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Phytochemical and Antimicrobial Studies Of *Capparis thonningii* And *Capparis tomemtosa*

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ABSTRACT- Extracts of leaves and stems of *Capparis thonningii* shum and *Capparis tomentosa* Lam (aerial parts) were subjected to preliminary phytochemical screening for the presence of plant secondary metabolites and *in vitro* antibacterial and antifungal studies. The results of the preliminary investigation revealed the presence of alkaloids, the steroidal nucleus, saponins and tannins in both plants. Cardiac glycosides were also indicated in trace amounts in *C. thonningii*. The leaf methanol extract of *C. thonningii* and ethanol extract of *C. tomentosa* aerial parts were investigated *invitro* antimicrobial activity using the agar disc diffusion technique. Six clinical strains of human pathogenic microorganisms, comprising 3 Gram positive, 1 Gram negative and 2 fungi were utilized in the studies. The extract of *C. tomentosa* displayed overwhelming concentration dependent antimicrobial properties, inhibiting the growth of *Staphylococcus aureus* and *Bacillus cereus*, far above that of ampicillin used in the study at a concentration of 1.0g/ml. The extract was insensitive to the 2 Gram negative bacteria in the assay, In the antifungal assay, the growth of *Aspergillus flavus* and *Candida albicans* used, were inhibited in the same manner comparable to griseofulvin the reference drug included in the study. The methanol extract of *C. thonningii* also displayed a concentration related antibacterial activity, inhibiting the growth of *S. aureus* comparable to ampicillin at 1.0g/ml. The extract was least active against to *Escherichia coli* with a mild activity at 1.0g/ml. The extract exhibited weak activities against *C. albicans* as well as *A. flavus*. Both plant extracts seem to justify their ethnomedical uses.

KEYWORDS- Antimicrobial activity, *Capparis tomentosa*, *Capparis thonningii*, Capparidaceae

INTRODUCTION

Ccpparis. thonnigii Shum. and *Capparis. tomentosa* Lam. both belong to the plant family Capparidaceae. They are climbing or scrambling pricky shrubs of the Savanna woodland indigenous to West Africa. In Nigeria, the leaf is used an anti-dote for snake-bites, swollen tonsils, sores and ulcers; the root is used as a cure for coughs and blood spitting in Ghana (1). On the other hand, *C. tomentosa* occurs throughout the dry savanna of the West African region, often on termite mounds, from Senegal through Niger to N. Nigeria and indeed across Africa. A decoction of the leafy stems in drought is a common treatment for veneral disease in Ivory Coast (2,3). The roots are used in Senegal for treating vaginal discharges and syphilis (4,5).

Plants of this family are known generally to contain glucosinolates. These mustard oils have skin irritant activity and may also have contact allergenic activity (6,7,8). The alkaloid, L-stachydrine has been isolated from *C. tomentosa* (9). A sulphur oil is present in the roots of this plant (10). Previous studies on the antimicrobial properties of plants of the Capparidaceae family have been reported (11,12,13). In continuation of our interest in this family the preliminary phytochemical, antibacterial and antifungal properties of *C. thonningii* and *C. tomentosa* are presented.

MATERIALS AND METHODS

Plant collection and authentication.

The leaves (92g) and stem (345g) of *C. thonningii* was collected at Olokemeji, Abeokuta and authenticated by Mr. T. K. Odewo of the Forestry Research Institute of Nigeria (FRIN) while aerial parts of *C. tomentosa* (300g) was collected and authenticated by Mr. G. Ibhanesebhor also of FRIN. Voucher specimen of *C. thonnigii* was deposited under FHI 105776 while that of *C. tomentosa* was deposited as FHI 14941 in the Herbarium of FRIN.

Plant preparation and extraction.

Air-dried aerial part of *C. tomentosa* (300g) was ground (Hammer mill). It was successively extracted in redistilled hexane and ethanol by maceration at room temperature (30^0C) for 72 hours respectively. The leaves and stem of *C. thonningii* were each air-dried

and extracted in redistilled methanol similar to that described above. The percentage yields of extracts were noted after removal of solvent.

Preliminary phytochemical screening.

Air-dried and powdered plant materials were screened for the presence of tannins, alkaloids, anthraquinones, cyanogenetic glycosides, saponin glycosides, and steroidal nucleus using the methods described by (13, 14).

