Antimicrobial Activity of Stem of *Capparis decidua* Edgew

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Summary

Capparis decidua Edgew commonly known as kair in Hindi is well known for curing a variety of ailments such as toothache, cough, asthma, intermittent fever and rheumatism. Petroleum ether, chloroform, ethyl acetate, ethanolic and aqueous extracts were screened for antimicrobial activity against bacteria and fungi.
The antimicrobial activity was carried out using different dilutions of different extracts (10mg/ml, 25mg/ml, 50mg/ml and 100mg/ml) against gram positive strains (*Staphylococcus aureus, Micrococcus luteus, Bacillus subtilus)* and gram negative ones (*Pseudomonas aeruginosa and Escherichia coli),* and Fungi like *Candida albicans* and *Aspergillus niger* by the cup-plate assay method and minimum inhibitory concentrations (MICs).
The minimum inhibitory concentration of chloroform extract against bacterial strains *staphylococcus aureus, Escherichia coli* was 12.5µg/ml and against *Micrococcus luteus, Pseudomonas aeruginosa and Bacillus subtilus* was 6.25µg/ml and the minimum inhibitory concentration of ethanolic extract against bacterial strains S*taphylococcus aureus, Micrococcus luteu* was observed 12.5µg/ml and against *Pseudomonas aeruginosa, Bacillus subtilus and Escherichia coli* was 6.25µg/ml. The minimum inhibitory concentration (MIC) of the both chloroform and ethanolic extract against *Candida albicans* was found 6.25µg/ml.
All the extracts have shown antimicrobial activity, but the chloroform and ethanolic extracts shows the significant zones of inhibition for all microorganisms studied and no MIC was found against Aspergillus *niger*.

Keywords: Antimicrobial activity, *Capparis decidua*, MIC, Microorganisms

Introduction

Medicinal plants are of important therapeutic aid for various ailments. It is estimated that an amount of 20,000 species from several families are useful for these purposes.[1] Furthermore, about 80% of the world population is dependent (wholly or partially) on plant-based drugs.[2] Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century.[3] Naturally occurring antimicrobials can be derived from plants, animal tissues, or microorganisms.[4] The shortcomings of the drugs available today propel the discovery of new pharmacotherapeutic agents in medicinal plants. [5]

In the present study we investigated the medicinal potential of the plant namely *Capparis decidua* belonging to the *Capparaceae* family. In the traditional system of medicine, the bark of the plant has been shown to be useful in the treatment of coughs, asthma and inflammation; roots used in fever and buds in the treatment of boils. In Unani, leaves act as appetizer, helps in cardiac troubles, fruits used in biliousness; Shoots along with shoots of Peganum harmala used as anti fertility drug; Ground stem and leaves used in alveolaris and pyorrhea; Root bark is used as anthelmintic and purgative; Wood coal used in muscular injuries.[6-8] In Sudan, *C. decidua* is used in swellings, jaundice and infection of joints.[9] *Capparis decidua* is rich sources of alkaloids, terpenoids, glycosides, sterols, some fatty acids and minerals. We herein report the antimicrobial activity of the crude extracts from the stem of *Capparis decidua* against a wide range of microorganisms which cause infectious diseases.

Material and Methods

Collection of plant material:

The stem of *Capparis decidua* was collected freshly from Loharu district Bhiwani (Haryana) in the month of August-September, 2008 depending upon its easy availability. It was authenticated by Dr. Minoo Parabia, Professor and Head, Department of Biosciences, Veer Narmad South Gujarat University against voucher specimen SA-1. The stem of *Capparis decidua* was subjected to shed drying and further crushed to powder, and then the powder was passed through the mesh 40.

Preparation of extracts:

The collected plant material was dried in the shade and ground to a powder. The dried and ground plant material (1.0 kg) was successively extracted with different solvents like petroleum ether, chloroform, ethyl acetate, ethanol and water for 72 hours each. The different solvent extracts were concentrated to dryness under reduced pressure. The obtained extracts were stored in a refrigerator at 4^0C until use.

Microbial strains:

Five strains of bacteria and two strains of fungi from the Microbial Type Culture Collection (MTCC, IMTECH), Institute of Microbial Technology Sector – 39A, Chandigarh – 160036, INDIA, were tested: *Pseudomonas aeruginosa* (MTCC 1688), *Staphylococcus aureus* (MTCC 737), *Bacillus subtilis* (MTCC 441), *Micrococcus luteus* (MTCC *106), *Escherichia coli* (MTCC 443), *Candida albicans* (MTCC 3017), and *Aspergillus niger* (MTCC 1344). All the strains were stored at freeze temperature until use.

Antimicrobial assays:

The antimicrobial activity was evaluated by Cup-Plate method.

I. **Culture media**: Nutrient agar (NA) (Himedia) containing bromocresol purple was used for the activation of *Bacillus* species, while NA was used for other bacteria. Sabouraud glucose agar (Himedia) was used for the activation of the fungi. The Nutrient agar was used in sensitivity assay. Nutrient broth was used for MIC determination.

