

ANTIMICROBIAL ACTIVITIES OF SUCCESSIVE SOLVENT EXTRACTS OF *ALBIZIA LEBBECK* AND *SOLANUM SEAFORTHIANUM* AGAINST SOME HUMAN PATHOGENIC MICROORGANISMS

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ABSTRACT

Objective: The continuous emergence of multidrug resistance bacteria and yeast, and the negative impact of synthetic preservatives have led to an increased interest in the use of plant extracts as alternative antimicrobial agents. In the present investigation, the antimicrobial activity of successive solvent extracts of *Albizia lebeck* and *Solanum seforthianum* has been evaluated against human pathogenic bacteria and yeast.

Methods: The disc diffusion method was employed for determination of the zone of inhibitions (ZOIs) and twofold broth dilution technique was employed for determination of minimal inhibitory concentrations, and minimal bactericidal/fungicidal concentrations.

Results: Among the successive solvent extracts tested, methanol extracts of both *A. lebeck* and *S. seforthianum* showed highest antibacterial activity with ZOIs ranged between 10.0 and 20.4 mm at 1 mg/disc followed by ethanol (ZOIs 8.1–17.6 mm). The petroleum ether, toluene, and chloroform extracts showed the least activity. The highest activity was observed against *Streptococcus faecalis*, whereas the least activity was observed against *Pseudomonas aeruginosa*.

Conclusion: The broad-spectrum antimicrobial activity of methanol extract of *A. lebeck* and *S. seforthianum* could be explored as antimicrobial agents for the management of pathogenic bacteria and yeast.

Keywords: *Albizia lebeck*, *Solanum seforthianum*, Antimicrobial activity, Successive solvent extracts, Disc diffusion techniques.

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INTRODUCTION

Infectious diseases caused by many pathogenic bacteria and yeast are a major problem despite the tremendous achievement in human health care. According to the WHO statement, infectious diseases are the world's leading cause of premature death, and also approximately 17 million people die from infectious diseases annually [1]. These populations include infants and children, elderly peoples, pregnant women, people taking certain kinds of medications and also immune suppressed patient, such as cancer patients, ICU patients, and diabetic patients [2,3]. Some of the multidrug-resistant (MDR) pathogenic microbes commonly reported worldwide are *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Candida albicans* [4,5]. Even though many pharmacological industries have produced a large number of new antibiotics in the last two decades, the continuous increase of drug-resistant pathogens is a big challenge for the treatment of infectious diseases [6]. Hence, there is a need to search for alternate effective and eco-friendly strategies. Medicinal plants have been used by humans since time immemorial for different ailments, and they also provided many valuable drugs on antimicrobial, antitumor, antidiabetic, antihypertensive, antimalarial, antioxidant, anti-inflammatory, antimutagenic, analgesic, and cardiovascular properties [7,8]. Medicinal plants are the richest bioresource of drugs of traditional medicines as well as modern medicines, and also they have provided many pharmaceutical intermediates and chemical entities for discovery and development of novel drugs [9,10]. Further, they possess great potential in the field of biomedicine as they effectively destroy several bacterial, fungal, and viral pathogens [11,12]. Among the estimated 250,000–500,000 plant species, only a smaller percentage (<10%) was subjected to pharmacological evaluation [13]. Considering these, the present investigation was undertaken and evaluates the antimicrobial activity of *Albizia lebeck* belongs to the family Leguminosae and *Solanum seforthianum* belongs to the family Solanaceae.

METHODS**Plant material collection and authentication**

Fresh leaves of *A. lebeck* and *S. seforthianum* were collected from the Southern part of Karnataka (India) during 2014–2017. The plant samples were authenticated by Dr. Seetharam, Professor, Department of Biological Sciences, Bengaluru University, Bengaluru, India, and the authenticated voucher specimens were deposited in the Department of Microbiology and Biotechnology, Bangalore University, Bengaluru, along with a proper voucher number (Voucher number: BUB/MB-BT/DCM/DST-SERB/1/28/2014-17).

