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Original Article

Phytochemical Screening and *in-vitro* Antimicrobial Activity of *Citharexylum spinosum* L. (Verbenaceae)

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ABSTRACT

Objectives: The present study was conducted to evaluate antibacterial and antifungal activity of leaf and bark extract of *Citharexylum spinosum* L. (Verbenaceae). **Methods:** Extraction of powdered leaf and bark material was carried out by maceration process. The extracts were screened for detection of phytochemicals by standard phytochemical analyses. Antibacterial and antifungal activities were evaluated by agar well diffusion and poisoned food technique, respectively. **Results:** Leaf extracts showed marked antibacterial activity than bark extract. Both extracts showed highest and least inhibitory activity against *Bacillus cereus* and *Shigella flexneri*, respectively. Both extracts caused >50% inhibition of mycelial growth of two seed-borne fungi viz. *Aspergillus niger* and *Bipolaris* sp. Preliminary phytochemical analysis detected the presence of saponins, alkaloids, flavonoids, sterols and triterpenoids in both the extracts. **Conclusions:** The plant *C. spinosum* can be used to treat infections caused by pathogenic bacteria and to manage seed-borne fungal diseases of plants.

Key words: *Citharexylum spinosum*, Maceration, Phytochemical, Antibacterial, Antifungal

1. INTRODUCTION

Citharexylum spinosum L., (synonym *C. quadrangulare* Jacq., *C. fruticosum* L.) belonging to the family Verbenaceae, is a large shrub or small tree (growing to a height of 12-15m and around 1m in diameter) with tetragonal braches and branchlets. In Greek, kithara means lyre and xylon refers to wood. The plant is known by the names fiddlewood, spiny fiddlewood and Florida fiddlewood and is native of tropical America. The plant is pan-tropical in distribution. The trunk of the tree is straight. Leaves are opposite, up to 28x11cm, elliptic-oblong, acute or obtuse at

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apex, entire or dentate; petiole is up to 2.4cm long. Flowers are minute, fragrant, white in color and occur in long drooping racemes. Calyx is small and corolla is white in color. Fruit is drupaceous with 2 pyrenes; pyrenes 2-celled and 2-seeded. Flowering occurs more or less throughout the year[1,2]. The plant *C. spinosum* is often grown as ornamental plant and shade plant. Wood is hard and is used for making fence, some instruments especially musical instruments and for general building construction[3,4]. The plant is used traditionally as antipyretic, diuretic and antiarthritic and for treating liver disorders[5]. In Trinidad and Tobago, the leaves of the plant are traditionally used as anthelmintic[6]. Phytochemicals viz. stigmasterol, spinoside, β-sitosterol, tunispinosides, verbascoside, durantoside I, methyl benzoate, lamiide, lamiidoside, duranterectoside C, and many others have been identified in the plant[7-12]. The plant is reported to exhibit antiallergic[13], radical scavenging and antioxidant[14,15], antibacterial[8], antifungal[16,17], allelopathic[11], anti-inflammatory[10], gastroprotective[10], and antipyretic[10] activity. In the present study, we screened the in vitro antibacterial and antifungal activity of leaf and bark extract of *C. spinosum*.

2. MATERIALS AND METHODS

Collection, identification and extraction

The plant materials were collected at Haniya, Hosanagara Taluk, Shivamogga district, Karnataka in the month of December 2017. The plant was identified by Dr. Vinayaka K.S, Principal, KFGC, Shikaripura. The leaf and bark were cleaned, dried under shade and powdered. Extraction of powdered materials was carried out by maceration technique. Methanol was used as extraction solvent. Solvents were evaporated and crude leaf and bark extracts were obtained[18]. Leaf and bark extracts were screened for detection of phytochemicals by standard tests[19].

Test bacteria

Four bacteria viz. *Bacillus cereus*, *Shigella flexneri*, *Salmonella typhimurium* and *Escherichia coli* were used for screening their susceptibility to extracts.

Antibacterial activity of leaf and bark extracts

Agar well diffusion method[18] was used to evaluate antibacterial activity of leaf and bark extracts (20mg extract/ml of DMSO) against test bacteria. Streptomycin (1mg/ml of sterile distilled water) was used as standard antibiotic. Zones of inhibition formed around the wells were measured using a ruler.

Test fungi

Two seed-borne fungi viz. *Aspergillus niger* and *Bipolaris* sp. were used to assess their susceptibility to extracts.

Antifungal activity of leaf and bark extracts

Poisoned food technique[18] was carried out to investigate antifungal activity of leaf and bark extracts. Test fungi were aseptically inoculated on control (without extract) and poisoned (1mg extract/ml of medium) potato dextrose agar medium. Diameter of fungal colonies was recorded in mutual perpendicular directions after an incubation period of 96 hours. Extent of reduction in mycelial growth (%) was calculated using the formula:

Reduction in mycelial growth (%) = (Dc – Dt / Dc) x 100, where 'Dc' and 'Dt' denotes the diameter of colonies in control and poisoned plates, respectively.

Statistical analysis

Experiments were conducted in triplicates. Results are presented as Mean±Standard deviation (S.D) of 3 trials.