Microorganisms.

Clinical strains of three human pathogenic bacteria made up of 3 Gram-positive (*Staphyloccocus aureus*, *Bacillus subtilis* and *B. cerues*) and 1 Gram-negative bacteria (*Escherichia coli*) were used for the antibacterial assay, while for the antifungal assay, one yeast (*Candida albicans*) and one mold (*Aspergillus niger*) were used for the studies. All the microorganisms were obtained from the laboratory stock of the Department of Pharmaceutical Microbiology of the University of Ibadan, Nigeria.

Media.

Nutrient broth, nutrient agar, sabouraud dextrose agar (SDA), tryptone soya broth, tryptone soya agar (Oxoid Laboratories, U.K) were used in the study. Methanol was used in solubilising the extracts and drugs and was used as the negative control in the studies.

Antimicrobial Agents.

Ampicillin, 1mg/ml, (Help Pharmaceuticals, Germany) was used as the standard reference drug for antibacterial assays while tioconazole cream and griseofulvin were used as the standard reference drugs for antifungal assays.

Preparation of bacterial cultures.

The agar cup diffusion method was used to test the fractions for antimicrobial activity. From stored slopes, 5ml single strength nutrient broth was inoculated. The tubes were well shaken and incubated at 37°C for 18-24 hours.

Preparation of fungal cultures

From stored slopes, 5ml single strength tryptone soya broth was inoculated. The tubes were well shaken and incubated at room temperature for 2-3 days.

Using sterile pipettes, 0.2ml of 1 in 100 dilution of the bacterial cultures were added to 20ml of the melted and cooled (45-50°C) nutrient agar. The contents were mixed by gentle swirling movements before being poured into clean, sterile petri dishes. After agar in plates solidified, 6 wells (7 mm each) were bored in each plate using an aseptic cork borer. 1000 mg/ml, 500 mg/ml and 250 mg/ml of each of the extracts reconstituted in methanol were filled into the wells with the aid of Pasteur pipettes. Diameters of zones of inhibition were determined as an indication of activity after incubating the plates at 37°C for 24 hours for bacteria and at 25°C for 72 h for fungi. When seeded with bacteria, each plate had wells filled with methanol (for *C. thonningii*), hexane and ethanol (for *C. tomentosa*). The antibacterial and antifungal studies were done using our previous procedures (11, 12,13). Ampicillin was used as reference drug for antibacterial studies and for antifungal studies; tioconazole (15) and griseofulvin (16) were utilized.

RESULTS

The extraction of *C. tomentosa* with hexane and ethanol gave yields of 0.14% and 1.77% respectively, while the percentage yields of the stem methanol and leaf methanol extracts of *C. thonningii* were 1.44% and 1.32% respectively. The results of the phytochemical screening indicated the presence of alkaloids, saponin glycosides, traces of cardiac glycosides, tannins and the steroidal components. The presence of free and combined anthraquinones were indicated in *C. tomentosa* (Table1). For the antimicrobial activity, the diameters of the inhibition zones were measured and recorded.

Table 1. Preliminary Phytochemical screening of extracts.

	C. thonningii Leaf	***C. thonningii*** Stem	***C. tomentosa***
Alkaloids	+++	+++	+++
Cardiac glycosides	+	+	-
Saponin glycosides	+++	+++	+++
Anthraquinones			
Free	-	-	-
Combined	-	+	++
Steroidal Nucleus	++	++	+++
Cyano glycosides	-	-	-

(-): Absent, (+): Slightly present, (++): Fairly present, (+++): Abundant

Table 2- Antimicrobial activity of extracts.

Extract	Concentration mg/ml	*S. aureus*	*B. subtillis*	*B. cereus*	*E. coli*	*C. albicans*	*A. flavus*
C. thonningii							
Leaf	250	+	+	ND	-	+	+
	500	++	-	ND	++	+	+
	1000	+++	+++	ND	++	+	+
Stem	250	+	+	ND	-	+	+
	500	++	-	ND	++	+	+
	1000	+++	+++	ND	++	+	+
C. tomentosa							
Hexane	250	-	ND	-	-	-	-
	500	-	ND	-	-	+	-
	1000	-	ND	-	-	++	+++
Ethanol	250	-	ND	-	-	-	-
	500	+	ND	+	-	+++	-
	1000	+++	ND	+++	-	+++	+++
Control							
Ampicillin	1mg/ml	+++	+++	++	++	ND	ND
Tioconazole	1%w/v	ND	ND	ND	ND	+++	+++
Griseofulvin	1%w/v	ND	ND	ND	ND	+++	+++
Methanol	-	-	-	-	-	-	-

