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Phytochemical analysis and *in vitro* antimicrobial activity of stem bark extracts of *Albizia lebbeck* (L.) Benth

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ABSTRACT

Antimicrobial activity of stem bark of Albizia lebbeck was studied against seven pathogenic bacteria and three fungal strains by agar well diffusion method. Antimicrobial activity was recorded for hexane, chloroform, methanol, ethanol and aqueous extracts. Alcohol (ethanol and methanol) extracts exhibited higher degree of antimicrobial activity compared to chloroform, hexane and aqueous extracts. Escherichia coli *was turned out be the most susceptible bacterium to the crude stem bark chemical constituents using the standard tetracycline and nystatin. Minimum inhibition concentration values of hexane, chloroform, methanol, ethanol and aqueous extracts determined by the agar dilution method ranged between 31.2 and 1000 µg. The study suggested that the stem bark extracts possess bioactive compounds with antimicrobial activity against the tested bacteria and fungi revealing a significant scope to develop a novel broad spectrum of antimicrobial drug formulation from A. lebbeck.*

INTRODUCTION

Albizia lebbeck (L.) Benth. (Fabaceae) is a deciduous tree well known in the Indian subcontinent, particularly the areas of Southeast Asia with marked dry season. It is often simply called "siris" and is widely used in Indian traditional system and folk medicine. Plant bark rough, grey, somewhat flaky; inner bark reddish. It is an astringent, also used by some cultures to treat boils, cough, flu, gingivitis, lung problems, and pectoral problems. It is used as a tonic to treat abdominal tumors [8]. Decoction of leaves and bark are protective against bronchial asthma and other allergic disorders [28]. Bark and seeds are astringent and are given in piles and diarrhea. Bark is also used in toothache and diseases of gum. Phytochemical investigation showed that the bark contains saponins, macrocyclic alkaloids, anthraquinone glycosides, tannins and flavonoids [24]. The albiziasaponin A, albiziasaponin B and albiziasaponin C have been isolated from the bark [22]. The main active constitute of bark extract is anthraquinone glycosides and is active against aerobes and mechanism of the action is the leakage of the cytoplasmic constituents [9]. There are also lots of data on therapeutic properties of anticonvulsive activity [15], nootrophic and anxiolytic activity [29], antiulcerogenic activity [7], antifertility effect [10,11], antispermatogenic, antiandrogenic activity [12], alleric rhinitis [23], analgesic and anti inflammatory effect [1], antioxidant activity [20],

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antidiuretic activity [27], antimicrobial activity of leaves [5,24], flowers [13], pods, seeds, flowers and roots [26], flowers and pods [21]. Based on few reports on antimicrobial activity of sapwood heartwood and bark [25], leaves and bark [18], of this plant the present study was carried out to phytochemical analysis and antimicrobial activity of different solvent and aqueous extracts of stem bark of *A. lebbeck*.

MATERIALS AND METHODS

Chemicals, Media and Antibiotics

The organic solvents i.e., hexane, chloroform, methanol, ethanol and dimethyl sulphoxide (DMSO) were obtained from Rankem company, India. Nutrient broth, Nutrient agar and Saboraud dextrose agar were obtained from Hi-media, Mumbai, India. The antibacterial agent Tetracyclin was obtained from Axiom Laboratories Ltd., India.

Stem bark collection

The stem bark of *A. lebbeck* was collected from Sudikonda forest, East Godavari district, Andhra Pradesh. The specimen was authenticated by Prof. Vatsavaya S. Raju, Plant Systematics Lab, Kakatiya University, Warangal and voucher specimen was deposited in the Herbarium, Botany department (BDH), Andhra University, Visakhapatnam.

Stem bark extract

The stem bark was dried in shade (25 - 28 ^{0}C) for a month. The dried stem bark was ground using a mechanical grinder. Sequential extraction of it was done using hexane, chloroform, followed by methanol and finally ethanol. The filtrates were concentrated by removing the solvents under reduced pressure at 40 ^{0}C using a rotary evaporator. The concentrated crude extracts were labeled and stored at 4 ^{0}C [2].

Simultaneously, the aqueous extract of the stem bark was prepared by adding boiled water to the powdered in a beaker on water bath, with occasional stirring for 4 hrs. The aqueous extract was then filtered and reduced under pressure. At the time of testing known quantity of crude (100, 200, 300 mg/ml) were dissolved in DMSO.

