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In vitro anti- bacterial activity of leaves extracts of *Albizia lebbeck* Benth against some selected pathogens

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ABSTRACT

Objective: To screen the anti-bacterial activity of *Albizia lebbeck* (*A. lebbeck*) Benth leaves extract against the selected bacterial pathogens *viz.*, *Bacillus subtilis* (MTCC441), *Escherichia coli* (MTCC443), *Klebsiella pneumonia* (MTCC 109), *Proteus vulgaris* (MTCC742), *Pseudomonas aeruginosa* (MTCC741), *Salmonella typhii* (MTCC733) and *Staphylococus aureus* (MTCC96). **Methods:** The leaves extracts of *A. lebbeck* was tested against bacteria by the agar disc diffusion method. **Results:** Results of the present study indicated that different extracts of *A. lebbeck* showed inhibitory effects against the pathogens. The present study results demonstrated that methanolic extracts of *A. lebbeck* conferred the widest spectrum activities that inhibited the growth of all studied pathogens with the maximum zone of inhibition. The methanolic extracts of *A. lebbeck* illustrated the highest zone of inhibition against the pathogens *Bacillus subtilis* (16 mm), *Escherichia coli* (22 mm), *Klebsiella pneumonia* (11 mm), *Proteus vulgaris* (18 mm), *Pseudomonas aeruginosa* (22 mm), *Salmonella typhii* (23 mm) and *Staphylococus aureus* (17 mm). The ethyl acetate extracts demonstrated maximum zone of inhibition against *Escherichia coli* (26 mm), *Pseudomonas aeruginosa* (22 mm) and *Klebsiella pneumonia* (16 mm). **Conclusions:** It is expected that this study would direct to the establishment of some active compounds that could be used to formulate new and more potent anti-bacterial drugs of natural origin.

1. Introduction

For thousand years, mankind has learnt about the benefits of plant to alleviate or cure illnesses. The plant kingdom constitutes a source of new chemical compounds which may be important owing to their potential use in medicine and other applications. Medicinal plants occupy an important position in the socio-cultural, spiritual and medicinal arena of rural people of India. India is richly endowed with indigenous plants which are used in herbal medicine to cure diseases and heal injuries[1]. These plants exhibit a wide range of biological and pharmacological activities such as anti-cancer, anti-inflammatory, diuretic, oxytocic, laxative, antispasmodic, antihypertensive, anti-diabetic, and anti-microbial functions. About 30% of the worldwide drug sales are based on natural products. It is estimated that there are about 2 500 000 species of higher plants throughout the world, and most of them have not been examined in detail for their pharmacological activities[2]. However, for some decades, there has been increasing interest in plant uses and the detection of their constituents with antibacterial activity. With the development of antimicrobials, microorganisms have adapted and become resistant to previous antimicrobial agents. Modern phytomedicine is a timely and original handbook paving the way to success in plant-based drug development, systematically addressing the issues facing a pharmaceutical scientist who wants to turn a plant compound into a safe and effective drug. Plant derived antimicrobial agents have been largely overlooked. Many commercially proven drugs used in modern medicines, were initially used in crude form in traditional or folk healing practices or for other purposes that suggested potentially useful biological activity[1].

Albizia lebbeck (*A. lebbeck*) Benth is widely distributed in India and is also found in South Africa and Australia. Barks are used in toothache, piles, diarrhea and diseases of the gum. Decoction of the leaves and barks are protective against bronchial asthma and other allergic disorders.

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Phytochemical investigations showed that the pod of the *A. lebbeck* contains 3′,5-dihydroxy-4′,7 dimethoxy flavone, and N-Benzoyl-L-phenyl alaninol. The beans of the plant contain albigenic acid-a new triterpenoid sapogenin. The plant also contains saponins, macrocyclic alkaloids, tannins, and flavonols. The decoction of *A. lebbeck* stem bark was found to be effective against bronchospasm induced by histaminic acid phosphate and showed di-sodium chromoglycate like action on mast cells. The active constitute of bark extract is anthraquinone glycosides. The main constituent from bark is active against aerobes and mechanism of action is that glycosides cause the leakage of the cytoplasmic constituents. Two new tri-o-glycoside flavonols kaempferol and quercetin were identified from the leaves of *A. lebbeck*. Albizziahexoside a new hexaglycosylated saponin was isolated from leaves of *A. lebbeck*. Lignins present in their cell walls have been oxidized with alkaline nitrobenzene. It is reported to have antiseptic, antimicrobial, anti-ovulatory, anti-fertility, anti-protozoal, anti-dysentric, anti-tubercular and anti-cancer activities[3-7]. With this background the present was aimed to screen the antibacterial properties of *A. lebbeck* leaves against the human pathogens.