Preparation of successive solvent extracts from selected plants

The plants, namely, *A. lebeck* and *S. seforthianum* showed promising antimicrobial activity in the aqueous extract were subjected to successive solvent extraction following the procedure of Ayoub and Hamid [14]. 50 g of shade-dried and finely powdered of each plant material was filled in the thimble and extracted successively with 200 ml of petroleum ether, toluene, chloroform, methanol and ethanol using a Soxhlet extractor to colorless extract obtained at the top of the extractor. Each solvent extract was concentrated separately under reduced pressure using a rotary flash evaporator (Superfit, Mumbai, India), and subjected for antimicrobial activity evaluation at desired different concentrations.

Antimicrobial activity evaluation**Test microorganisms**

Nine human pathogenic microorganisms, namely, *E. coli* (National Collection of Industrial Microorganisms [NCIM] 2065), *K. pneumoniae* (NCIM 2957), *Proteus vulgaris* (NCIM 2027), *Pseudomonas aeruginosa* (NCIM 5031), *Salmonella typhi* (NCIM 2051), *S. aureus* (NCIM 2079), *Streptococcus faecalis* (NCIM 5025), *C. albicans* (NCIM 3471), and

Cryptococcus neoformans (NCIM 3541) were collected from NCIM, Pune, India. All the tested bacteria and yeast were maintained on Mueller-Hinton agar (MHA) and malt extract-glucose-yeast extract-peptone-agar (MGYPB), respectively. 24 h old bacterial and 48 h old yeast cultures were used as test organisms.

Antimicrobial activity assay

The antibacterial and anti-yeast activities of successive solvent extracts of *A. lebbek* and *S. seaforthianum* were evaluated against human pathogenic seven bacterial species and two yeast species by disc diffusion method following the procedure of Wikler, [15]. Briefly, 100 µl of each bacterial inoculum (10^8 CFU/ml) and yeast inoculum (10^6 CFU/ml) were separately spread on MHA for bacterial species and MGYPB for yeast species and allowed to dry for 10 min. Four layered 6 mm sterilized paper discs (Whatman No. 1) impregnated separately with different successive solvent extract (1 mg/disc) was placed on the surface of pre-inoculated MHA and MGYPB plates. The plates were kept for 1 h for extract diffusion at 4°C; then all the treatments were incubated at 37°C for 24 h for bacteria and 28±2°C for 48 h for yeast. The disc impregnated with the same amount DMSO served as a negative control. The synthetic erythromycin (15 µg/disc) and fluconazole (25 µg/disc) served as a positive control for bacteria and, yeast, respectively. After incubation, antimicrobial activity was determined by measuring the diameter of zone of inhibitions (ZOIs) in millimeters (mm).

Determination of minimum inhibitory concentrations (MICs) and minimum bactericidal/fungicidal concentrations (MBCs/MFCs)

The MICs and MBCs of methanol extracts of *A. lebbek* and *S. seaforthianum* were determined using a broth microdilution technique following the procedure of Prakash *et al.* [16] with some modification. Briefly, 200 µl of twofold serially diluted each methanol

extract in MHB and MGYPB (0.031–2.0 mg/ml) was added separately to the wells of a sterile 96-well microtiter plate, inoculated with 15 µl of bacteria (10^8 cells/ml), yeast (10^6 cells/ml), and fungal spore suspension (10^8 spores/ml), respectively. The inoculated plates were incubated at 37±2°C for 24 h for bacteria and 30±2°C for 48 h for yeast. After the incubation, 50 µl of iodinitrotetrazolium chloride (INT-2 mg/ml) was added into each well of a microtiter plate and incubated further for another 30 min at respective temperatures as mentioned above. The pale yellow-colored INT was reduced to a pink color which indicated the presence of microbial viability, while the yellow color remained the same indicates the microbial growth inhibition. The lowest concentration required to reduce the intensity of the pink color was considered as MIC, and the lowest concentration at which yellow color remains unchanged considered as MBC/MFC.

Statistical analysis

The experiments were performed in triplicate and values were expressed as means±standard error. Analysis of variance was conducted, and the differences between values were tested for deviation significance by ANOVA with the SPSS 20 program (IBM, Armonk, NY, USA). Differences at $p \leq 0.05$ were considered statistically significant.

