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Journal of Medicinal Plants Research

Full Length Research Paper

Biological activity of extracts from *Capparis* decidua L. twigs

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Capparis decidua L. (family Capparaceae) is widely used in Sudan for curing variety of ailments. The objective of this study was to evaluate the antimicrobial, antigiardial, antimalarial, antioxidant activities and cytotoxicity of plant extracts from the twigs of C. decidua. Extracts were evaluated for their effectiveness against four bacterial strains including both Gram-positive (Bacillus subtilis and Staphylococcus aureus) and Gram-negative (Escherichia coli and Pseudomonas aeruginosa) bacteria as well as fungal species (Candida albicans and Aspergillus niger) using disc diffusion method. Antibacterial effects of twigs extracts showed different degrees of inhibition profiles against tested bacteria. The ethyl acetate extract showed the highest activity against S. aureus (21 mm), B. subtilis (20 mm) and P. pneumoniae (18 mm) while the n-butanol extract displayed best inhibition against P. pneumoniae (18 mm) and E. coli (16 mm). All extracts showed high antifungal activity against A. niger and C. albicans with inhibition zone ranged from 17 to 22 mm. Antimalarial activity assay showed that all extracts were less potent than chloroquine drug against P. falciparum. Antigiardial activity was determined against Giardia lamblia where the chloroform and ethyl acetate extracts possessed potent antigiardial activity after 24 h at concentration 500 µg/ml with respectively 91 and 89% mortality comparable to that exhibited by metronidazole drug (89%). The antioxidant potential of extracts was determined on the basis of their scavenging activity of the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical and ferric-reducing antioxidant power (FRAP). The petroleum ether and n-butanol extracts showed moderate DPPH scavenging activity, while the ethyl acetate and chloroform extracts showed low activity. All extracts were inactive in the FRAP assay. All extracts were nontoxic against brine shrimps and vero cell lines suggesting that they were safe for traditional use.

Key words: Capparis decidua, antimicrobial activity, antigiardial activity, antimalarial activity, antioxidant activity, cytotoxicity.

INTRODUCTION

Populations in developing countries may only have access to local traditional medicines as their critical source of primary healthcare (Cordell, 2011). Plant-based

traditional medicine represents primary or perhaps only form of accessible primary health care in many parts of rural Africa (Mahomoodally, 2013). Infectious diseases

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> have remained a major cause of death and disability worldwide and account for one-third of all deaths in the world. The World Health Organisation (WHO) estimates that nearly 50,000 people die each day throughout the world from infectious diseases (Chanda and Rakholiya, 2011).

Capparis decidua L. belongs to family Capparaceae, vet an important medicinal plant of Sudan. The tree is typical of deserts and semi-deserts of northern and central Sudan, especially on sandy soils and in low rainfall savanna on clays spreading to the borders of Republic of Southern Sudan, sometimes mixed with Acacia seyal or Balanites aegyptiaca (El-Amin, 1990). The plant and its parts are widely used by traditional healers and tribal people in Sudan for curing variety of ailments. Paste of young leaves and branches are applied as plaster on boils and swelling, and as antiinflammatory, astringent, stomachic, laxative, antidote for skin diseases (AL-Yahva 1986; Atigur et al., 2004). Decoction of fresh twigs is taken against jaundice and the fumigation of the stems is used as anti-rheumatic. The stems are also used as a poultice for swelling and joint pains and against head-ache (El-Ghazali et al., 1994, 1997). The roots are used to relieve fever, rheumatism jaundice (ELKamali and Elkhalifa, and 1999). Pharmacological studies of plant for the exploration of biological activities play important part in science of traditional medicine.

The objective of this study was to evaluate the antimicrobial, antigiardial, antimalarial, antioxidant activities and cytotoxicity of plant extracts (petroleum ether, chloroform, ethyl acetate and butanol) from the twigs of *C. decidua*.

MATERIALS AND METHODS

Plant material

Twigs of *C. decidua* were collected from Goz Abu kelab, White Nile State, Sudan in July, 2012. Voucher specimen No. ACD12 was deposited in the Herbarium of Botany Department, Faculty of Science, University of Khartoum.

Preparation of crude extracts

Twigs of the plant were air dried in shade and ground to powder using a pestle and mortar. 100 g of powder was extracted sequentially with petroleum ether, chloroform, ethyl acetate and butanol at room temperature for 48 h. Extracts were first filtered through Whatman No. 4 filter paper. After filtration, the extracts were vacuum concentrated.