Microbial strains and Growth conditions

Seven bacterial strains namely *Bacillus subtilis* (MTCC 2763), *Escherichia coli* (MTCC 2960), *Klebsiella pneumoniae* (MTCC 4032), *Pseudomonas aeruginosa* (MTCC 6642), *Proteus vulgaris* (MTCC 1771), *Staphylococcus aureus* (MTCC 7443), *Streptomyces pneumoniae* (MTCC 1935) and three fungal strains *Aspergillus niger* (MTCC 4325), *Candida albicans* (MTCC 4748) and *Saccharomyces cerevisiae* (MTCC 4742) were procured from IMTECH, Chandigarh, India. Broth and agar were prepared according to the manufacturer's instructions.

Before testing, the bacterial suspension was transferred to nutrient broth and cultured at 37 ^{0}C. Inoculates were prepared by adjusting the turbidity of the medium to match the 0.5 MC farland standard. The fungal cultures were maintained on Saboraud dextrose agar, incubated at 25 ^{0}C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in 100 ml of sterile normal saline and then suspension was stored in refrigerator till used.

Determination of Antimicrobial activity

Antibacterial and antifungal activity of stem bark extracts of *A. lebbeck* were determined using agar well method [3] (Aniel kumar et al 2010). For susceptibility test, 100 µl of inoculums, equivalent to 10 CFU was mixed with 6 ml of nutrient agar (to ensure even distribution of bacteria) and poured immediately into the sterile petriplates. The petriplates were left to solidify for 10 minutes. A sterilized 6 mm borer was used to make wells in the centre of the divided areas. About 50 µl each extract was then pipette into the wells. Petriplates with bacteria and test extracts was incubated at 37 ^{0}C for 16-18 hr after which the inhibition zone (IZ) was measured using an antibiotic zone reader scale (HiAntibiotic ZoneScale-c).

For the antifungal activity, the same method as for bacteria was adopted of nutrient agar, Saboraud dextrose agar was used. The inoculated medium was incubated at 25 ^{0}C for two days for the *C. albicans*, *S. cerevisiae* and three days for *A. niger*. About 500 µg of nystatin was dissolved in 1

ml of sterile de ionized water. About 10 µl of 0.5 mg/ml nystatin (equivalent to 5 µg dose) and 10 µl of DMSO was pipette into wells. For bacteria multidrug antibiotic disc was used (Axiom Laboratories Ltd., India). The experiments were conducted in triplicates each and diameter of the IZ surrounding each well was recorded.

The extracts that exhibited IZ were subjected to minimum inhibition concentration (MIC) assay by using serial two-fold dilution [16]. A quantity of 0.6 g of each extract was dissolved in 300 ml sterile nutrient broth which yields initial concentration of 2000 µg/ml. Subsequently, two-fold serial dilution was made from the stock to obtain 1000, 500, 250, 125, 62.5, 31.2 µg/ml concentrations. One ml of standardized inoculums of each test organism was introduced into each extract nutrient broth mixture and then incubated at 37^0C. The lowest concentration inhibiting growth was regarded as the MIC of the extracts. For the fungi, the inoculated medium was incubated at 25^0C for two (*C. albicans*, *S. cerevisiae*) to three (*A. niger*) days.

Statistical analysis

Each experimental data from triplicates was subjected to one way ANOVA using Minitab version 15. A significant level of 0.05 was used for all statistical analyses.

RESULTS AND DISCUSSION

The antimicrobial activity of the five different solvent extracts of *A. lebbeck* revealed that the ethanol extract had significant activity against all the tested microorganisms followed by methanol extract, while the chloroform and hexane extracts possessed moderate activity and the aqueous extract of least activity (Table 1). The results of the present study are significant at level of $p>0.05$.

