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Phytochemical screening and antimicrobial activity of *Capparis zeylanica* stem extract

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Abstract
Medicinal plants are alternative rich source of antibacterial agents. The present study was an attempt to isolate the phytoconstituents present in the ethyl acetate fraction of *Capparis zeylanica* and to assess *in vitro* antimicrobial activity of different fractions of *Capparis zeylanica. Capparis zeylanica* belongs to Capparidaceae, found useful in various system of medicine. It possesses numerous pharmacological and phytochemical properties. The stems are shade dried powdered and subjected to extraction and fractionation. The fractions were subjected to column chromatography followed by isolation and characterisation of active components using TLC and spectrophotometric methods. The present research lead to the separation of β-sitosterol, α-amyrin and kaempferol a flavonoid derivative were identified and characterized by physical methods**.** The methanol, ethyl acetate, hexane and hydroalcoholic extracts of stems of *Capparis zeylanica* were evaluated for antimicrobial activity against gram+ve, gram-ve bacteria and fungi. Zone of inhibition diameters were observed. This activity was evaluated using disc diffusion method with a standard antibiotic and anti-fungal drug, penicillin and fluconazole. The antibacterial activity was not present in hexane fraction. Methanolic extract showed moderately good activity. However, highest antibacterial activity was found in Ethyl acetate fraction. The anti-fungal activity of the extract and fractions was moderately active compared to standard.

Keywords: *Capparis zeylanica*, phytochemical screening, antibacterial activity and antifungal activity

Introduction
Human beings have relied on natural products as a resource of drugs for thousands of years. Plant-based drugs have formed the basis of traditional medicine systems that have been used as traditional medicines for their primary health care [1]. There are 119 chemicals, derived from 90 plant species, which can be considered as important drugs in one or more countries [2, 3]. These plant-derived medicines not only validate the traditional knowledge but also can contribute in the development of better allopathic drugs. Bacterial ability to outsmart current drugs demands the continual supply of new drugs [4-6]. It has been a big challenge to find compounds with strong antibacterial potency and low toxicity. Exploration of plant-based extracts and compounds for the importance of antibacterial potency can help to fulfill such demand [6]. *Capparis zeylanica* Linn. (*C. zeylanica*) (*Capparis horrida* Linn, *Capparis brevispina* DC.) is known as Indian caper belonging to family Capparidaceae. It is a rigid, wiry and much branched shrub and widely distributed in Bangladesh, India, Srilanka and Malaysia [7]. Plants are 2–3m in height. Almost all the parts *i.e.* root, bark, fruits, leaves and seeds are used for different purposes. The root and bark of the plant are bitter and useful as tonic, expectorant, anthelmintic, emmenagogue, analgesic and also used in rheumatism, paralysis, tooth ache, enlarged spleen [8, 9]. In Unani medicine, the decoction of the root bark is prescribed as deobstruent to liver and spleen and as an anthelmintic and anti-inflammatory agent [10]. The phytochemical screening of this plant reported the presence of various phytochemicals like steroids, alkaloids, glycosides and flavonoids. So, in the present study we made an attempt to isolate different compounds from ethyl acetate fraction of *C. zeylanica* stems and to evaluate the antimicrobial activity of different fractions of *C. zeylanica*. The present paper reports a comparative potency of extracts obtained in different solvents from stem of *C. zeylanica* to inhibit Gram-positive, gram-negative bacteria and fungi.

Materials and methods
Plant collection and identification
The plant specimen was collected from Kondapalli near Vijayawada and voucher specimen was authentified by Department of Botany, ANU.
The bacterial strains of *Bacillus pumilus* (*B.P*), *Bacillus subtilis* (*B.S*), *Proteus vulgaris (P.V),*

Escherichia coli (*E.C*) and fungal strains *Aspergillus niger* (*A.N*), *Penicillium chrysogenum* (*PC*) were obtained from Biotechnology Laboratory, Chebrolu Hanumaiah Institute of Pharmaceutical Sciences.

