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Efficacy of Aqueous and Methanol extracts of *Caesalpinia sappan* L. and *Mimosa pudica* L. for their potential Antimicrobial activity

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Abstract

Aqueous and methanol extract of two medicinal plants of *Caesalpinia sappan* L. and *Mimosa pudica* L. were evaluated for their antimicrobial activities against *Staphylococcus aureus* NCIM 5021, *Bacillus subtilis* NCIM 2010, *Escherichia coli* NCIM 2118, *Pseudomonous aeruginosa* NCIM 5029, *Klebsiella pneumoniae* NCIM 2707, *Proteus vulgaris* NCIM 2027, *Candida albicans* NCIM 3102 and *Aspergillus niger* NCIM 545. The antibacterial activity of aqueous and methanol extracts was determined by agar disk diffusion and broth dilution method. The plant extracts were more active against Gram positive bacteria than against gram negative bacteria. The most susceptible bacteria were *S.aureus*, followed by *B.subtilis*, while more resistant bacteria were *S.aureus*, followed by *E.coli*. From the screening experiment, *Caesalpinia sappan* L. showed the best antibacterial activity; hence this plant can be further subjected to isolation of the therapeutic antimicrobials and further pharmacological evaluation.

Key words: methanol, disc diffusion method, microorganisms

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Introduction

According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases[1]. Several screening studies have been carried out in different parts of the world. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world. Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, recently much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine[2]. *Mimosa pudica* Linn. is a creeping annual or perennial herb often grown for its curiosity value, as the compound leaves fold inward and droop when touched and reopens within minutes. It belongs to the Fabaceae family. *Mimosa pudica* is native to Brazil, but is now a pan tropical weed. This plant has a history of use for the treatment of various ailments and the most commonly used plant part for this purpose is the root, but flowers bark and fruit can also be utilized. Several research works have been carried out to study about the phytochemical components of *Mimosa pudica* and also about the antimicrobial activity of the plant[3].

Caesalpinia sappan Linn. a small thorny tree, 6-9 m high is found in India, Peru, Malaya etc. It is being used traditionally for large number of ailments and reported to have a wide variety of medicinal properties. Its anticonvulsant, anti-inflammatory, anti-proliferative, antimicrobial, anticoagulant, antiviral, immunostimulant and antioxidant activities have been reported. According to Ayurveda, the heartwood is useful in vitiated conditions of Pitta, burning sensation, wounds, ulcers, leprosy, skin diseases, diarrhea, dysentery, diabetes etc. A decoction of the heartwood is commonly used in Kerala, India for its antithirst, blood purifying, anti-diabetic properties and the plant is one of the ingredients in many traditional Ayurvedic formulations[4.] In the present work, two different medicinal plants were evaluated for their antimicrobial properties.

Materials and methods

Collection of Plant materials

The two plants used in the present study were *Caesalpinia sappan* L. (bark) and *Mimosa pudica* L. (leaves) were collected from Kerala State in the month of December 2009. The collected plant samples were washed thoroughly in running tap water to remove the surface microflora and other adherents.

Crude Extraction

Aqueous extraction

100 grams of dried plant material was extracted in distilled water for 6 h at slow heat. Every 2 h it was filtered through 8 layers of muslin cloth and centrifuged at 5000 g for 15 min. The supernatant was collected. This procedure was repeated twice and after 6 h the supernatant was concentrated to make the final volume one-fifth of the original volume[2.]

Solvent extraction

100 grams of dried plant material was extracted with 200 ml of methanol kept on a rotary shaker for 24 h. Thereafter, it was filtered and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fifth of the original volume[2.] It was stored at 4^{o}C in airtight bottles for further studies.

Phytochemical Analysis

Phytochemical screening of ethyl acetate extract for the presence of these secondary metabolites: Alkaloids (Draggendorff's, Hager's test, Wagner's test & Mayer's test), Glycosides (Baljet test & Legal test), Carbohydrates (Fehling's test, Benedict's test & Molisch's test), Saponins (Foam test & Blood Haemolysis test), Steroids (Libermann Burcherd test), Tannins (Ferric Chloride test) and Proteins (Biurette test)[5] .