Ethanol extract exhibited the highest IZ against *E. coli* while methanol was against *E. coli, K. pneumoniae, S. pneumoniae*, and chloroform extract against *K. pneumoniae* and *P. aeruginosa*. Hexane extract exhibited the highest zone of inhibition against *S. aureus* and *C. albicans* whereas aqueous extract against *S. pneumoniae*. *E. coli, P. aeruginosa, P. vulgaris* and *C. albicans* were sensitive organisms to the all the solvent extracts. *A. niger* was the resistant to the chloroform, hexane and aqueous extracts while *B. subtilis, K. pneumoniae*, and *S. aureus* were resistant to the aqueous extract and *S. pneumoniae* to the hexane extract of *A. lebbeck* stem bark. On comparison with standard antibiotics, ethanol extract was showed broad spectrum of antimicrobial activity except *P. aeruginosa* and *S. cerevisiae*, they exhibited less IZ values than antibiotics. Methanol extract was also showed broad spectrum antimicrobial activity against *B. subtilis, E.coli, S. pneumoniae* and *A. niger*.

Table 1: Antimicrobial activity of different extracts of *A. lebbeck* stem bark.

Or	Zone of inhibition (mm)															Controls	
	Methanol extract			Ethanolextract			Chloroform extract			Hexane extract			Aqueous extract				
	100	200	300	100	200	300	100	200	300	100	200	300	100	200	300	T/N	D
BS	12±0.4	14±0.1	21±0.4	16±0.9	18±0.2	20±0.5	–	–	12±0.3	–	–	10±0.3	–	–	–	18^T	–
EC	13±0.0	17±0.8	23±0.0	15±0.8	20±0.3	26±0.3	11±0.6	12±0.5	18±0.0	–	10±0.7	13±0.1	11±0.5	12±0.2	14±0.0	22^T	–
KP	12±0.4	16±0.4	23±0.3	16±0.4	21±0.4	24±0.3	12±0.4	16±0.4	20±0.0	–	–	12±0.1	–	–	–	24^T	–
PA	14±0.5	16±0.8	18±0.4	15±0.2	17±0.6	19±0.2	16±0.2	18±0.4	20±0.3	11±0.4	13±0.3	15±0.2	10±0.3	12±0.3	14±0.6	25^T	–
PV	12±0.3	14±0.7	16±0.9	13±0.5	17±0.4	24±09	12±0.7	14±0.3	16±0.3	–	10±0.2	12±0.7	–	10±0.2	12±0.4	22^T	–
SA	–	10±0.4	12±0.0	14±0.5	21±0.4	25±0.8	–	–	14±0.1	–	12±0.4	16±0.2	–	–	–	24^T	–
SP	13±0.4	19±0.4	23±0.8	14±0.0	19±0.4	24±0.1	11±0.1	13±0.4	19±0.0	–	–	–	12±0.7	16±0.4	21±0.2	22^T	–
AN	10±0.0	16±0.2	19±0.4	12±0.3	16±0.7	22±0.4	–	–	–	–	–	–	–	–	–	18^N	–
CA	14±0.3	16±0.2	18±0.1	17±0.1	21±0.2	25±0.2	12±0.9	13±0.3	19±0.1	10±0.7	12±0.3	16±0.2	12±0.4	14±0.0	19±0.8	23^N	–
SC	12±0.9	13±0.7	15±0.9	14±0.3	16±0.1	18±0.5	–	10±0.6	12±0.2	–	10±0.2	12±0.5	–	–	–	20^N	–

All the values of inhibitory activity for the extracts tested are significant at 0.05 levels.
Or: Organisms; T: Tetracyclin; N: Nystatin; D: DMSO
BS: *Bacillus subtilis*; EC: *Escherichia coli*; KP: *Klebsiella pneumoniae*; PA: *Pseudomonas aeruginosa*; PV: *Proteus vulgaris*; SA: *Staphyloccocus aureus*; SP: *Streptomyces pneumoniae*; AN: *Aspergillus niger*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae.*

From the MIC values (Table 2), it was observed that *E.coli* showed the least MIC value for both ethanol and methanol extracts, the same MIC value was exhibited by *K. pneumoniae* and *S. aureus* for ethanol extract. The chloroform extract showed the least MIC value against *P. aeruginosa* while hexane extract against *S. aureus* and aqueous extract against *C. albicans*. The preliminary phytochemical analysis revealed the presence of alkaloids, amino acids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, glycosides, phenols, saponins, steroids, tannins and terpenoids in the different solvent and aqueous extracts of *A. lebbeck* stem bark (Table 3).