(ND)=not done, (+++) = high activity (>20mm), (++) = relatively high activity (15-19mm), (+)= low activity(11-14mm), (-) = no inhibition(<10mm)

DISCUSSION

The leaf and stem extracts of *C. thonningii* displayed concentration dependent antibacterial activities and this was comparable to that of the refernce drug ampicillin at 1g/ml as shown in Table 2. Only the ethanol extract of the aerial parts of *C. tomentosa* inhibited the growth of bacteria at concentrations of 1000 mg/ml and 500 mg/ml respectively. The hexane extract of *C. tomentosa* was not sensitive to the bacteria at the test concentrations (see Table 2). The leaf and stem extracts of *C. thonningii* showed inhibitory activity against *C. albicans* and *A. flavus*. The ethanol extract of *C. tomentosa* displayed the highest antifungal activity and its activity at 1000 mg/ml and 500 mg/ml was higher than that of the reference antifungal drug, griseofulvin (Table 2). The results of this study confirm the use of these plants as remedies for cough, sores, ulcers and venereal diseases. Other plants of the family Capparidaceae have been investigated for their antimicrobial activity. The leaves, stem bark and roots of *Ritchiea capparoides var. longipedicellata* were found to possess antifungal properties (16). *Cleome viscosa* also showed significant inhibitory activity against castor-oil-induced diarrhoea in rats (17). There is absolute need for bioactivity-guided fractionation and isolation of the active components in the plant extracts. The ethanol extract of *C. tomentosa* had impressive antibacterial and antifungal properties and could lead to the discovery of new antibiotics. This becomes more relevant as the current antibiotics in use are fast loosing effectiveness due to emergence of resistant microorganisms. The isolation of components of the aerial parts of *C. tomentosa* ethanol extract is in progress as very potent antimicrobial agents.

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PHCOG MAG.: Web watch

Dear Colleagues,

We will have in Cabo Frio, from November 30th up December 2nd, 2006 a very special multi-professional meeting entitled “New Trends in Health, Prevention and Quality of Life” (http://www.cepuerj.uerj.br/eventos1.htm). It will be a relevant event for different professionals and graduate and undergraduate students to discuss subjects related with the health, prevention and quality of life. The participants will discuss topics about Cardiology, Traumatic and Orthopedic, Pneumology, Oncology, Neurology, Rheumatology, Aging, Prosthesis and Orthesis, Esthetic and Dermato-functional, Urogenital procedures, Gynecology and Obstetric procedures, Disorders of the Movement, Therapy of the movement, Physiotherapy and the Sports, Traditional Chinese Medicine and Laboratories and Images Techniques. Some of these topics will be presented as round-tables and the other as posters or courses.

The organizing Committee has tried to involve in this meeting several professionals to exchange experiences. Biologists, dentistries, Physical Educators, nurses, nutritionists, physicians and physiotherapists will discuss and integrate ideas about the new trend in health, prevention and quality of life. New methods, research reports and multidisciplinary exchanges are the main focus of the various sessions. Various colleagues from different States in Brazil and from some countries in the world are well represented in this first entitled “New Trends in Health, Prevention and Quality of Life”.

Cabo Frio is one of the most pleasant city in the Rio de Janeiro State and the participants will find special beaches and sites to be, as well as, several facilities, as banks, restaurants, bars (with music) and several places to shop local souvenirs and gifts and to enjoy. Cabo Frio is about 160 km from Rio de Janeiro city. It is very easy to go to Cabo Frio from Rio de Janeiro in special and frequent buses that are in a Terminal located downtown and very close to the International Airport of the Rio de Janeiro. Please, visit the home page of our scientific event in the address http://www.cepuerj.uerj.br/eventos1.htm you will find, in Portuguese, the title of the event "Abordagens em Saúde, Prevenção e Qualidade de Vida". Click in it and you will find an option to choose "Português" and English.

We are looking forward tom see you in Cabo Frio.

On behalf of the Organizing Committee
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