2. Materials & methods

2.1. Collection of plant materials

A. lebbeck Benth were collected from natural habitats in and around the Rasipuram, Nammakal, Tamil Nadu (India) and authenticated by Dr. E. G. Wesely and voucher specimen were deposited in the Department of Biotechnology, Muthayammal College of Arts and Science, Rasipuram, Nammakal, Tamil Nadu India.

2.2. Preparation of crude extract

Leaves samples of *A. lebbeck* Benth were air and shade dried for two weeks and pulverized to powder using mortar. The dried and powered leaves materials (50 g) were extracted successively with 250 mL of petroleum ether, Ethyl acetate and Methanol by using Soxhlet extractor for 8 h at a temperature not exceeding the boiling point of the solvent. The aqueous extracts were filtered using Whattman filter paper (No.1) and then concentrated in vacuum at 40℃ using rotary evaporator. The residues obtained were stored in a freezer -70℃ until further tests[8].

2.3. Evaluation of antibacterial activities

The crude extracts were used for bioassay against both gram negative and gram positive bacteria *viz.*, *Bacillus subtilis* (MTCC441), *Escherichia coli* (MTCC443), *Klebsiella pneumonia* (MTCC 109). *Proteus vulgaris* (MTCC742), *Pseudomonas aeruginosa* (MTCC741), *Salmonella typhii*(MTCC733), and *Staphylococus aureus* (MTCC96). Inoculums were prepared from the 24 hours old culture of standard bacterial isolates in nutrient broth. Nutrient agar plates were prepared and the inocula were seeded by spread plate method. The agar disc diffusion method was used for the antibacterial evaluations. The sterile discs were loaded with 50, 100, 200 and 500 μL of plant extracts. The plates were incubated at 37℃ for 24-48 h. Antibacterial activity was evaluated by measuring the inhibition zone in millimeter in diameter and tabulated[9]. All the samples were done in triplicate. Both positive and negative controls were determined, for negative control the three solvents (petroleum ether, ethyl acetate and methanol) were also used to determine their effect on test organisms. While seven common antibiotics *viz.*, tetracyclin, streptomycin, erythromycin, lincomycin, ryphampicin, norflaxicin and gentamycin discs were also used to compare the effectiveness of the plants extracts with that of the antibiotics.

3. Results

A total of three extracts *viz.*, petroleum ether, ethyl acetate and methanol were screened for the anti-bacterial activity against the selected pathogens. The anti-bacterial activity of the leaves extracts of *A. lebbeck* were illustrated in Table 1. The present study results demonstrated that methanolic extracts of *A. lebbeck* conferred the widest spectrum activities that inhibited the growth of all studied pathogens with the maximum zone of inhibition (Table 1). The methanolic extracts of *A. lebbeck* illustrated the highest zone of inhibition against the pathogens *Bacillus subtilis* (16

Table 1.
Anti-bacterial activity of leaves extracts of *A. lebbeck* Benth against the pathogens (mm).

Organism	Zone of inhibition (mm)																		
	Solvent extract (μg/mL)												Antibiotics (μg/mL)						
	PE(mg)				EA(mg)				M(mg)				T	S	E	L	R	Nx	G
	50	100	200	500	50	100	200	500	50	100	200	500	50	100	200	500	50	100	200
Escherichia coli	-	-	-	-	5	10	22	26	3	9	15	22	20	-	-	17	13	-	-
Bacillus subtilis	-	-	-	-	3	5	11	13	2	4	7	16	23	15	-	-	-	14	20
Proteus vulgaris	-	-	-	-	-	-	-	-	3	7	13	18	23	19	24	24	18	19	-
Klebsiella pneumonia	-	-	-	-	-	8	11	16	3	6	8	11	16	-	-	-	-	14	20
Pseudomonas aeruginosa	-	13	14	15	15	18	21	22	15	16	20	22	-	15	--	-	-	-	16
Salmonella typhii	-	-	-	-	-	3	9	19	5	12	21	23	-	-	-	-	-	-	-
Staphylococus. aureus	-	-	-	-	-	-	-	-	-	4	9	17	-	-	-	-	-	-	-