RESULTS

The antimicrobial activity of successive solvent extracts of *A. lebbek* and *S. seaforthianum* was evaluated and the obtained results are presented in Tables 1 and 2. The negative control DMSO did not show antimicrobial activity against the tested bacteria and yeast. Among the successive solvent extracts tested, methanol extracts of both *A. lebbek* and *S. seaforthianum* showed highest antimicrobial activity with ZOIs ranged between 10.0 and 20.4 mm at 1 mg/disc followed by ethanol extract (ZOIs 8.1–17.6 mm). Tukey's HSD data analysis revealed that

Table 1: Antimicrobial activity of successive solvent extracts of *Albizia lebbek* against some human pathogenic bacteria and yeast

Test microbes	Solvent extracts (1 mg/disc)					Methanol extract (mg/ml)		Erythromycin/Fluconazole
	PE	TE	CE	ME	EE	MIC	MFC	
<i>Escherichia coli</i>	7.2±0.4	6.8±0.3	10.6±0.5	11.8±0.6	10.1±0.6	0.25	1.0	11.36±0.5
<i>Klebsiella pneumoniae</i>	9.0±0.7	8.2±0.4	12.1±0.7	13.5±0.7	9.7±0.5	0.125	0.5	15.27±0.8
<i>Proteus vulgaris</i>	8.6±0.5	6.3±0.3	7.8±0.6	10.8±0.8	9.2±0.7	0.25	2.0	9.48±0.4
<i>Pseudomonas aeruginosa</i>	6.7±0.6	0±0.0	7.2±0.3	10.0±0.4	8.9±0.6	0.5	2.0	11.32±0.3
<i>Salmonella typhi</i>	9.5±0.6	8.5±0.7	13.4±0.8	13.9±0.4	11.4±0.5	0.125	0.5	20.18±0.7
<i>Staphylococcus aureus</i>	10.2±0.8	9.8±0.6	14.6±0.5	15.3±0.5	13.4±0.6	0.062	0.25	18.62±0.6
<i>Streptococcus faecalis</i>	12.8±0.5	10.5±0.7	17.2±0.7	19.8±0.3	17.6±0.4	0.031	0.25	20.95±0.8
<i>Candida albicans</i>	10.6±0.3	8.4±0.5	9.5±0.6	14.7±0.9	13.7±0.5	0.125	0.5	15.45±0.6
<i>Cryptococcus neoformans</i>	12.3±0.4	10.2±0.5	11.4±0.8	16.5±0.7	15.2±0.6	0.062	0.25	17.69±0.5

PE: Petroleum ether extract, TE: Toluene extract, CE: Chloroform extract, ME: Methanol extract, EE: Ethanol extract. Erythromycin (15 µg/disc) and Fluconazole (25 µg/disc) served as a positive control for bacteria and yeast, respectively. Data given are the mean of three replicates±standard error ($p < 0.05$). No activity was observed around DMSO control discs, MIC: Minimum inhibitory concentrations, MFC: Minimum fungicidal concentrations

Table 2: Antimicrobial activity of successive solvent extracts of *Solanum seaforthianum* against some human pathogenic bacteria and yeast

Test bacteria	Solvent extracts (1 mg/disc)					Methanol extract (mg/ml)		Erythromycin/fluconazole
	PE	TE	CE	ME	EE	MIC	MFC	
<i>Escherichia coli</i>	0.0±0.0	0.0±0.0	0.0±0.0	11.2±0.4	9.5±0.3	0.25	2.0	11.36±0.5
<i>Klebsiella pneumoniae</i>	0.0±0.0	0.0±0.0	8.8±0.5	12.8±0.5	9.2±0.6	0.25	1.0	15.27±0.8
<i>Proteus vulgaris</i>	0.0±0.0	0.0±0.0	0.0±0.0	10.6±0.6	8.1±0.3	0.5	2.0	9.48±0.4
<i>Pseudomonas aeruginosa</i>	0.0±0.0	0.0±0.0	0.0±0.0	13.4±0.7	9.8±0.7	0.25	2.0	11.32±0.3
<i>Salmonella typhi</i>	0.0±0.0	0.0±0.0	7.8±0.4	14.5±0.6	9.0±0.3	0.25	1.0	20.18±0.7
<i>Staphylococcus aureus</i>	0.0±0.0	0.0±0.0	9.8±0.8	15.3±0.5	11.5±0.8	0.125	0.5	18.62±0.6
<i>Streptococcus faecalis</i>	0.0±0.0	0.0±0.0	10.2±0.4	20.4±0.8	15.2±0.4	0.062	0.25	20.95±0.8
<i>Candida albicans</i>	8.2±0.4	6.4±0.5	8.6±0.5	13.2±0.7	12.1±0.5	0.125	0.5	15.45±0.6
<i>Cryptococcus neoformans</i>	9.8±0.6	8.0±0.7	9.5±0.8	15.7±0.6	13.4±0.6	0.062	0.25	17.69±0.5