The medicinal plants are rich in secondary metabolites which include alkaloids, glycosides, flavonoids, steroids, related active metabolites, which are of great medicinal value and have been extensively used in the drug and pharmaceutical industry. Recently, a number of studies have been reported on the phytochemistry of medicinal plants, particularly on the stem bark [4,6,17,19]. Interestingly, in the present investigation, stem bark of *A. lebbeck* has been screened for the presence of alkaloids, flavonoids, phenols, saponins, steroids, tannins and terpenoids. The results also revealed that all the solvent extracts possessed antimicrobial activity. Of which alcohol (methanol and ethanol) extracts showed the higher degree of antimicrobial activity on selected microorganisms. This is accordance with the previous study reported that methanol was the most effective solvent for plant extraction than hexane and water[14]. This may be as a result of the presence of a few compounds extracted into the solvent and they may not have been enough inhibitory activity on pathogens.

It was reported that ethyl acetate extracts of leaves of *A. lebbeck* showed activity against *E. coli. S. aureus, P. aeruginosa* and *B. cereus* [24] whereas flowers of methanol extract found to be

Table 2: MIC of the different extracts of *A. lebbeck* stem bark.

Organisms	Hexane extract	Chloroform extract	Methanol extract	Ethanol extract	Aqueous extract
B. subtilis	>1000	>1000	250	62.5	>1000
E. coli	>1000	>1000	31.2	31.2	>1000
K. pneumoniae	>1000	125	62.5	31.2	>1000
P. aeruginosa	1000	62.5	500	250	250
P. vulgaris	1000	1000	1000	500	1000
S. aureus	62.5	>1000	1000	31.2	>1000
S. pneumoniae	>1000	1000	62.5	125	>1000
A. niger	>1000	>1000	125	1000	>1000
C. albicans	125	1000	500	125	125
S. cerevisiae	>1000	>1000	1000	500	>1000

Table 3: Preliminary phytochemical constituents of *A. lebbeck* stem bark.

Phytochemical constituents	Hexane extract	Chloroform extract	Methanol extract	Ethanol extract	Aqueous extract
Alkaloids	+	+	+	+	+
Aminoacids	+	+	+	+	+
Anthraquinone	-	-	-	+	-
Carbohydrates	-	+	+	+	+
Cardiac glycosides	-	+	+	+	-
Flavonoids	-	-	-	+	+
Glycosides	-	+	+	+	-
Phenols	-	-	+	+	+
Saponins	-	+	+	+	+
Steroids	+	+	+	+	-
Tanins	-	-	+	+	+
Terpenoids	+	+	+	+	-

effective against *E. coli, K. pneumoniae, P. aeruginosa, S. aureus* and *P. mirabilis* [13]. Pods, seeds, flowers and roots of *A. lebbeck* crude methanol extracts active against bacteria such as *E. coli, S. typhi, S. aureus, P. aeruginosa, P. mirabilis, B. subtilis* and fungal species such as *A. niger, A. parasiticus, A. effusus, C. albicans, F. solani* [26]. Hydroalcohol extract of flowers and pod showed activity against *S. typhi, S. sonnei, E. coli, K. aurogenes* and *K. pneumoniae* [21], while sapwood, heart wood and bark from *A. lebbeck* showed weak to moderate activity against *B. subtilis, B. cereus, S. aureus, P. aeruginosa, E. coli, S. marcescens, A. tumefaciens* [25] and Bobby et al[5] reported that methanol extract of *A. lebbeck* leaves found IZ against *B. subtilis, E. coli, K. pneumoniae, P. vulgaris, P. aeruginosa, S. typhi, S. aureus*. Methanol and aqueous extracts of leaves and bark showed MIC values against *E.coli* and *S. aureus* [18] whereas in the present study also stated that alcohol (methanol and ethanol) extracts found to be effective against all the tested bacteria and fungi.

A. lebbeck is already considered as medicinal plant. The plant is said to be a source of many bioactive principles acting against some human ailments and in our study stem bark extracts exhibited the high degree of antimicrobial activity against all tested bacteria and fungal strains. The present study also suggests that stem bark posses bioactive compounds responsible for exerting antimicrobial action against infectious diseases caused by bacteria and fungi. Therefore, it is concluded that alcohol extracts of stem bark of *A. lebbeck* brings to light the scope to develop a novel broad spectrum of antimicrobial drug formulation.

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