T-Tetracyclin; S-Streptomycin; E-Erythromycin; L- Lincomycin; R-Ryphampicin; Nx-Norflaxicin; G-Gentamycin; PE-Petrolium ether, EA-Ethyl acetate, M-Methanol.

mm), *Escherichia coli* (22 mm), *Klebsiella pneumonia* (11 mm), *Proteus vulgaris* (18 mm), *Pseudomonas aeruginosa* (22 mm), *Salmonella typhii* (23 mm) and *Staphylococus aureus* (17 mm). The ethyl acetate extracts demonstrated maximum zone of inhibition against *Escherichia coli* (26 mm), *Pseudomonas aeruginosa* (22 mm) and *Klebsiella pneumonia* (16 mm).

4. Discussion

The plants known as medicinal, are rich in secondary metabolites which includes alkaloids, glycosides, flavonoids, insecticides, steroids, related active metabolites are of great medicinal value and have been extensively used in the drug and pharmaceutical industry[10]. Recently, a number of plants have been reported for antimicrobial properties across the world[11-15]. Several workers have reported that many plants possess antimicrobial properties including the parts which include flower, bark, stem, leaf, *etc*. In the present investigation, the leaves extracts of *A. lebbeck* a medicinally important plant from India have been screened for antimicrobial potential. The results of the present confirm that the antibacterial activity of *A. lebbeck* and supplemented the bio-efficacy studies on plants and plant extracts. It has shown that when solvents like ethanol, hexane and methanol are used to extract plants, most of them are able to exhibit inhibitory effect on both gram positive and gram negative bacteria[16]. Johnson *et al*[12] reported that methanol was the most effective solvent for plant extraction than hexane and water. In the present study we used methanol, petroleum ether and ethyl acetate for extraction. Of which the methanol extracts of *A. lebbeck* showed the highest rate of inhibition against the selected pathogens. The present study confirmed observations with maximum activity by Johnson *et al*. In addition, ethyl acetate and petroleum ether extracts of *A. lebbeck* showed zone of inhibition against the pathogens with varied diameter. The result of the present revealed that all these three leaves extracts possessed antimicrobial activity and they can be used as broad spectrum antibiotics since they were active against both gram positive and gram negative bacteria *viz*., *Bacillus subtilis, Escherichia coli, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhii* and *Staphylococus aureus*. The results of the present study suggested that the leaves extracts of *A. lebbeck* can be used in the treatment of gastrointestinal infection and diarrhoea and skin diseases in man also[17]. Haripriya *et al*[11] observed that petroleum ether extracts of *Selaginella involvens* showed higher antibacterial activity against *Escherichia coli* and *Pseudomonas*. Similarly in the present study also, ethyl acetate and methanol extracts showed the maximum zone of activity against *Bacillus subtilis, Escherichia coli, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhii* and *Staphylococus aureus*. The present study result revealed that the leaves extracts of *A. lebbeck* can be used in the treatment of boils, sores and wounds, since *Pseudomonas aeruginosa* have been implicated as causative agents of these diseases[18]. Rahul *et al*[3] studied the anti-bacterial activity of *A. lebbeck* leaves extracts against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus cereus* only. But in present study we screened the antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhii* and *Staphylococus aureus*. Our results supported and supplemented the previous observations. The previous phytochemical studies on leaves of *A. lebbeck* showed that high degree of flavonoids, tannins and saponins. They were known to show medicinal activity as well as physiological activity.

It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin. Studies are in progress to further evaluate the mechanisms of action of *A. lebbeck* extracts on some organisms associated with human diseases. Methanol and ethyl acetate extracts exhibited slightly higher efficacy than petroleum ether extracts, so ethyl acetate and alcoholic extracts were suggested to use as natural antibiotic administration for the diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

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