ISSN: 2520-7695 (Print) and XXXX-XXXX (Online)
Medical and Health Sciences Research Journal
Med. Health Sci. Res. J., Jan-Apr 2017, 1(1): 66-69
Journal Homepage: http://www.mhsrjournal.com/
http://www.wollegajournals.com/

Original Research

Antimicrobial Activity of *Capparis zeylanica* L. and *Capparis sepiaria* L.

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Abstract

The present study was conducted to determine antimicrobial activity of leaf extract of two Capparidaceae members namely *Capparis zeylanica* L and *Capparis sepiaria* L. Antibacterial and antifungal activity of leaf extracts was screened by Agar well diffusion and Poisoned food technique respectively. The extracts exhibited varied antibacterial activity against test bacteria and high inhibitory activity was observed against Gram positive bacteria when compared to Gram negative bacteria. *Staphylococcus epidermidis* and *Escherichia coli* were susceptible to highest and least extent to leaf extracts respectively. Among leaf extracts, *C. zeylanica* extract exhibited stronger antibacterial activity when compared to *C. sepiaria*. Poisoning of medium with leaf extracts resulted in suppression of mycelial growth of test fungi to considerable extent. The extract of *C. zeylanica* displayed marked antifungal activity when compared to *C. sepiaria*. Least inhibitory activity was observed against *Fusarium* sp.

Article Information

Article History:
Received : 20-02-2017
Revised : 24-04-2017
Accepted : 26-04-2017

Keywords:
Capparis zeylanica L.
Capparis sepiaria L.
Agar well diffusion
Poisoned food technique

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INTRODUCTION

The genus *Capparis* L. encompasses shrubs or climbing shrubs (about 250 species) which are often armed with stipular spines and found distributed in tropical and subtropical regions. The genus includes perennial flowering shrubs that are known by the common name caper shrubs or caper bushes. The leaves are simple, entire and rarely reduced. Flowers are bisexual, bracteates, axillary or supra-axillary, solitary or in rows, in racemes or umbels. Sepals and petals are 4 in number and are free. Stamens are many, ovary on a gynophore, 1-celled. Fruit is a berry, globose or ellipsoid. Many species are medicinally important and are widely used in various traditional medicine systems including Ayurveda (Mishra *et al*., 2007; Sini *et al*., 2011; Bhat, 2014). *C. zeylanica* L. is a climbing or straggling shrub with tomentose branches armed with recurved stipular spines. It is called Anthundikai in Kannada and Govinda phala in Sanskrit. It is frequent along the hedges. Leaves are ovate-elliptic. Flowers are 3.5-5cm across, white, fading to pink or purple, in supra-axillary rows of 2-6 flowers, often developing before leaves. Flowering occurs between December to April. Fruits are said to be edible (Bhat, 2014). Several phytochemicals have been identified from different parts of the plant. The plant is used in traditional medicine and is reported to possess several biological activities (Mishra *et al*., 2007; Chopade *et al*., 2008; Sini *et al*., 2011; Balekari and Veeresham, 2015). *C. sepiaria* L. is an armed straggling shrub with tomentose branches and recurved thorns. Leaves are simple, ovate-oblong or oblong-lanceolate, 1.5-2 x 0.5-1 cm, glabrous in both sides with entire margin. Flowers are white and present in axillary, shortly peduncled umbels. Fruit is globose, 1 seeded and 0.8cm long. The plant is useful in skin diseases. The plant is used traditionally and is reported to exhibit several bioactivities. A number of compounds have been identified from various parts of the plant (Singh, 1988; Pullaiah and Mohammed, 2000; Manjunatha *et al*., 2004; Mishra *et al*., 2007; Selvamani *et al*., 2008; Kalpana and Prakash, 2015). In the present study, we determined and compared antibacterial and anifungal potential of leaf extract of *C. zeylanica* and *C. sepiaria*.

MATERIALS AND METHODS

Collection and Extraction of Plant Material

The plants were collected at outskirts of Shikaripura during January 2017. The plants were identified by referring standard flora (Manjunatha *et al*., 2004; Bhat, 2014). The leaves were removed from the plants and washed well using clean water and dried under shade. The shade dried leaves were powdered in a blender and extracted by maceration process using methanol as solvent. The powders were left in methanol for 48 hours with occasional stirrings. The contents were filtered through Whatman No. 1 filter paper and the filtrates were subjected to evaporation at 40^{o}C (Kekuda *et al*., 2016). The crude leaf extracts were stored in refrigerator.