Antimicrobial screening

The antimicrobial activity was determined by the paper disc diffusion method[6] using Mueller-Hinton agar plates (MHA) (for all the bacteria) and potato dextrose agar plates (PDA) (for the fungi) previously inoculated with 24 h old Nutrient broth (NB) culture (0.5 McFarland Standard) for the bacteria or spores (10^6 spores/ml for the fungi) suspension in Potato Dextrose Broth (PDB) of the test organisms, respectively. Sterilized paper discs (6 mm), soaked in a known concentration of the crude extracts (500 mg/ml per disc) in DMSO were applied over each of the culture plates previously seeded with the 0.5 McFarland (for bacteria) and 10^6 spores/ml (for fungi). Antibiotic discs of Ciprofloxacin (1 mg/ml) was used as positive control for bacteria, fluconazole (1 mg/ml) was used for fungi and sterilized paper discs without extracts or antibiotics were used as negative controls for both the bacteria and fungi. The experiment was performed in triplicate. Incubations were at 37^{o}C for 24 - 48 h for bacteria and *C. albicans* and at room temperature for 72 h for the other filamentous fungi. Following incubation the zones of inhibition formed were measured and the mean diameter obtained. Overall, cultured bacteria with halos equal to or greater than 7 mm and fungi with 10 mm halos were considered susceptible to the tested extract[7].

Determination of MIC

The minimum inhibitory concentration (MIC) of the crude extracts was also determined using the same method except that the paper discs were soaked in different concentrations of the crude extracts dispersed in water (0.2-1.0ml). After incubating at 24 h at 37°C, the MIC of each sample was determined by measuring the optical density in the spectrophotometer (620 nm), and comparing the result with those of the non inoculated NB and PDB[7] .

Results and Discussion

The preliminary phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, carobohydrtaes and proteins (Table 1). The observed antibacterial activity is attributed to the presence of bioactive compounds in the extracts of plants tested. The presence of these bioactive compounds in crude extracts is known to confer antibacterial activity against disease-causing microorganisms[8] offer protection to plants themselves against pathogenic microbial infections[9.]

Table.1. Phytochemical screening of *Caesalpinia sappan* L. (bark) and *Mimosa pudica* L. (leaves)

Phytochemical constituents	*C. sappan*	*M.pudica*
Alkaloids	+	+
Stroids	+	-
Tannins	+	-
Flavonoids	+	+
Terpenoids	+	-
Carbohydrates	+	+
Proteins	+	+

The antibacterial activity of *C. sappan* (bark) and *M. pudica* (leaves) were assayed by the agar disc diffusion and broth dilution method against six bacterial and two fungal strains. Methanol extracts from the two plants were found to have highest antibacterial activity, whereas the aqueous extracts were less effective in inhibiting bacterial growth. Among the six bacterial strains tested for antibacterial activity, *S.aureus* was most susceptible with inhibition zones ranging from 9-20 mm and *K.pneumoniae* was least susceptible organism to the plant extracts. Further, the methanol extracts of *C.sappan* was found to be most effective than *M.pudica* (Table 2).

Table.2. Antimicrobial activity of *Caesalpinia sappan* (bark) and *Mimosa pudica* (leaves)

Microorganisms	***Caesalpinia sappan* (bark)**		***Mimosa pudica* (leaves)**	
	Methanol	**Aqueous**	**Methanol**	**Aqueous**
Staphylococcus aureus	20	15	15	9
Bacillus subtilis	14	10	12	8
Escherichia coli	15	8	10	-
Pseudomonous aeruginosa	12	7	11	7
Klebsiella pneumoniae	10	-	8	-
Proteus vulgaris	12	10	10	7
Candida albicans	10	-	8	-
Aspergillus niger	8	-	-	-

Methanol and aqueous of *C.sappan* inhibited gram-positive strains *S. aureus, B. subtilis,* gram-negative strains *K. pneumonia*, *E.coli* and *P.vulgaris* with MIC ranging from 0.14 to 0.82 mg/ml and 0.22 to 0.86 mg/ml, respectively. On the other hand, methanol and aqueous of *M.pudica* inhibited gram-positive strains *S. aureus, B. subtilis,* gram-negative strains *K. pneumonia*, *P.vulgaris* and *P.aeruginosa* with MIC ranging from 0.44 to 0.88 mg/ml and 0.71 to 0.83 mg/ml, respectively. The methanolic extracts of the screened plants showed moderate antifungal activity against *C.albicans* and *A.niger* (Table 3 &4). *C.albicans* were more susceptible than *A.niger.*

