Phytochemical and Antimicrobial Properties of Extracts of *Combretum racemosum*

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Abstract

***Combretum racemosum* (P. Beauv.) (Combretaceae), a straggling shrub widespread across Africa is traditionally reputed to be anthelmintic and antimicrobial for genito-urinary and gastrointestinal infections. The methanol and ethyl acetate crude extracts obtained from the whole plant were evaluated invital to determine inhibition of human pathogenic micro organisms made up of five bacteria and three fungi. The extracts inhibited the eight test organisms to different degrees.**

All the bacteria strains were sensitive to both extracts at concentration ranging from 25 to 125 mg/ml using the agar broth cup diffusion procedure. The sensitivity of *Salmonella typhii*, *Escherica coli* and *Pseudomonas aeruginosa* (gram negative) to both extracts were not concentration dependent, whereas sensitivity of *Bacillus subtilis* and *Staphylococcus aureus* (gram positive) were concentration dependent with activity being higher at higher concentrations of ethyl acetate extract. Only the methanol extract exhibited intrinsic antifungal properties on *Candida albicans*, *Asperigillus niger* and *Dermatophyte* sp. with activity comparable to that of the reference drug tioconazole trosyd. Preliminary phytochemical screening of both extracts indicated the presence of alkaloids, steroids, cardiac glycosides, saponins and tannins.

INTRODUCTION

Combretum racemosum commonly known as Christmas rose, belongs to the family Combretaceae. The plant has been used for several years in African traditional medical practices and as a condiment in soups. It is a shrub indigenous to the tropical and pan tropical regions. In addition to its anthelmintic and antimicrobial properties, the plant is also used for the treatment of haemorrhoids, convulsive coughing, tuberculosis, toothache and male sterility (Burkill, 1985; Oliver-Bever, 1986).

Plants belonging to the family Combretaceae are reputed for anthelmintic and antimicrobial activities. Substantial work has been done in this plant family (Adjanohun and Ake Assi, 1972; Bouquet and Debray, 1974; Burkill, 2000; Jossang et al., 1996; Walker, 1953) but there is no report of any antimicrobial study on *C. racemosum.*

In continuation of our studies on biological activities of medicinal plants (Ajaiyeoba et al., 2000; Onocha et al., 2003), we now report on phytochemical and antimicrobial properties of extracts of *Combretum racemosum.*

MATERIALS AND METHODS

Collection, Authentication and Extraction of Plant Material

The whole plant material of *C. racemosum* was collected from the University campus and authenticated by Mr. Felix Usang of the Forest Research Institute (FRIN), Ibadan where a voucher specimen was deposited under file number FHI106430.

The airdried plant material (whole plant, 946 g) was extracted in hexane, ethylacetate and methanol for 48 hours. On concentration the resultant hexane (18 g), ethylacetate (28 g) and methanol (20 g) extracts were stored in the refrigerator for further use.

Phytochemical Screening

Preliminary phytochemical screening for various secondary metabolites such as anthraquinones, tannins, cardiac glycosides, alkaloids, saponin glycosides and the steroidal nucleus were done for the ethyl acetate and methanol plant extracts using the usual procedures (Harborne, 1991).

Antimicrobial Assay

1. Microorganisms. Cultures of five human pathogenic bacteria made up of three gram negative and two gram positive bacteria were used for the in vitro antibacterial assay. The species used were *Salmonella typhii*, *Escherica coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus*. For the antifungal assay, three fungi were utilized: *Candida albacans*, *Aspergillus niger*, and *Dermatophyte* sp. All the microorganisms were obtained from the laboratory stock of the department of Microbiology, University of Ibadan, Ibadan.

2. Media. Nutrient broth, nutrient agar, sabourand dextrose agar (SDA), and tryptone soya agar (Oxford Laboratories, UK) were used in the assays. Methanol and ethylacetate (Merck) were also used in solublising the extracts/drugs and as a negative control in the assays.

3. Antimicrobial Agents. Ampicillin, 12.5 μg/ml (Lab Oftalmiso, Spain), and tioconazole cream 1 mg/ml (Pfizer Inc., New York) were included as standard reference drugs in the study.

4. Antimicrobial Activity Determination. The cup agar broth diffusion procedure (Zwadyk, 1972) was used as an overnight broth culture of 1-2 x 10^7 CFU of each bacterium was used to seed sterile molten agar medium maintained at 45°C. Sterile tryptone soya agar plate was similarly seeded with fungi. When seeded plants had solidified, five wells (10 mm) respectively, were bored in each plate (7 mm, diameter) with an aseptic cork borer when seeded plates had solidified. 200 mg/ml of extract was reconstituted in methanol (or ethyl acetate) and 80 μl dispensed into each of the wells with the aid of a Pastuer pipette. Diameters of zones of inhibition were determined after incubating plates at 37°C for 24 h (bacteria) and at 25°C for 72h (fungi). When seeded with bacteria, each plate had wells filled with methanol (or ethylacetate) as well as ampicillin and for fungi, tioconazole. This method is similar to previous procedures (Kavanagh, 1977).

Antimicrobial studies were done in triplicates and diameters of zones of inhibition (mm) are expressed as the mean and standard errors on means. Student's "T" tests was used to test probability at $P< 0.05$.