Antibacterial Activity of Leaf Extracts

Antibacterial activity of *C. zeylanica* and *C. sepiaria* was evaluated by using Agar well diffusion method against a panel of 7 bacteria (Gram positive bacteria- *Staphylococcus aureus* NCIM 5345, *Staphylococcus*

epidermidis NCIM 2493, *Bacillus subtilis* NCIM 2063 and *Bacillus cereus* NCIM 2016; Gram negative bacteria- *Escherichia coli* NCIM 2065, *Pseudomonas aeruginosa* NCIM 2200 and *Salmonella typhimurium* NCIM 2501). The broth cultures of test bacteria were prepared by inoculating pure cultures of test bacteria into sterile Nutrient broth tubes followed by incubating the tubes at 37^{o}C for 24 hours. The broth cultures were swabbed uniformly on sterile Nutrient agar plates, wells of 8mm were punched in the inoculated plates and the wells were filled with leaf extracts (20mg/ml of Dimethyl sulfoxide [DMSO]), reference antibiotic (Chloramphenicol, 1mg/ml of sterile distilled water) and DMSO. The plates were incubated at 37^{o}C for 24 hours and the zones of inhibition were measured (Chopade *et al*., 2008; Kekuda *et al*., 2012). The experiment was done in triplicate and the results are recorded as Mean ± Standard deviation.

Antifungal Activity of Leaf Extracts

We evaluated antifungal activity of *C. zeylanica* and *C. sepiaria* by Poisoned food technique (Junaid *et al*., 2014; Kekuda *et al*., 2016) against 6 fungi namely *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Alternaria* sp., *Curvularia* sp. and *Fusarium* sp. isolated previously from moldy grains of sorghum. The control (without extract) and poisoned Potato dextrose agar (PDA; 1mg extract/ml of medium) plates were inoculated aseptically with well sporulated cultures of test fungi. The plates were incubated at room temperature for 4 days and the colony diameter of test fungi was measured in mutual perpendicular directions. Antifungal potential of leaf extracts, in terms of inhibition of mycelial growth of test fungi (%), was calculated using the formula:

Inhibition of mycelial growth (%) = (C – T / C) x 100,

where C and T refers to colony diameter of test fungi on control and poisoned plates respectively. The experiment was done in triplicate and the results are recorded as Mean ± Standard deviation.

RESULTS

The result of antibacterial activity of leaf extract of *C. zeylanica* and *C. sepiaria* is shown in Table 1. The extracts exhibited inhibitory activity against all test bacteria. Among extracts, extract of *C. zeylanica* displayed marked inhibition of test bacteria when compared to *C. sepiaria*. *S. epidermidis* and *E. coli* were inhibited to high and least extent respectively. Among bacteria, Gram positive bacteria were susceptible to extracts to higher extent when compared to Gram negative bacteria. Reference antibiotic caused marked antibacterial activity when compared to extracts while DMSO did not cause inhibition of any test bacteria.

Table 1: Antibacterial activity of *C. zeylanica* and *C. sepiaria*

Test bacteria	Zone of inhibition in cm			
	C. sepiaria	***C. zeylanica***	**Antibiotic**	**DMSO**
S. aureus	1.50±0.00	1.67±0.06	3.20±0.00	0.00±0.00
S. epidermidis	1.67±0.06	1.90±0.00	3.39±0.06	0.00±0.00
B. cereus	1.50±0.00	1.77±0.06	3.43±0.06	0.00±0.00
B. subtilis	1.57±0.06	1.70±0.00	3.53±0.12	0.00±0.00
E. coli	1.00±0.10	1.17±0.06	2.33±0.06	0.00±0.00
S. typhimurium	1.17±0.06	1.43±0.06	2.90±0.00	0.00±0.00
P. aeruginosa	1.33±0.12	1.53±0.06	2.97±0.06	0.00±0.00