II. **Chemicals for antimicrobial assay:** Ciprofloxacin and Nystatin (Central Drug House (P). LTD., New Delhi-110002., India) were used as positive reference standards (RA) for all bacterial and fungi strains respectively. The dimethylsulfoxide (DMSO) (Qualigenis) was used as solvent for the tested samples.

III. **Preparation of inoculums**: Bacterial inoculums were prepared by growing freeze-dried cells in Nutrient Broth for 24 h at 37^0C. Slants were prepared by streaking of these cell suspensions and sub culturing was done by using the same broth to provide initial cell counts of about 10^4 CFU/ml did sub culturing and incubated at 37^0C for required time. The filamentous fungi were grown on sabouraud dextrose agar (SDA) slants at 25^0C for seven days and the spores were collected using sterile doubled distilled water and homogenized.

IV. ***Preparation of test sample:*** The petroleum ether, chloroform, ethyl acetate extracts ethanolic and aqueous extracts were dissolved in 10% aqueous dimethylsulfoxide (DMSO) to obtain the different concentrations (10 mg, 25 mg, 50 mg and 100 mg per ml). Negative controls were used 10% aqueous dimethylsulfoxide (solvent control). Ciprofloxacin and Nystatin were used as positive reference standards having a concentration 5 µg per ml for all bacterial and fungi strains.

V. **Cup-plate method assay [10]:** Petri plates were prepared by pouring 30 ml of Nutrient Agar Medium for all the bacteria. The test organism was inoculated on solidified agar plate with the help of micropipette and spreaded and allowed to dry for 10 min. Three wells or cavities were made in agar containing each Petri dish by a sterilized steel borer. To these cavities standard and test compound solutions were filled. All the work was carried out under aseptic conditions for microbial assay. The plates for the bacteria were incubated at 37^0C ± 1^0C for 24 hours. The fungal strains *Candida albicans* and *Aspergillus niger* were incubated at 25^0C for 72 hours and seven days respectively. The antimicrobial potential of test compound was determined on the basis of mean diameter of zone of inhibition around the wells in millimeters. Each assay was carried out in the form triplicate three times. The results are shown in the Tables 1 & 2.

VI. **Minimum Inhibitory Concentration (MIC):** The experiment was according to two fold serial dilution method. The stock solution of test solutions (extracts) was prepared at concentration of 100µg/ml in nutrient broth and serially diluted up to five times. Six assay tubes were taken for screening of minimum inhibitory concentration of each strain. In the first tube 1ml of the sterilized nutrient broth was inoculated and then 1ml of the test compound solution was added and thoroughly mixed to concentration of 50µg/ml. Further dilutions of this solution were made by inoculating 1ml from first tube into second assay tube serially and 0.1 ml of each test inoculums were added in each tube and were done in duplicate. The procedures were conducted under aseptic conditions.

The inoculated tubes were kept at 37^0C ± 1^0C at 24 hours for bacterial assay and seven days for fungi (*Aspergillus niger*) & three days for fungi (*Candida albicans*) at 25^0C ± 0.1^0C during the incubation period. After the incubation period, tubes were removed and observed for any deposits or turbidity in the solution and shaken to suspend bacteria and fungi that might have been settled down. These concentrations were observed & assumed as minimum inhibitory concentration (MIC). The results are shown in the Tables 3 & 4.

Results and Discussion

Petroleum ether, chloroform, ethyl acetate, ethanolic and aqueous extracts were screened against bacteria and fungi. Among the tested extracts petroleum ether and ethyl acetate extracts shows activity against *Pseudomonas aeruginosa, Staphylococcus aureus*, *Bacillus subtilis* and aqueous extract against *Pseudomonas aeruginosa, Bacillus subtilis.* The chloroform and ethanolic extract shows activity against all used bacterial strains

(*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *and Micrococcus luteus*) [Table 1].

Table 1: Antibacterial activity of extracts of *Capparis decidua*

Extracts	Conc. (mg/ml)	Cup-plate method (inhibition zone, mm)				
		S. A.	M. L.	B. S.	P. A.	E. Coli
Petroleum ether	10	-	-	-	2.33 ± 0.058	-
	25	-	-	-	4.67 ± 0.058	-
	50	-	-	5.67 ± 0.058	5.67 ± 0.058	-
	100	2.67 ± 0.058	-	8.0 ± 0.1	8.67 ± 0.058	1.33 ± 0.058
Chloroform	10	-	-	3.0 ± 0.1	6.33 ± 0.057	-
	25	-	-	5.33 ± 0.115	10.33 ± 0.058	-
	50	4.33 ± 0.115	3.65 ± 0.058	7.67 ± 0.058	12.0 ± 0.1	-
	100	5.67 ± 0.058	5.33 ± 0.058	9.67 ± 0.058	14.33 ± 0.115	2.33 ± 0.058
Ethanolic	10	2.67 ± 0.115	-	-	10.33 ± 0.058	-
	25	5.33 ± 0.058	-	-	11.66 ± 0.058	1.67 ± 0.058
	50	6.33 ± 0.058	4.33 ± 0.058	3.67 ± 0.058	15.33 ± 0.115	3.67 ± 0.058
	100	8.33 ± 0.058	8.0 ± 0.10	7.67 ± 0.153	21.33 ± 0.058	5.67 ± 0.058
Ethyl acetate	10	-	-	-	6.33 ± 0.058	-
	25	-	-	5.33 ± 0.058	10.33 ± 0.058	-
	50	3.67 ± 0.058	-	8.0 ± 0.10	12.0 ± 0.1	1.23 ± 0.058
	100	5.67 ± 0.058	-	9.67 ± 0.058	14.33 ± 0.058	3.3 ± 0.058
Aqueous	10	-	-	5.0 ± 0.1	5.67 ± 0.058	-
	25	-	-	7.0 ± 0.1	9.33 ± 0.058	-
	50	-	-	9.33 ± 0.058	11.67 ± 0.058	-
	100	-	-	11.69 ± 0.058	13.33 ± 0.058	-
Ciprofloxacin	5 µg/ml	26 ± 0.051	14 ± 0.068	32 ± 0.024	25 ± 0.035	22 ± 0.056