3. RESULTS AND DISCUSSION

Plants contain a wide range of chemical substances (metabolites) termed as phytochemicals that falls into two categories viz. primary and secondary metabolites. Plant secondary metabolites are important as they provide protection against herbivores and pathogens. Due to advancement in analytical techniques, a huge number of secondary metabolites have been isolated from plants and their chemical nature is characterized[19-23]. Medicinal values of plants are due to many of secondary metabolites as experimental results have revealed the potent biological activities of plant secondary metabolites such as antibacterial, antioxidant and anticancer activity[24-28]. Various methods are available for extraction of powdered plant materials. In the present study, we extracted powdered leaf and bark material of *C. spinosum* by maceration process using methanol as extraction solvent. Several studies have shown that methanol can efficiently extract a variety of plant secondary metabolites[21,29-32]. Preliminary phytochemical analysis of leaf extract of *C. spinosum* revealed the presence of all phytochemicals in leaf extract. In the bark extract, all phytochemicals except tannins and glycosides were detected (Table 1). Mohammed et al.[10] revealed the presence of phytochemicals viz. glycosides, flavonoids, sterols, triterpenes, saponins and tannins.

Table 1: Phytochemicals in leaf and bark extract of *C. spinosum*

Phytochemical	Leaf extract	Bark extract
Saponins	+	+
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	-
Glycosides	+	-
Sterols	+	+
Triterpenoids	+	+
'+' Detected; '-' Not detected		

Antibacterial activity of leaf and bark extracts

Need for alternative strategies for therapy against infectious bacteria arose due to drawbacks, such as development of resistance in pathogenic bacteria, high cost and adverse effects on the health, which are associated with the use of antibiotics. Plants and their metabolites are one among the potential alternatives being used in disease therapy. It is shown from several studies that crude extract and isolated components from plants possess antibacterial activity against a wide range of bacteria[18,19,24,33-36]. Agar well diffusion

methods is one of the widely used antibacterial screening assays[37]. In this method, the formation of zones of inhibition of bacterial growth around the wells is considered positive for antibacterial activity. In the present study, both leaf and bark extracts of *C. spinosum* exhibited inhibitory activity against all test bacteria as evidenced by the zones of inhibition formed around wells (Table 2). Overall, leaf extract caused higher inhibition of test bacteria when compared to bark extract. Leaf and bark extracts showed highest and least inhibitory activity against *B. cereus* and *S. flexneri* respectively. The susceptibility of test bacteria to both the extracts was in the order: *B. cereus* > *S. typhimurium* > *E. coli* > *S. flexneri*. Reference antibiotic displayed marked inhibition of test bacteria when compared to extracts. No inhibitory activity was observed in case of DMSO. The study of Mar and Pripdeevech[8] revealed the antibacterial activity of essential oil from the flowers of *C. spinosum*.

Table 2: Antibacterial activity of leaf and bark extract of *C. spinosum*

Test bacteria	Zone of inhibition (cm)			
	Leaf extract	Bark extract	Antibiotic	DMSO
B. cereus	1.93±0.05	1.60±0.00	3.00±0.00	0.00±0.00
E. coli	1.60±0.00	1.43±0.05	3.53±0.05	0.00±0.00
S. flexneri	1.40±0.00	1.40±0.00	3.30±0.00	0.00±0.00
S. typhimurium	1.63±0.05	1.50±0.00	3.13±0.05	0.00±0.00

Antifungal activity of leaf and bark extracts

Interest in scientific community on plants with antifungal activity was intensified due to the adverse effects of synthetic fungicides. Indiscriminate use of fungicides often results in environmental pollution and emergence of resistant strains of phytopathogenic fungi. Numerous studies have shown the potential of crude solvent extracts and isolated constituents of plants to exhibit antifungal activity against a range of phytopathogenic fungi including seed-borne fungi[17,18,38-42]. Poisoned food technique is one among the widely used antifungal screening methods and suppression of mycelial growth of test fungi in poisoned plate is considered positive for antifungal activity[37]. In the present study, we employed poisoned food technique to evaluate antifungal activity of *C. spinosum*. Poisoning of potato dextrose agar medium with leaf and bark extract (1mg extract/ml of medium) resulted in considerable suppression of mycelial growth of both the fungi indicating antifungal activity. Both the fungi were inhibited to >50% by both the extracts. Among extracts, marked antifungal potential was shown by leaf extract when compared to bark extract. The extent of inhibition of *A. niger* by leaf and bark extract was 61.74% and 58.83%, respectively while the inhibition of *Bipolaris* sp. by leaf and bark extract was 66.00% and 53.33%, respectively (Table 3). In an earlier study, methanol extract obtained from the wood of *C. spinosum* was shown to inhibit fungi viz. *Penicillium selerotigenum*, *Paecilomyces variotii*, and *Aspergillus niger*[16]. Aqueous and organic extracts of *C. spinosum* were effective in causing inhibition of phytopathogenic fungi viz. *Fusarium culmorum*, *F. graminearum*, *Aspergillus flavus*, *A. niger* and *A. fumigatus*[17].

Table 3: Antifungal activity of leaf and bark extract of *C. spinosum*

Treatment	Colony diameter in cm	
	A. niger	***Bipolaris* sp.**
Control	5.83±0.05	4.50±0.00
Leaf extract	2.23±0.05	1.53±0.05
Bark extract	2.40±0.00	2.10±0.00

4. CONCLUSION

From the results, it can be concluded that the leaf and bark extract of *C. spinosum* exhibits antimicrobial activity. The observed antimicrobial activity of extract could be due to the presence of phytochemicals such as alkaloids, flavonoids, saponins and triterpenoids in the extracts. The plant can be used against infectious agents in an appropriate formulation. Isolation of active principles and their biological activity determinations are to be carried out.

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