PE: Petroleum ether extract, TE: Toluene extract, CE: Chloroform extract, ME: Methanol extract, EE: Ethanol extract. Erythromycin (15 µg/disc) and Fluconazole (25 µg/disc) served as a positive control for bacteria and yeast respectively. Data given are the mean of three replicates±standard error ($p < 0.05$). No activity was observed around DMSO control discs, MIC: Minimum inhibitory concentrations, MFC: Minimum fungicidal concentrations

the petroleum ether, toluene and chloroform extracts showed the least activity against tested bacteria and yeast. The obtained results confirm that the active compounds which are responsible for antimicrobial activity are more soluble in the methanol extract followed by ethanol but least soluble in toluene.

The methanol extract which showed the highest antimicrobial activity was selected for MICs and MBCs/MFCs value determination using the broth dilution technique. The MICs and MBCs/MFCs value ranged between 0.031 and 0.5 and 0.25 and 2.0 mg/ml, respectively. Among the bacteria tested, *Streptococcus faecalis* was observed as more susceptible bacteria with least MIC and MBC values, whereas *E. coli* and *P. aeruginosa* were as observed more resistant bacteria with highest MICs and MBCs values.

DISCUSSION

The present study clearly confirms that Gram-positive bacteria are more susceptible than Gram-negative bacteria; this is due to the presence of extra outer lipopolysaccharides cell wall in Gram-negative bacteria, which restrict extract penetration into the bacterial cell. On a comparative evaluation with synthetic antibiotics, the antibacterial activity of methanol extracts of *A. lebbeck* and *S. seaforthianum* was comparable with the synthetic antimicrobial agent's erythromycin and fluconazole. The review of literature revealed that the extracts of *A. lebbeck* and *S. seaforthianum* showed antimicrobial, antioxidant, antidiabetic, antimalarial, antiarthritic, and anti-allergic properties [17-27]. Ali *et al.* [17] from Bangladesh evaluated the antimicrobial and antioxidant activity of petroleum ether, ethyl acetate and methanol extracts of *A. lebbeck* against *B. subtilis*, *S. aureus*, *V. mimicus*, *S. typhi*, *S. dysenteriae*, *C. arrizae*, and *A. niger*. Malla *et al.* [21] reported that the methanolic extract of stem bark of *A. lebbeck* showed antimicrobial activity against *E. coli* and *S. aureus*. Siahpoosh *et al.* [20] reported that the methanolic extract of *A. lebbeck* showed moderate antioxidant activity. Xavier *et al.* [27] reported that the methanol extracts of *S. seaforthianum* showed promising inhibitory activity. Although the antimicrobial activity has been previously reported, there are no reports on antimicrobial activities of successive solvent extracts of *A. lebbeck* and *S. seaforthianum* against a broad range of bacteria and yeast. We are the 1st time to report broad-spectrum antimicrobial activity of successive solvent extracts of *A. lebbeck* and *S. seaforthianum*.

CONCLUSION

The continuing emergence of MDR human pathogenic microbes and undesirable side effects caused by many currently available synthetic drugs have been collectively necessitated to search for new and broad-spectrum alternative antibacterial agents. The promising antimicrobial activities of methanol extract of *A. lebbeck* and *S. seaforthianum* might be a prospective source as alternative antimicrobial agents for the management of pathogenic microorganisms.

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AUTHORS' CONTRIBUTIONS

Design, experimental part of the work and writing of the manuscript were done by the first author Dr. Venkatesh, H. N. The design of the work and correction of the manuscript were done by the corresponding author Dr. D. C. Mohana.

CONFLICTS OF INTEREST

No potential conflicts of interest were reported by the authors.

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