Table 2 and Figure 1 show the result of antifungal potential of leaf extract of *C. zeylanica* and *C. sepiaria*. Considerable reduction in the mycelial growth of test fungi was observed in plates poisoned with leaf extracts. Here also, extract of *C. zeylanica* exhibited marked inhibitory effect when compared to *C. sepiaria*. Extract of *C. sepiaria* showed highest and least inhibitory activity against *A. niger* and *A. fumigatus* and *Fusarium* sp. respectively. *Curvularia* sp. and *Fusarium* sp. were inhibited to highest and least extent respectively by extract of *C. zeylanica*. Among *Aspergillus* species, *A. niger* displayed highest susceptibility to extracts followed by *A. flavus* and *A. fumigatus*.

Table 2: Colony diameter of fungi in plates poisoned with *C. zeylanica* and *C. sepiaria*

Treatment	Colony diameter in cm					
	A. niger	***A. flavus***	***A. fumigatus***	***Curvularia* sp.**	***Alternaria* sp.**	***Fusarium* sp.**
Control	5.63±0.12	4.20±0.17	3.83±0.06	4.37±0.15	5.07±0.12	4.17±0.12
C.sepiaria	2.07±0.12	2.27±0.12	2.33±0.06	1.63±0.12	2.33±0.15	2.53±0.06
C.zeylanica	1.63±0.06	1.97±0.06	1.83±0.06	1.07±0.12	1.57±0.12	2.40±0.00

DISCUSSION

Interest in plants with antibacterial potential has been triggered in recent years due to drawbacks associated with the use of antibiotics such as high cost, side effects and emergence of resistant pathogens in hospital as well as community settings. Antibacterial activity determination of a number of plants highlighted potential inhibitory activity of plants against a wide range of pathogenic bacteria including drug resistant bacteria (Modi *et al*., 2011; Paul *et al*., 2013; Sandrasagaran *et al*., 2014; Valle *et al*., 2015). In the present study, we screened antibacterial activity of leaf extract of *C. zeylanica* and *C. sepiaria* by agar well diffusion. This method is one of the extensively used in vitro methods used to evaluate antibacterial activity of plants (Chopade *et al*., 2008; Balouiri *et al*., 2016). Overall, *C. zeylanica* was more effective in causing inhibition of test bacteria when compared to *C. sepiaria*.

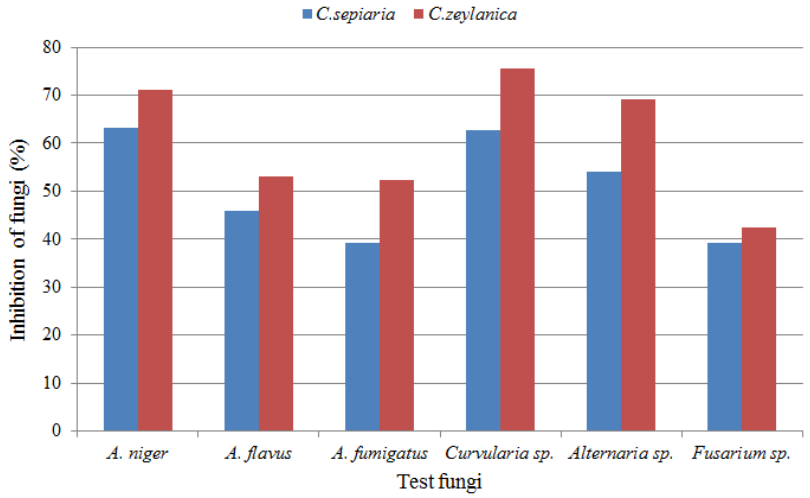

Figure 1: Inhibition of test fungi (%) by *C. zeylanica* and *C. sepiaria*

The leaf extract of *C. zeylanica* showed marked antibacterial activity against Gram positive bacteria when compared to Gram negative bacteria. *S. epidermidis* was inhibited to high extent while other Gram positive bacteria were susceptible to more or less similar extent. Among Gram negative bacteria, *P. aeruginosa* was inhibited to higher extent while least inhibitory activity was observed against *E. coli*. Various parts of *C. zeylanica* were shown to exhibit antibacterial activity. Petroleum ether, chloroform, ethanol and water extract of root of *C. zeylanica* exhibited *in vitro* antibacterial activity (Chopade *et al*., 2008). In a study by Priya *et al*. (2012), ethyl acetate extract from leaves of *C. zeylanica* exhibited marked antibacterial activity against reference bacteria and clinical isolates. Pendyala *et al*. (2016) revealed moderate and high antibacterial activity of methanol and ethyl acetate extract of stem respectively. Recently, Haque *et al*. (2016) showed antibacterial activity of various solvent extracts of leaf, root and stem of *C. zeylanica* against Gram positive and Gram negative bacteria. It was observed that chloroform and methanolic extract displayed marked antibacterial activity.