Fig 1: Image of plant *C. zeylanica*

Preparation of the extracts

The extraction of stems of *Capparis zeylanica* was done by maceration using 80% alcohol and 20%water for 14 days. Then the extracted drug was then fractionated successively using hexane and ethyl acetate by mother liquor method. These fractions were subjected for preliminary phytochemical screening, using various qualitative tests for the detection of phytochemical constituents present in all three fractions. The ethyl acetate fraction on TLC over silica gel showed five distinct spots whereas the methanol soluble showed three spots. Since ethyl acetate extract showed positive for more constituents when compared to other extracts, it was subjected to column chromatography.

Column Chromatography of Ethyl acetate fraction of *C. zeylanica* extract

A normal Column Chromatography (CC) was used to fractionate compounds from fractions using silica gel as a stationary phase. 90 g of silica gel for column chromatography (CC) was mixed with hexane to form slurry and stirred using a stirring rod. 25g of the ethyl acetate extract was mixed with 1 g of silica gel and the mixture was dried in room temperature. Initially, hexane was gradually added into the column to remove fats, waxes and some chlorophyll. The polarity was increased by addition of EtOAC (0% -100%). The total volume used was 200 ml. 50 fractions were collected and left to dry at room temperature. Fractions were mixed according to the results obtained from TLCs. The use of concentrated sulfuric acid and vanillin as a spraying reagent revealed the presence of sterols in fraction 7 and 8. The crystals were named CZEA1. Another two compounds CZEA-2 and CZEA-3 were isolated from 11-15 and 35-40 fractions of column chromatography. All compounds were crystallized and the melting points of three compounds were noted. All the compounds were further subjected to TLC using several solvent systems to calculate the R*f* values.

Antimicrobial evaluation

Antimicrobial activity was evaluated using disc diffusion method with a standard antibiotic, 10 units/disc of penicillin. The ethyl acetate, hexane, hydroalcoholic fractions and methanolic extract were dissolved in Dimethyl Sulfoxide (DMSO) each in two concentrations of 125mg/ml and 250mg/ml to study the antimicrobial activity. The test organisms are seeded into clean test tube contains sterile nutrients agar media (20ml) for bacterial screening. 1µl of inoculums is mixed uniformly with 20 µl of sterile melted nutrient agar and cooled to 40°c and then poured into sterile petri dishes. When the agar solidifies, holes of uniform diameter are made using sterile borer. Stock solutions of the extracts at doses of 125 and 250mg/ml were prepared in Dimethyl Sulfoxide. Reference standard Penicillin was used at a dose of 10mg/ml in sterile water. 0.05ml each, of extract and reference standard were added into the well. DMSO was used as the negative control in the study. The bacterial plates were incubated at 37 °C for 24hrs and diameters of zones of inhibition were determined as an indication of activity. Antifungal activity was determined similarly but the test organisms are seeded into clean test tube containing sterile potato dextrose agar media (20ml) for fungal screening. Antifungal drug fluconazole was used as standard.

Results

Phytochemical Studies

Preliminary phytochemical screening was done for Hexane, ethyl acetate and hydro-alcoholic fractions of CM using various qualitative tests. The results of phytochemical screening are presented in Table 1. From the positive tests for steroids given by CZEA1, it is assumed to be a compound containing steroidal nucleus. CZEA1 is white crystalline substance with melting point 134-136 °C and Rf value 0.55 (EtAc/Hex: 1/3). Based on Rf value it is confirmed as β-sitosterol.

CZEA2, was isolated from hexane: ethyl acetate mixture (90:10) and was crystallized with a mixture hexane and ethyl acetate. It showed positive color reaction for tri-terpenoids with Liebermann burchard test. It is identified as α-amyrin based on its melting point (m.p) 186-187 ºC. Identity was further confirmed by Co-TLC with authentic sample.

CZEA3, was isolated from Hexane: ethyl acetate (50:50) fractions and was crystallized twice from ethyl acetate. Yellow needles were obtained with melting point 278 ºC. It showed positive color reaction for flavonoids with Shinoda's test. Its acetate was prepared where colourless feathery needles with m.p 184 ºC were obtained. From the above data, the properties of the original compound and its derivatives agreed with that of kaempferol and the identity was confirmed by comparison with authentic sample by Co-TLC. The Rf values of all compounds were presented in Table 2.