Phytochemical analysis

Qualitative preliminary phytochemical analysis was performed initially with different chemical reagents to detect the nature of phytoconstituents and their presence in twigs. The presence of sterols/terpenes, flavonoids, tannins, alkaloids, lignins, saponins and coumarins were evaluated by standard qualitative methods of Trease and Evans (2002).

Antimicrobial activity

Test strains and culture media

Standard strains of microorganism were used in this study and were obtained from Medicinal and Aromatic Institute of Research, National Research Center, Khartoum. The bacterial species used were the Gram-negative bacteria; *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) and the Grampositive bacteria; *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923). Fungal species were *Candida albicans* (ATCC 7596) and *Aspergillus niger* (ATCC 9763). Bacteria were grown in Mueller Hinton Agar and fungi were grown in Sabouraud Dextrose Agar. The concentration of bacterial suspensions were adjusted to 10⁸ cells/mL, and that of fungal suspensions to 10⁷ cells/ml.

Antibacterial assay

Antibacterial activity of extracts was evaluated by the disc diffusion method (Kil et al., 2009). Extracts solutions (100 mg/ml) were prepared by diluting with 5% dimethyl sulfoxide (DMSO). The test microorganisms were seeded into respective medium by spread plate method. After solidification, filter paper discs with a diameter of 6.0 mm were impregnated with 10 μ l of crude extracts followed by drying off. DMSO was used as a negative control, while gentamicin (10 μ g/disc) was used as a positive control. Antibacterial discs were dispensed onto the surface of the inoculated agar plates and Petri plates were incubated for 24 h at 37°C. Diameters of clear zone of inhibition produced around the discs were measured and recorded.

Antifungal assay

Antifungal activity was also evaluated by the disc diffusion method (Mothana and Lindequist, 2005). Paper discs were impregnated with 10 μ I of extracts at 100 mg/ml followed by drying off. DMSO was used as a negative control, while nystatin (10 μ g/disc) was used as a positive control. Antifungal discs were dispensed onto the surface of the inoculated agar plates, after which the plates were incubated at 27°C for 48 h. After the colonies grew, the zones of inhibition around the discs were measured and recorded.

Antigiardial activity

Giardia lamblia were taken from patients of Ibrahim Malik Hospital (Khartoum). All positive samples were examined by wet mount preparation and were transported to the laboratory in nutrient broth medium. Trophozoites of *G. lamblia* were maintained in RPMI 1640 medium containing 5% bovine serum at $37\pm1^{\circ}$ C and were employed in the log phase of growth.

In vitro susceptibility assay

In vitro susceptibility assay was done according to the method of Cedillo-Rivera et al. (2002). Five milligram from each extract was dissolved in 50 μ l of DMSO at Eppendorf tube containing 950 μ l distilled water in order to reach concentration of 5 mg/ml. The concentrates were stored at -20°C for further analysis. Assay was performed in 96-well microtiter plate. Serial dilutions of each extract were obtained by diluting successively 20 μ l of extract solution with 20 μ l complete RPMI medium solution to achieve the required concentrations. Then, 80 μ l of culture medium which was complemented with parasite was added to each well containing 20

 μ I of sample. The final volume in the wells was 100 μ I. Metronidazole was used as positive control in concentration 312.5 μ g/mI, whereas untreated cells were used as a negative control (culture medium plus trophozoites). For counting, samples were mixed with Trypan blue in equal volume. The final number of parasites was determined with haemocytometer three times for counting after 0, 24, 48 and 72 h. The mortality % of parasite for each extracts activity was carried out according to the following formula:

Mortality of parasite (%) = $\frac{\text{Control negative - sample}}{\text{Control negative}} \times 100$

Only 100% inhibition of the parasite was considered, when there was no motile parasite observed.

Antimalarial activity

Plasmodium falciparum K1 parasite strain was obtained from Khartoum Hospital (Khartoum). Samples were maintained at 5% hematocrit (human type O-positive red blood cells) in complete RPMI 1640 medium (RPMI 1640 medium supplemented with 25 mM HEPES, 370 µM hypoxanthine, 40 µg/ml gentamycin, 0.25 µg/ml Fungizone and 0.5% (wt/vol) AlbuMax II) in 60 mm Petri dish according to the modified candle jar method (Trager and Jensen, 1978). The culture was routinely monitored through Geimsa staining of the thin smears. Standard drug (chloroquine) and extracts at different concentrations (range from 1 to 1000 µg/ml) were prepared in distilled water (chloroquine) DMSO (test extracts) and then diluted to achieve the required concentrations. The synchronized culture with parasitaemia of 1.5 and 3% haematocrit were incubated in 96-well microtitre plate predisposed with multiple concentrations of extracts for 48 h at 37°C in candle jar. Blood smears from each well were fixed in methanol, stained with Giemsa's stain and the numbers of infected RBCs per 5000 cells were counted. The antimalarial activity of the test extract was expressed as 50% inhibitory concentration (IC₅₀) determined from dose-response curve by non-linear regression analysis (curve-fit) using Graph Pad Prism (version 4) software. Crude extracts with IC_{50} values > 50 ug/ml were considered to be inactive (Kraft et al., 2003).