Extract obtained from *C. sepiaria* leaf has also displayed antibacterial activity in this study but to a lesser extent when compared to *C. zeylanica* leaf. The nature of inhibition of Gram positive and Gram negative bacteria by leaf extract of *C. sepiaria* was similar to that of antibacterial activity displayed by *C. zeylanica* i.e., *C. sepiaria* extract displayed marked inhibition of Gram positive bacteria and highest and least inhibitory activity was observed against *S. epidermidis* and *E. coli* respectively. In a previous study, the chloroform and ethanol soluble fractions from stem of *C. sepiaria* have displayed antibacterial activity (Satyanarayana *et al*., 2010). In another study, Sundaram *et al*. (2011) revealed the concentration dependent antibacterial potential of aqueous and ethanolic extract of leaf of *C. sepiaria* against Gram negative enteric bacteria. Kalpana and Prakash (2015) found marked antibacterial activity of leaf and fruit extracts of *C. sepiaria* against Gram positive and Gram negative bacteria with highest activity being displayed by fruit extract.

Indiscriminate use of synthetic fungicides in order to manage fungal diseases of plants has created environmental pollution and adverse effects against non-target organisms. Besides, these fungicides are costly and cannot be afforded by many farmers. Higher plants have been considered as potential alternates for these chemical agents and many studies have shown marked antifungal activity of plants against various fungal pathogens (Albera *et al*., 2011; Junaid *et al*., 2014; Rodino *et al*., 2014; Neela *et al*., 2014; Kekuda *et al*., 2016). Poisoned food technique is one among the various *in vitro* antifungal methods being used to evaluate antifungal potential of plants (Junaid *et al*., 2014; Rodino *et al*., 2014; Neela *et al*., 2014; Kekuda *et al*., 2016). In the present study, we evaluated antifungal activity of *Capparis* species by Poisoned food technique against 6 seed-borne fungi. When compared to *C. sepiaria*, extract of *C. zeylanica* exhibited marked suppression of mycelial growth of test fungi as evidenced by considerable reduction in the colony diameter on poisoned plates. It was observed that 3 and 5 out of 6 test fungi were inhibited to more than 50% by extract of *C. sepiaria* and *C. zeylanica* respectively. *Fusarium* sp. was least susceptible to extracts. In a previous study, aqueous extract of leaves of *C. zeylanica* exhibited marked antifungal effect against *Trichophyton rubrum* when compared to other solvent extracts (Priya *et al*., 2012). Pendyala *et al*. (2016) revealed the potential of stem extract of *C. zeylanica* to inhibit *A. niger* and *Penicillium chrysogenum*. The study of Chopade *et al*. (2008) revealed lack of antifungal activity of root of *C. zeylanica* against *A. niger* and *Candida albicans*.

CONCLUSIONS

Both *C. zeylanica* and *C. sepiaria* showed antibacterial and antifungal activity. Among the *Capparis* species selected, marked inhibitory activity was shown by *C. zeylanica*. From the results of this study it can be concluded that the selected plants can be exploited as sources of antimicrobial agents which can be used against microbial infections. Formulations prepared using these plants can be used against bacterial infections and seed mycoflora and other phytopathogenic fungi. Further studies on purification of secondary metabolites from leaves and their antimicrobial activity have to be carried out.

Conflict of Interest

None declared.

Acknowledgements

Authors thank Head of the Department of Microbiology and Principal of S.R.N.M.N College of Applied Sciences for providing facilities to conduct work. Authors thank N.E.S, Shivamogga for moral support. Authors thank Dr. Vinayaka K.S for assisting in collection and identification of plant.

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