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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF VARIOUS FRUIT PODS AGAINST HUMAN PATHOGENS

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ABSTRACT: Traditional plant-based remedies continue to be an important therapeutic aid for treating many infections. In the current study, an attempt was made to know the antibacterial activity of different pod extracts of plants like *Albizia lebeck*, *Cesalpina pulcherima*, *Leucenea leucocephala*, & *Tecoma stans* against six different human pathogens *E. coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Proteus vulgaris*. The pods were collected, washed, shade dried and powdered. The powders of the four different pods were extracted with methanol and water and screened for their phytochemical constituents by standard protocols and were shown to contain carbohydrates, flavonoids, tannins, phenols, saponins. All the extracts obtained were tested against the six pathogenic bacteria by cup plate method. Out of all the extracts, methanolic extracts of all the pods showed promising activity against all six pathogens. Methanolic extracts of *C. pulcherima* (2.2 cm) & *T. stans* (2.13 cm) exhibited greater activity than standard (2.0 cm) when tested against *E. coli*. The antibacterial activity of extracts may be linked to the presence of flavonoids, saponins, and tannins.

INTRODUCTION: The Indian sub-continent is a large repository of medicinal plants that are used in traditional medical treatments, use of medicinal plants as a source of medicine has been an ancient practice. The alternative medicines in the traditional systems are derived from herbs, minerals, and organic matter, while for the preparation of herbal drugs only medicinal plants are used.

According to WHO, 75% of the world's population are using herbs for basic healthcare needs ¹. The herbal parts used in herbal therapy, include seeds, berries, roots, leaves, fruits, bark, flowers and sometimes the whole plant. In the present study antimicrobial activity of fruit pods of *Albizia lebeck*, *Leucenea leucocephala*, *caesalpina pulcherima*, *Tecoma stans* were assessed. Studies on various parts of these plants are already available. There is meagre information available on the antimicrobial activities of these fruit pods.

Albizia lebeck fruit pods are 12-35 cm long × 3-6 cm broad, light yellowish-brown colored that contain 5-15 rounded, free moving seeds ². *Leucenea leucocephala* fruit pods are 14-16 cm × 1.5-2 cm pendant, brown at maturity ³. *Caesalpina*

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pulcherima pods are 12 cm long⁴. *Tecoma stans* fruits are large elongated somewhat flattened capsules (10-30 cm long & 5-20 cm wide)⁵. These fruits turn from green to brown in color as they mature and finally split open to release numerous papery seeds.

MATERIALS AND METHODS:

Plant Materials (Fruit): The fruit of *Albizia lebbek*, *Lucaenia leucocephala*, *Cesalpinia pulcherrima* & *Tachoma stans* were collected during summer from the village Manakondur, Karimnagar district. All the mentioned fruits were dried under shade and then ground into a fine powder and stored separately to perform the experiments.

Organisms: Medicinally important bacterial strains like *Bacillus subtilis* (MTCC7086), *Escherichia coli*, *Enterobacter aerogenes* (MTCC7016), *Pseudomonas aerogenosa* (MTCC7083), *Staphylococcus aureus* (MTCC7405) and *Proteus vulgaris* (MTCC744) have been used which were procured from MTCC (IMTECH), Chandigarh, India.

Methods:

Extraction Procedure: All the dried fruits (*Albizia lebbek*, *Lucaenia leucocephala*, *Cesalpinia pulcherrima* and *Tecoma stans*) that were procured, were subjected to Soxhlet extraction individually using methanol and distilled water.

After extracting, the solvents were distilled off and the obtained products were collected and put in a desiccator for moisture evaporation. All the extracts were then qualitatively analyzed

individually to know the phytochemical constituents present in them.

Phytochemical Screening: The products obtained were screened for various phytochemical constituents like carbohydrates, amino acids, proteins, steroids, flavonoids, glycosides, tannins, phenols, alkaloids & saponins^{6,7,8,9}.

Anti-Bacterial Activity:¹⁰

A) Preparation of Pure Cultures: Appropriate amounts of the culture from the stocks were inoculated into freshly prepared media. The process was carried out in Lamina Air Flow chamber following aseptic conditions. They were allowed to incubate for about 24-48 to obtain the growth of the organism.