Antioxidant activity

DPPH radical scavenging activity

Antioxidant activity of the extracts was estimated using 1, 1diphenyl-2-picrylhydrazyl (DPPH) method (Yamaguchi et al., 1998). Test samples were dissolved separately in 5% DMSO to get test solution of 1 mg/ml. Assay was performed in 96-well, microtiter plates. 140 μ l of 0.6 x 10⁶mol/l DPPH were added to each well containing 70 μ l of sample. The mixture was shaken gently and left to stand for 30 min in dark at room temperature. The absorbance was measured spectrophotometrically at 517 nm using a microtiter plate reader (Synergy HT Biotek[®], logiciel GEN5). Ascorbic acid was used as a reference antioxidant compound. The ability to scavenge DPPH radical was calculated by the following equation:

$$I\% = [(A_{blank} - A_{sample}) / A_{blank}] \times 100,$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test sample), and A _{sample} is the absorbance of the extracts/reference.

Ferric-reducing antioxidant power assay

Ferric-reducing antioxidant power assay (FRAP) of the samples was tested using the assay of Oyaizu (1986). Different concentrations of the extracts were mixed with 2500 μ l of phosphate buffer (pH 6.6) and 2500 μ l of potassium ferricyanide. Later, the mixture was incubated at 50°C for 20 min and, then, trichloroacetic acid (10%) was added. After the mixture was shaken vigorously, this solution was mixed with distilled water and ferric chloride (0.1%). After 30 min of incubation, absorbance was read at 700 nm. Increased absorbance of the reaction meant increased reducing power and compared to that of chlorogenic acid as the reference.

Cytotoxicity

Brine shrimp lethality test

Artemiasalina (shrimp eggs) was placed in natural sea water, and eggs hatched within 48 h, providing a large number of larvae (nauplii). The tested sample (20 mg) was dissolved in 2 ml of solvent. From this solution 5, 50 and 500 μ l were transferred to vials forming concentrations of 10, 100 and 1000 μ g/ml respectively. The solvent was allowed to evaporate overnight. Volume was made to 5 ml with seawater. Ten larvae were placed in each vial using a Pasteur pipette. Vials were incubated at 25 to 27°C for 24 h under illumination. Etoposide was used as positive control and number of survived larvae was counted. Data was analyzed by Finney Probit Analysis computer program to determine LC₅₀ values with 95% confidence intervals (McLaughlin, 1998).

Microculture tetrazolium (MTT) assay

The experiment was performed according to method described by Berridge et al. (2005). Vero cells (normal, African green monkey kidney) were cultured in a 96 -well plate for overnight CO2 environment at 37°C. Supernatant was removed, and 50 µl of serially diluted extracts (range from 0.01 to 100 µg/ml) and 150 µl complete medium DMEM supplemented with 5% (v/v)) fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 µl/ml) were added to each well. After incubation, the culture medium was aspirated carefully and 50 µl of 3-(4, 5-dimethylthiazol) -2, 5diphenyle-tatrazolium bromide (MTT) solution (2 mg/ml PBS) was added to each well and further incubated for 4 h. MTT solution was aspirated, and 100 µl of DMSO was added to dissolve the blue insoluble MTT formazan produced by mitochondrial dehydrogenase. The plate was agitated at room temperature for 15 min then read at 540 nm by using micro-plate readers. The optical density was measured at 540 nm and the percentage of viable cells was calculated as relative ratio of optical densities.

Statistical analysis

All experiments were performed in triplicates and data were presented as means \pm S.D. Statistical analysis for all the assays results were done using Microsoft Excel program.