Table 1: Preliminary phytochemical analysis of fractions of *Capparis zeylanica*

S. No.	Test for	Hexane fraction of CZ	Ethyl acetate fraction of CZ	Hydro-alcoholic fraction of CZ
1	Alkaloids	-ve	+ve	-ve
2	flavonoids	-ve	+ve	-ve
3	Carbohydrates	-ve	-ve	+ve
4	Proteins	-ve	+ve	-ve
5	Tannins	-ve	-ve	+ve
6	Steroids	-ve	+ve	+ve
7	Fixed oils	+ve	-ve	-ve

Table 2: R_f values of compounds isolated

S. No.	Compound	Solvent system	R_f value	Compound
1	CZEA1	Hexane: Ethyl acetate (3:1)	0.55	β-Sitosterol
2	CZEA2	Pet-ether: Chloroform(9:1)	0.82	α-Amyrin
3	CZEA3	n-Butanol: Acetic acid: Water (4:1:5)	0.64	Kaempferol

Antimicrobial activity of different fractions of *C. zeylanica*

The fractions of *C. zeylanica* (*n*-hexane, Ethyl acetate and hydro-alcoholic) were responsive to both Gram-positive and Gram-negative bacteria to *B. pumilus, B. subtilis, E. coli* and *P. vulgaris* with clear inhibition of zones (Table 1). The results showed that the highest inhibition zones were recorded for the ethyl acetate fraction of *C. zeylanica* at a dose of 250mg/ml which were 22mm, 22mm, 21 mm and 22mm respectively. The results of anti-fungal activity indicate that the highest inhibition zones were observed with fluconazole, moderate anti-fungal activity was observed with methanolic extract and ethyl acetate, hydro alcoholic fractions of *C. zeylanica* The findings indicated that stem extract of *C. zeylanica* was more potent and relatively equivalent to the standard antibacterial agents and less potent against fungi when compared with standard drugs. This probably explained the use of the extract of this plant in traditional medicines against a number of infections.

Table 3: Antibacterial activity of *Capparis zeylanica* on gram +ve and gram –ve bacteria

S. No.	Extract of *C. zeylanica*	Zone of inhibition (mm)			
		B.s	***B.p***	***E.c***	***P.v***
1	Methanolic extract (125 mg/ml)	16	16	18	16
2	Methanolic extract (250mg/ml)	21	21	20	21
3	Hexane fraction (125,250mg/ml)	-	-	-	-
4	Ethyl acetate fraction (125mg/ml)	18	17	18	17
5	Ethyl acetate fraction (250mg/ml)	22	22	21	22
6	Hydro alcoholic fraction (125mg/ml)	15	15	17	16
7	Hydro alcoholic fraction (250mg/ml)	20	19	18	20
8	Penicillin (10 units/ml)	21	22	20	23
9	DMSO	-	-	-	-

B.s = Bacillus subtilis; B.p =Bacillus pumilis; E.c = Escherichia coli; P.v = Proteus vulgaris;

Table 4: Antifungal activity of *Capparis zeylanica*

S. No.	Extracts of *C. zeylanica*	Zone of inhibition (mm)	
		A.n	*p.c*
1	Methanolic extract (125mg/ml)	-	-
2	Methanolic extract (250mg/ml)	11	13
3	Hexane fraction(125, 250mg/ml)	-	-
4	Ethyl acetate fraction(125mg/ml)	-	-
5	Ethyl acetate fraction(250mg/ml)	12	14
6	Hydroalcoholic fraction(250mg/ml)	11	12
7	Fluconazole(10mg/ml)	22	18
8	DMSO	-	-

A.n = Aspergillus niger; P.c = Penicillium chrysogenum; - = No activity

Discussion

The antibacterial activities were found very much promising. Among all the fractions (*n*-hexane, ethyl acetate and hydro-alcoholic) of the stem of *C. zeylanica*, methanolic extract and ethyl acetate fraction showed good antibacterial activity and moderate anti-fungal activity. The present findings of *C. zeylanica* extracts demonstrate that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases [12]. These findings support the traditional knowledge of local users and it is a preliminary, scientific, validation for the use of the plants for antibacterial activity to promote proper conservation and sustainable use of such plant resources [13-14]. This study reveals that *C. zeylanica* will be possible source for new antibacterial substances against important pathogens of medically and veterinary importance.

Conflicts of interest statement

We declare that we have no conflict of interest.

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