Table.3. MIC of *C.sappan* bark

Organisms	Methanol (mg/ml)					Aqueous (dilutions) ml				
	0.2	**0.4**	**0.6**	**0.8**	**1.0**	**0.2**	**0.4**	**0.6**	**0.8**	**1.0**
S. aureus	0.3	0.28	0.24	0.22	0.19	0.82	0.80	0.78	0.74	0.72
B. subtilis	0.34	0.29	0.24	0.19	0.14	0.42	0.40	0.38	0.36	0.34
E. coli	0.48	0.44	0.40	0.36	0.31	0.86	0.82	0.78	0.74	0.72
P. aeruginosa	0.82	0.78	0.74	0.70	0.65	0.83	0.80	0.77	0.77	0.76
K. pneumoniae	0.38	0.34	0.30	0.26	0.22	0.59	0.57	0.56	0.53	0.51
P.vulgaris	0.48	0.42	0.36	0.32	0.26	0.60	0.58	0.56	0.54	0.51
C.albicans	0.286	0.284	0.282	0.280	0.278	0.489	0.487	0.485	0.483	0.481
A. niger	0.107	0.105	0.103	0.101	0.100	0.328	0.326	0.324	0.322	0.320

Table.4. MIC of *M.pudica* leaves

Organisms	Methanol (dilutions) ml					Aqueous (dilutions) ml				
	0.2	**0.4**	**0.6**	**0.8**	**1.0**	**0.2**	**0.4**	**0.6**	**0.8**	**1.0**
S. aureus	0.88	0.86	0.84	0.82	0.80	0.84	0.83	0.82	0.81	0.80
B. subtilis	0.52	0.50	0.48	0.46	0.45	0.51	0.49	0.47	0.45	0.44
E. coli	0.85	0.83	0.81	0.79	0.78	0.86	0.83	0.81	0.79	0.76
P. aeruginosa	0.82	0.80	0.78	0.76	0.74	0.83	0.81	0.78	0.76	0.74
K. pneumoniae	0.75	0.77	0.76	0.75	0.73	0.80	0.77	0.74	0.71	0.70
P.vulgaris	0.80	0.78	0.76	0.74	0.71	0.81	0.79	0.77	0.76	0.74
C.albicans	0.398	0.397	0.396	0.395	0.394	0.581	0.579	0.577	0.575	0.573
A. niger	0.300	0.280	0.260	0.240	0.220	0.487	0.484	0.481	0.478	0.477

In general, the methanol extract of the tested plants was most effective in inhibiting the bacterial growth suggesting that polar solvent methanol was most successful in extracting secondary metabolites responsible for the antibacterial property than aqueous extracts[10] . The extracts of *C.sappan* showed the presence of tannins and alkaloids. Several plants, rich in tannins have been shown to possess antimicrobial activities against a number of microorganisms. For example Banso and Adeyemo[11] , investigated the antibacterial activity of leaf extract of *Dichrostachys cinerea* and reported the presence of tannins, alkaloids and glycosides. Amongst the grampositive and gram-negative bacteria, gram-positive bacterial strains were more susceptible to the extracts when compared to gram negative bacteria. This may be attributed to the fact that these two groups differ in their structure of the cell wall components [12] . The ability of tannin compounds to cause the bacterial colonies to disintegrate, probably results from their interference with the bacterial cell wall; thereby inhibiting the microbial growth

Extracts of *C.sappan* in this study demonstrated a broad-spectrum of activity against both gram-positive and gram-negative bacteria and fungi. The broad-spectrum antibacterial activities of the plant extract, possibly due to the identified alkaloids and tannins, further confirm its use as a health remedy in folklore medicine. Bioactive substances from this plant can therefore be employed in the formulation of antimicrobial agents for the treatment of various bacterial and fungal infections including gonorrhea, Isolation, identification and purification of these phytoconstituents and determination of their respective antimicrobial potencies and toxicological evaluation with the view to formulating novel chemotherapeutic agents should be the future direction for investigation.

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