RESULTS AND DISCUSSION

Phytochemical screening

Preliminary phytochemical analysis of twig extracts of *C. deciduas* revealed presence of sterols/terpenes, flavonoids, tannins, alkaloids, lignin, saponins and

Miene ennenione	Mean diameter of growth inhibition zone of different extracts				
Micro organisms	Petroleum ether	Chloroform	Ethyl acetate	Butanol	Standard drug*
B. subtilus	15±0.33	14±0.01	20±0.40	17±0.03	25±0.02
S. aureus	16±0.70	16±0.08	21±0.20	17±0.01	17±1.30
E. coli	15±0.70	15±0.70	15±0.70	16±0.70	17±1.00
P. aeruginosa	14±0.70	17±1.4	18±0.01	18±0.57	18±0.01
C. albicans	18±0.75	20±0.10	19±0.60	17±0.70	14±0.01
A. niger	22±0.01	18±0.70	18±0.03	17±0.01	18±0.10

Table 1. Antimicrobial activity of twigs extracts of C. decidua.

*gentamicin (10 μ g/disc) was used as a positive control for bacteria, and nystatin (10 μ g/disc) as a positive control for fungi. Values are presented in mean ± SD (n =3).

coumarins. Several chemical researches have been previously carried out on *C. deciduas*. Sterols (Rathee et al., 2010a, 2010b), flavones (Saxena and Goutam, 2008), oxygenated heterocyclic constituents (Gupta and Ali 1997), alkaloids (Ahmad et al., 1985) and isothiocyanate glucosides (Juneja et al., 1970) have been reported in different parts of this plant.

Antimicrobial activity

The antibacterial activity of the petroleum ether, chloroform, ethyl acetate and n-butanol extracts from twigs of *C. decidua* was determined against the Gram positive *B. subtilis* and *S. aureus* and the Gram negative *E. coli* and *P. aeruginosa* and two fungi; *A. niger* and *C. albicans* using the disc diffusion method. Results are presented in Table 1.

Different extracts showed variable activity against the tested bacteria. Generally, the ethyl acetate and nbutanol extracts respectively showed higher antibacterial activity than the petroleum ether and chloroform extracts. The ethyl acetate extract showed the highest activity against S. aureus (21 mm), B. subtilis (20 mm) and P. pneumonia (18 mm) while the n-butanol extract displayed best inhibition against P. pneumonia (18 mm) and E. coli(16 mm). Interestingly, the ethyl acetate, n-butanol (18 mm) and chloroform (17 mm) extracts demonstrated antibacterial activity against *P. aeruginosa* comparable to that obtained for gentamicin at 10 µg/disc (18 mm). These results supported the findings of Eldeen and Van Staden (2007) who reported the antibacterial activity of dichloromethane, ethyl acetate and ethanol extracts of C. decidua twigs against B. subtilis, S. aureus, E. coli and P. pneumoniae and those of Nour and El-Imam (2013) on the methanol extract of stems.

All extracts exhibited high antifungal activity against *A. niger* and *C. albicans* with inhibition zone rangeing from 17 to 22 mm. Results of antifungal activity against *C. albicans* in this study were higher than those obtained by Nour and El-Imam (2013) who reported that the methanol extract was active while other tested extracts (chloroform and water) showed weak activity and they also found that

all extracts were ineffective against *A. niger*. These differences in results might be attributed to different extraction solvents which influence the biological activity (Sinero et al., 2008).

Antigiardial activity

The activity of different twig extracts of C. decidua against G. lambelia was investigated using three different concentrations and results are presented in Figure 1. All extracts possessed antigiardial activity and their activity was variable according to concentration of extract and exposure time. The petroleum ether extract was effective against G. lambilia and showed highest activity after 72 h where it gave 84% mortality for the highest concentration (500 µg/ml) compared with positive control, metronidazole drug, which gave 92% mortality. The antigiardial activity of the chloroform and ethyl acetate extracts was concentration dependent and the percentage mortality (91 and 89% respectively), after 24 h and at concentration 500 µg/ml, was comparable to that exhibited by the positive control (89%). The highest antigiardial activity of the n- butanol extract after 48 and 72 h gave mortality of 79%, at concentration 500 µg/ml. Thus, it was clear that, at concentration 500 µg/ml, the chloroform and ethyl acetate extracts possessed potent antigiardial activity after 24 h while, all extracts were highly effective after 72 h. Plants from Sudan were shown to possess antigiardial activity belonging mainly to the family Cucurbitaceae. Elhadi et al. (2013) found that seeds of Cucurbita maxima had potent antigiardial activity while Hassan et al. (2011) demonstrated that crude extracts as well as Cucurbitacin E and Cucurbitacin L 2-O- β -glucoside isolated from C. lanatus var. citroides possessed also strong antigiardial activity. To the best of our knowledge this is the report on the antigiardial activity of C. decidua.