B) Activity Testing: Nutrient agar medium was prepared, autoclaved & poured into sterile Petri plates. Allowed the plates to solidify. The antimicrobial activity was tested using the "Agar well diffusion technique". 100 µl of the culture was taken and inoculated onto the media using a micro-pipette. It was then evenly spread using a sterile glass spreader. Using a gel-borer, wells were made in the plates. All the extracts were dissolved in dimethyl sulphoxide (DMSO) and 50 µl of each extract was introduced into wells using a micro-pipette. They were incubated for 24 h and then observed for 'zone of clearance' and were compared against a standard drug "Streptomycin". All the steps were carried out in Laminar Air Flow chamber following aseptic conditions. Triplicates were maintained for each organism and for each extract while performing the experiment.

RESULTS:

TABLE 1: PHYTOCHEMICAL SCREENING OF METHANOLIC AND AQUEOUS EXTRACTS OF FRUIT PODS

S. no.	Phytochemical constituents	ME				AQ			
		AL	LL	CP	TS	AL	LL	CP	TS
1	Carbohydrates	+	+	+	+	+	+	+	+
2	Proteins	-	+	-	+	-	-	-	-
3	Amino acids	-	+	-	+	+	+	+	-
4	Steroids	-	-	-	+	-	-	-	+
5	Flavonoids	+	+	+	+	+	-	+	-
6	Glycosides	+	-	+	+	-	-	-	-
7	Tannins	+	+	+	+	-	+	-	-
8	Phenols	+	+	+	+	-	+	-	+
9	Saponins	+	+	-	+	+	+	-	+

ME: Methanolic extract; AQ: Aqueous extract; AL: *Albizia lebbek*; LL: *Lucaenia leucocephala*; CP: *Cesalpinia pulcherrima*; TS: *Tecoma stans*.

TABLE 2: ANTIBACTERIAL ACTIVITY OF METHANOLIC & AQUEOUS EXTRACTS OF FRUIT PODS AGAINST SIX HUMAN PATHOGENS

S. no.	Microorganism	ME				AQ				Standard
		AL	LL	CP	TS	AL	LL	CP	TS	
1	<i>Proteus vulgaris</i>	1.23	0.6	1.4	1.06	1	0.7	1.03	0.6	1.5
2	<i>Staphylococcus aureus</i>	-	-	0.76	-	-	-	-	-	2.6
3	<i>Enterobacter aerogenes</i>	0.23	0.26	0.96	1.66	-	-	-	-	2.7
4	<i>Escherichia coli</i>	-	1.86	2.2	2.13	-	1.63	-	0.73	2
5	<i>Bacillus subtilis</i>	0.53	2.26	0.46	1	0.2	1.36	0.46	-	1.6
6	<i>Pseudomonas aerogenosa</i>	-	1.46	1.26	2.13	0.83	-	-	-	1.8

ME: Methanolic extract; AQ: Aqueous extract; AL: *Albizia lebbek*; LL: *Leucenea leucocephala*; CP: *Caesalpina pulcherima*; TS: *Tecoma stans*; Standard: Antibiotic Streptomycin.

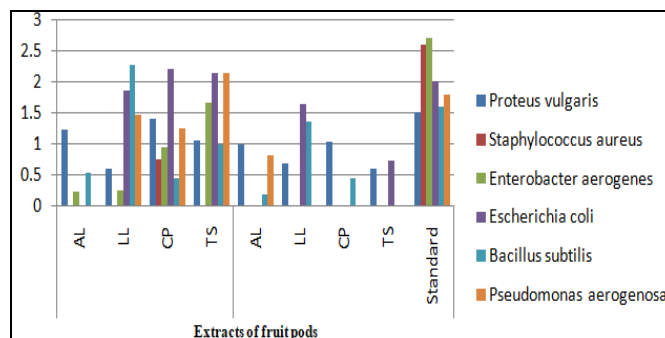


FIG. 1: ANTIBACTERIAL ACTIVITY OF POD EXTRACTS AGAINST PATHOGENS. ME: Methanolic extract; AQ: Aqueous extract; AL: *Albizia lebbek*; LL: *Leucenea leucocephala*; CP: *Caesalpina pulcherima*; TS: *Tecoma stans*; Standard: Antibiotic Streptomycin.