RESULTS AND DISCUSSION

The results of the phytochemical screening of the hexane, ethylacetate and methanol extracts of the whole plant are presented in Table 1. Preliminary phytochemical screening of all extracts indicated the presence of alkaloids, steroids, cardiac glycosides, saponins and tannins.

The antibacterial ties of the ethylacetate and methanol extracts at concentrations ranging from 25 to 125 mg/ml is presented in Table 2. The bacteria used were clinical strains of *Salmonella typhii*, *Escherica coli*, *Pseudomonas aeruginosa* (gram negative), *Bacillus subtilis* and *Staphylococcus aureus* (gram positive).

Only the ethylacetate and methanol extracts inhibited the 5 test organisms to different degrees. All the bacteria strains were sensitive to both extracts at concentration ranging from 25 to 125 mg/ml using the agar broth cup diffusion procedure. However, the sensitivity of *Salmonella typhi*, *Escherica coli* and *Pseudomonas aeruginosa* (gram negative) to both extracts were not concentration dependent, whereas sensitivity of *Bacillus subtilis* and *Staphylococcus aureus* (gram positive) were concentration dependent, activity being higher at higher concentrations of ethyl acetate extract.

The result of the antifungal activities of the methanol extract at concentrations ranging from 25 to 125 mg/ml is presented in Table 3. Three clinical strains of human

pathogenic fungi were used namely: *Candida albicans*, *Asperigillus niger* and *Dermatophyte* sp. Only the methanol extract exhibited intrinsic antifungal properties on *Candida albicans*, *Asperigillus niger* and *Dermatophyte* sp. with activity comparable to that of the reference drug, tioconazole trosyd, against *Candida albicans*. It is note worthy that the methanol extract in addition, inhibited the growth of the other two fungi (*Asperigillus niger* and *Dermatophyte* sp.) while the reference drug was inactive to both.

Conclusively, the antibacterial activity of both extracts (ethylacetate and methanol) and the antifungal activity of the methanol extract further confirms the use of the plant in African ethnomedicine for the treatment of genito-urinary and gastro-intestinal infections, hemorrhoids, convulsive coughing, tuberculosis, toothache and male sterility (Bouquet and Debray, 1974; Burkill, 1985; Burkill, 2000; Jossang et al., 1996; Oliver-Bever, 1986).

The need for development of new antibiotics due to the increasing number of anti-biotic resistant organisms, and more importantly from natural sources cannot be overemphasized. *Combretum racemosum* provides a good opportunity for drug development in this area.

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Tables

Table 1. Phytochemical screening of the hexane, ethylacetate and methanol extracts of *Combretum racemosum* (whole plant).

Secondary metabolites	Extracts (whole plant)		
	Hexane	Ethylacetate	Methanol
Alkaloids	-	+	+++
Anthraquinones	-	-	-
Tannins	-	-	++
Steroids	+++	+++	+++
Cardiac glycosides	++	-	-
Saponin glycosides	-	+	+++
Reducing sugars	-	+	++

- absent; + low concentration; ++ medium concentration; +++ high concentration

Table 2. Antibacterial activities of the ethylacetate and methanol extracts of *Combretum racemosum* (whole plant).

Extracts	Extract conc./Ref./ control (mg/ml)	Diameters of zones of inhibition of bacteria (mm, P<0.05)				
		S. typhii	*E. coli*	*P. aeruginosa*	*B. subtilis*	*S. aureus*
Ethylacetate	25	13±0.5	10±0.1	11±0.5	9±1.0	-
	50	13±0.1	11±0.1	13±0.8	9±0.5	-
	75	13±0.8	13±0.8	13±0.2	15±0.4	9±0.4
	100	13±1.0	13±0.1	13±0.1	15±0.1	13±0.5
	125	15±0.2	13±0.2	13±0.8	15±0.4	13±0.6
	Ethylacetate	-	-	-	-	-
	Ampicillin	27±0.5	17±0.5	27±0.2	27±0.1	17±0.5
Methanol	25	9±0.7	-	-	-	8±0.5
	50	10±0.2	-	9±0.3	8±0.3	8±0.6
	75	10±0.2	9±0.4	9±0.1	8±0.5	8±0.2
	100	10±1.0	11±0.5	11±0.7	9±0.8	9±0.1
	125	-	11±0.6	13±0.6	11±0.4	9±0.1
	Methanol	-	-	-	-	-
	Ampicillin	27±0.5	19±0.3	31±0.1	27±0.0	25±o.6

Table 3. Antifungal activities of the methanol extract of *Combretum racemosum* (whole plant).

Conc. of extract (mg/ml)/ Ref./Control	Diameters of zones of inhibition of fungi (mm, P<0.05)		
	Candida albican	*Asperigillus niger*	*Dermatophyte* sp.
25	15±0.3	14±1.0	10±0.6
50	15±0.4	24±0.3	16±0.5
75	20±0.5	20±0.4	20±0.3
100	20±0.3	18±0.3	20±0.4
125	26±0.6	20±0.5	20±0.2
Methanol	-	-	-
Tioconazole trosyd	28±0.5	-	-