extracts, whereas terpenes, alkaloids, coumarins and steroids were detected in eight, three, three and one of ten plant extracts, respectively.

4. Discussion and conclusions

The antimicrobial activities of ten methanol extracts from ten medicinal plants against 38 microorganisms examined in the present study and their potency were quantitatively assessed by the presence or absence of inhibition zone diameters (Table 2), and MIC values (Table 3). The results showed that the plant extracts have inhibition effect on the growth of Gram-positive bacteria and yeasts, and no activity against Gram-negative bacteria. The phytochemistry screening revealed the presence of components (alkaloids, coumarins, flavonoids, and terpenes) with antimicrobial activity in all extracts (Cowan, 2000). The extract of *P. africana* was the most active against *Staphylococcus* spp. and yeast whereas the extracts of *O sulcata* and *M. Africana* was active against *Staphylococcus* spp. and all Gram-positive bacteria tested respectively.

Our data showed that there was no uniform response within or between the bacterial strains of the same species and *C. albicans* isolates in terms of susceptibility to antimicrobial

compounds in the methanol extract of medicinal plants studied. These kinds of differences in susceptibility among the microorganisms against antimicrobial substances in plants extracts may be explained by the differences in cell wall composition. For the bacteria, Gram-negative bacteria have an outer phospholipidic membrane arraying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes, while porins constitute a selective barrier to the hydrophilic solutes with an exclusion limit of about 600 Da (Nikaido and Vaara, 1985). The Gram-positive bacteria should be more susceptible, having only outer peptidoglycan layer which is not an effective permeability barrier (Scherrer and Gerhardt, 1971). The antifungal compounds of the plants assayed are not well known; however, the presence of flavonoids and terpenes and certain degree of lipophicity might determine toxicity by the interactions with the membrane constituents and their arrangement (Tomas-Barberan et al., 1990).

The results were encouraging and may suggest that methanol extract of some medicinal plants possess compounds with antibacterial and anticandidal properties which can be used as antimicrobial agents in new drugs for therapy of infectious diseases.

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Table 1: List of medicinal plants used

Family name	Botanic name	Voucher number	Site of collection	Part used	Disease
Asteraceae	*Crepis cameroonica* Bab. c. ex Hutch. et Dalz	22072/SRF/CAM	Obili (Yaounde)	leaf	Nose and ocular infections, diarrhoea
Fabaceae	*Crotalaria retusa* Linn	23781/SRF/CAM			Eczema
Ochnaceae	*Lophira lanceolata* Van Tiegh ex Keay	3512/SRFK/CAM	Balamba (Bafia)	Leaf	Toothache, dermatosis, wound, conjunctivitis
Clusiaceae	*Mammea Africana* Sabin	17276/SRF/CAM		Stem	Scabies, constipation, abortion, syphilis, gonorrhoea,
Ochnaceae	*Ochna afzelii* R. Br. Ex Oliv	8493, H.N.C. Yde	Nkolafamba	Stem	Toothache, respiratory track infection
Ochnaceae	*Ouratea elongate* Oliv F. T. A.	56132, H.N.C., Yde	Mont Kala	Leaf	Gastritis, rheumatism
Ochnaceae	*Ouratea flava* Schumach et Thonning ex StapF	27056, H.N.C., Yde	Mont Elonden	Leaf	Gastritis, rheumatism
Ochnaceae	*Ouratea sulcata* Van Tiegh ex Keay	10133/SRF/CAM	Kribi	Leaf	Gastritis, rheumatism
Euphorbiacea	*Plagiostyles africana* Prain	5723/SRF/CAM	Mont Cameroun	Leaf	Ocular infection, chest complaint
Apocynaceae	*Voacanga Africana* StapF	9227/SRF/CAM	Obala	Seed	Orchitis, carious toot, gonorrhoea