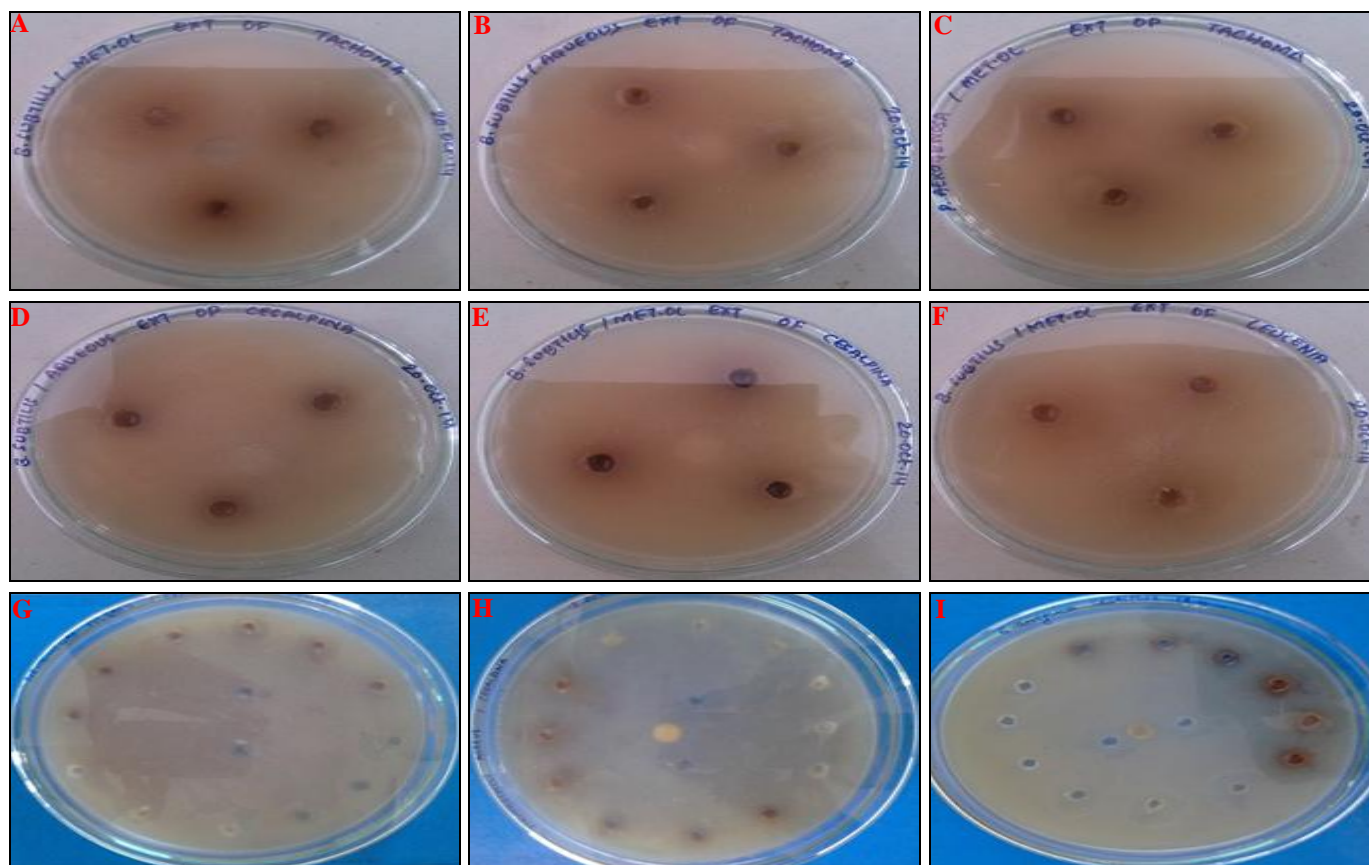


FIG. 2: ZONES OF INHIBITION BY FRUIT POD EXTRACTS AGAINST HUMAN PATHOGENS. A) Activity of methanolic extract of *Tecoma stans* against *Bacillus subtilis*. B) Activity of aqueous extract of *Tecoma stans* against *Bacillus subtilis*. C) Activity of methanolic extract of *Tecoma stans* against *P. aeruginosa*. D) Activity of aqueous extract of *Caesalpina pulcherima* against *Bacillus subtilis*. E) Activity of methanolic extract of *Caesalpina pulcherima* against *Bacillus subtilis*. F) Activity of all extracts against *proteus vulgaris*. G) Activity of all extracts against *Staphylococcus aureus*. H) Activity of all extracts against *Enterobacter aerogenes*.

DISCUSSION: Phytochemical screening was done for both methanolic and aqueous fruit pod extracts of *Albizia lebbeck*, *Leucenea leucocephala*, *Ceasalpina pulcherima*, and *Tecoma stans*. The phytochemical analysis revealed the presence of flavonoids in all the extracts except aqueous extract of *Leucenea leucocephala*. Presence of glycosides was observed with methanolic extracts of *A. lebbeck*, *C. pulcherima* & *T. stans*. Methanolic extracts of all the pods had tannins whereas aqueous extract of only *L. leucocephala* pods had tannins. Phenols were present in methanolic extracts of *Albizia lebbeck*, *Leucenea leucocephala*, *T. stans* and aqueous extracts of *Leucenea leucocephala* & *Tecoma stans*. Presence of saponins was clear in methanolic & aqueous pod extracts of *Albizia lebbeck*, *Leucenea leucocephala*, *Tecoma stans*, but absent in the extracts of *Caesalpina pulcherima* pods.

Antibacterial activity of methanolic and aqueous pod extracts of *Albizia lebbeck*, *Leucenea leucocephala*, *Ceasalpina pulcherima*, and *Tecoma stans* was carried out by agar cup plate method. The activity was tested against six test organisms, of which four are gram-negative bacteria (*E. coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, & *Proteus vulgaris*) and