S. A. –*Staphylococcus aureus*, M. L. – *Micrococcus luteus*, B. S. – *Bacillus subtilus*,

P. A. – *Pseudomonas aeruginosa*, E. coli **-** *Escherichia coli*,

- Sign shows no zone of inhibition

Petroleum ether, ethyl acetate and aqueous extract showed no activity against any of both fungi. The ethanolic and chloroform extract shows activity against *Candida albicans* but no activity against *Aspergillus niger* [Table 2]. The results of different extract were correlated with standard drug and shows that the chloroform and ethanolic extract shows good activity against all bacteria and one fungus. The petroleum ether, ethyl acetate and aqueous extract having no activity against fungi.

Table 2: Antifungal activity of extracts of *Capparis decidua*

Fungi	**Conc. (mg/ml)**	**Extracts (zone of inhibition, mm)**					
		P	**C**	**E. A**	**E**	**Aq.**	**N 5µg/ml**
Candida albicans	10	-	1.34 ± 0.058	-	1.33 ± 0.058	-	12.2 ± 0.087
	25	-	2.71± 0.059	-	2.67 ± 0.058	-	
	50	-	3.68 ± 0.058	-	3.67 ± 0.058	-	
	100	-	5.54 ± 0.059	-	5.33 ± 0.058	-	
Aspergillus niger	10	-	-	-	-	-	11.47 ± 0.065
	25	-	-	-	-	-	
	50	-	-	-	-	-	
	100	-	-	-	-	-	

P- Petroleum ether, C – chloroform, E. A- Ethyl acetate, E – Ethanolic, Aq – Aqueous and N – Nystatin; - Sign shows no zone of inhibition

The minimum inhibitory concentration of chloroform extract against bacterial strains was observed - *staphylococcus aureus, Escherichia coli* – 12.5µg/ml, *Micrococcus luteus, Pseudomonas aeruginosa, and Bacillus subtilus* – 6.25µg/ml and the minimum inhibitory concentration of ethanolic extract against bacterial strains was observed - S*taphylococcus aureus, Micrococcus luteu* – 12.5µg/ml, *Pseudomonas aeruginosa, Bacillus subtilus and Escherichia coli* – 6.25µg/ml [Table 3]. The minimum inhibitory concentration (MIC) of the both chloroform and ethanolic extract against *Candida albicans* was found – 6.25µg/ml and no minimum inhibitory concentration were found against *Aspergillus niger* [Table 4].

Table 3: The results showed the MIC for antibacterial activity

Microorganism	**Extract**	**Serial dilution (µg/ml)**					
		50	25	12.5	6.25	3.12	1.56
Staphylococcus aureus	CHF	-	-	-	+	+	+
	Eth.	-	-	-	+	+	+
Micrococcus luteus	CHF	-	-	-	-	+	+
	Eth.	-	-	-	+	+	+
Pseudomonas aeruginosa	CHF	-	-	-	-	+	+
	Eth.	-	-	-	-	+	+
Bacillus subtilus	CHF	-	-	-	-	+	+
	Eth.	-	-	-	-	+	+
Escherichia coli	CHF	-	-	-	+	+	+
	Eth.	-	-	-	-	+	+

- No growth; + Growth; CHF – Chloroform extract; Eth. – Ethanolic extract

Stock solution = 100 µg/ml

Table 4: The results showed the Minimum Inhibitory Concentration for antifungal activity

Microorganism	**Extract**	**Serial dilution (µg/ml)**					
		50	25	12.5	6.25	3.12	1.56
Candida albicans	CHF	-	-	-	-	+	+
	Eth.	-	-	-	-	+	+
Aspergillus niger	CHF	+	+	+	+	+	+
	Eth.	+	+	+	+	+	+

- No growth; + Growth

In conclusion, although the activities displayed by the ethanolic & chloroform extracts of *Capparis decidua* stems are significant, the results reported here, render this species interesting for future research.

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