Table 2: Disc diffusion tests

Organism	Plant species [a]									
	Cc	Cr	Ll	Ma	Oa	Oe	Of	Os	Pa	Va
Staphylococcus aureus 1	-[b]	-	-	10	-	-	-	13	9	-
S. aureus 2	8	-	-	13	-	-	-	12	12	7
S. aureus 3	-	-	-	10	-	-	-	10	12	-
S. aureus 4	9	10	9	13	9	-	-	11	12	-
S. epidermidis	10	10	8	12	9	-	-	12	13	-
S. aureus ATCC 25923	-	9	-	11	-	-	-	11	12	-
Enterococcus hirae	-	-	NT	12	-	-	-	-	-	11
Candida albicans 1	9	10	9	9	8	10	10	NT	12	7
C. albicans 2	8	10	8	-	11	-	13	NT	10	10
C. albicans 3	-	8	9	-	10	8	8	NT	14	9
C. albicans 4	10	10	8	-	10	8	10	NT	14	-
C. albicans 5	7	9	-	-	9	-	8	NT	16	-
C. albicans 6	10	10	10	11	10	-	11	NT	15	-
C. albicans 7	10	8	9	-	8	7	8	NT	16	-

[a] Cr: *Crepis cameroonica;* Cr; *Crotalaria retusa;* Ll: *Lophira lanceolata;* Ma: *Mammea africana;* Oa: *Ochna afzelii;* Oe: *Ouratea elongata;* Of: *Ouratea flava ;* Os: *Ouratea sulcata;* Pa: *Plagiostyles africana;* Va: *Voacanga africana*

[b] No inhibition zone

Table 3: Minimum inhibitory concentrations (MICs)

Organism	Plant species [a]										Antimicrobial [b]	
	Cc	Cr	Ll	Ma	Oa	Oe	Of	Os	Pa	Va	PG	Eco
Staphylococcus aureus 1	- [c]	-	-	>1024	-	-	-	>1024	512	-	2	NT
S. aureus 2	-	-	-	>1024	-	-	-	>1024	512	-	<0.25	NT
S. aureus 3	-	-	-		-	-	-	>1024	128	-	4	NT
S. aureus 4	>1024	-	>1024	>1024	>1024	-	-	>1024	64	-	0.25	NT
S. aureus 5	>1024	>1024	>1024	>1024	>1024	-	-	>1024	64	-	0.25	NT
S. aureus ATCC 25923	>1024	>1024	>1024	>1024	>1024	-	-	>1024	>1024	-	2	NT
Enterococcus hirae	>1024	-	>1024	>1024	>1024	-	-	>1024	>1024	-	1	NT
Candida albicans 1	>1024	>1024	>1024	>1024	>1024	>1024	>1024	-	>1024	>1024	NT*	>1024
C. albicans 2	-	-			>1024	-	-	-	-	-	NT	256
C. albicans 3	-	-	>1024	>1024	>1024	-	-	-	>1024	1024	NT	256
C. albicans 4	-	-			>1024	-	-	-	>1024	-	NT	-
C. albicans 5	-	>1024		>1024	>1024	-	-	-	>1024	-	NT	>1024
C. albicans 6	>1024	>1024	>1024	>1024	>1024	-	>1024	-	>1024	-	NT	>1024
C. albicans 7	>1024		>1024		>1024	-	>1024	-	1024	-	NT	>1024

[a] Cr: *Crepis cameroonica;* Cr: *Crotalaria retusa;* Ll: *Lophira lanceolata;* Ma: *Mammea africana;* Oa: *Ochna afzelii;* Oe: *Ouratea elongate;* Of: *Ouratea flava ;* Os: *Ouratea sulcata;* Pa: *Plagiostyles africana;* Va: *Vouacanga Africana*

[b] Eco: Econazole; PG: Penicillin G

[c] Minimum inhibitory concentration not determined because inhibition zone diameters by disc diffusion test were < 8 mm.

* NT: not tested

Table 4: Phytochemical screening

Plant species	Component				
	Alkaloids	Coumarins	Flavonoids	Steroids	Terpenes
Crepis cameroonica	+	-	+	-	+
Crotalaria retusa	+	-	+	-	+
Lophira lanceolata	-	-	+	-	+
Mammea africana	-	+	+	-	-
Ochna afzelii	-	+	+	-	+
Ouratea elongata	-	-	+	-	+
Ouratea flava	-	-	+	-	+
Ouratea sulcata	-	-	+	-	+
Plagiostyles africana	-	+	+	+	-
Voacanga africana	+	-	+	-	+