Antimalarial activity

The antimalarial activity of petroleum ether, chloroform,

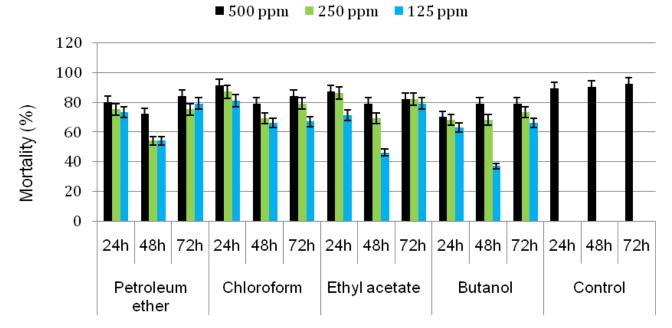


Figure 1. Antigiardial activity of twigs extracts of C. decidua against G. lambelia. Values are presented in mean ± SD (n = 3).

Table 2.	Antimalarial	activity	of	twigs	extracts	of	С.
deciduas	against Plas	modium	fa	lciparu	ım.		

Extract	IC₅₀ (µg/ml)
Petroleumether	233.11±6.5
Chloroform	7599.46±9.7
Ethylacetate	68.504±10.5
n- Butanol	136.4±9.9
Control (Chloroquine)	0.008±0.1

Values are presented in mean \pm SD (n =3).

Table 3. Antioxidant activity of twigs extracts of *C. deciduas.*

Extract	DPPH (%)	Iron chelating
Petroleum ether	48±0.02	Inactive
Chloroform	23±0.05	Inactive
Ethyl acetate	35±0.06	Inactive
Butanol	43±0.10	Inactive
Control*	92±0.04	96±0.04

*Ascorbic acid was used as a positive control forDPPH assay and chlorogenic acid for iron chelating assay. Values are presented in mean \pm SD (n =3).

ethyl acetate and n- butanol extracts of twigs of *C. decidua* was screened against *P. falciparum* and results are presented in Table 2. The antimalarial activity of different stem extracts of *C. decidua* was variable from moderate activity in the ethyl acetate extract (IC_{50} 68.5

 μ g/ml), to weak activity in both the n-butanol (IC₅₀ 136.4 μ g/ml) and petroleum ether (IC₅₀ 233 μ g/ml) extracts, to no activity in the chloroform extract (IC₅₀> 500 μ g/ml). These results were in agreement with the findings of Ali et al. (2002) who reported that the methanolic extract of *C. decidua* was less potent against both the chloroquine-sensitive *P. falciparum* K1 and NF54 strains.

Antioxidant activity

The *in vitro* antioxidant activity of the petroleum ether, chloroform, ethyl acetate and n-butanol extracts from twigs of *C. decidua* was evaluated using DPPH and FRAP assays. Results are shown in Table 3. The petroleum ether and n-butanol extracts showed moderate DPPH scavenging activity while those of ethyl acetate and chloroform revealed weak DPPH scavenging activity. All extracts were inactive in the FRAP assay. Previous study on *C. decidua* revealed that leaves, flowers and fruits have potent antioxidant activity, reducing different types of radicals as well as ferric reducing antioxidant power (Zia-UI-Haq et al., 2011).

Cytotoxicity

Cytotoxicity of petroleum ether, chloroform, ethyl acetate and butanol extracts of twigs of *C. decidua* were evaluated by brine shrimp lethality test and against vero cell lines using MTT assay (Table 4). All extracts displayed moderate toxicity to brine shrimps with LD_{50} value ranged from 262 and 228 µg/ml. Moreover,

IC ₅₀ (μg/ml)			
Brine shrimps	Vero cell lines		
262± 8.5	618±9.8		
228±7.0	421±7.7		
228±4.5	4315± 3.7		
262±3.6	22585±9		
7.45±0.1	9.3±0.01		
	Brine shrimps 262± 8.5 228±7.0 228±4.5 262±3.6		

Table 4. Cytotoxicity of twigs extracts of C. deciduas.

*Etoposide was used as a positive control for brine shrimps assay.Triton-x100 was used as the control for MTT assay. Values are presented in mean \pm SD (n =3).

cytotoxicity of extracts against vero cell lines revealed IC_{50} value ranged from 421 to 22585 µg/ml indicating that all extracts were virtually non-toxic (IC_{50} >90 ppm) (Khalighi-Sigaroodi et al., 2012).

Conclusion

The results obtained support some of the traditional uses of *C. deciduas* and may offer potential leads to new active natural products. Further phytochemical research is needed to identify the active principles.

Conflict of Interests

The authors have not declared any conflict of interests.

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