two are gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*). All methanolic extracts had shown antibacterial activity against *Proteus vulgaris* & *Enterobacter aerogenes*. Methanolic extract of *Albizia lebbeck* had exhibited maximum activity with a zone of inhibition of 1.23 cm almost near to the standard antibiotic used (Streptomycin) whose zone of inhibition was 1.5 cm.

Methanolic extract of *Leucenea leucocephala* had shown maximum zone of inhibition (2.26 cm) against *Bacillus subtilis*, which is greater than that of standard (1.6 cm). Similarly, methanolic extract of *Tecoma stans* had greater activity (2.13 cm) than the antibiotic streptomycin *Tecoma stans* (1.8 cm) used as standard. Methanolic extracts of *Caesalpina pulcherima* (2.2 cm) & (2.13 cm) exhibited greater activity than standard (2.0cm) when tested against *E. coli*. Methanolic & aqueous of all extracts had not shown activity against *S. aureus* except methanolic extract of *Caesalpina pulcherima* pods which had shown a negligible activity.

Methanolic pod extracts had exhibited good activity in comparison with aqueous pod extracts. The extracts that showed antibacterial activity have flavonoids, glycosides, tannins, phenols & saponins as their phytochemical constituents. Phytochemical constituents such as alkaloids, flavonoids, phenols, tannins, saponins are secondary metabolites of plants that serve a defense mechanism against invasion by many microorganisms, insects¹¹. Flavonoids are known to be synthesized by plants in response to microbial infection¹². Saponins show their anti-microbial activity by causing leakage of proteins and certain enzymes from cell¹³. Tannins interfere with protein synthesis by binding to proline-rich proteins¹⁴. The antimicrobial activity may be due to the presence of phytochemical constituents like phenolic compounds & flavonoids present in plants as secondary metabolites. Further work may be needed to determine the precise active principle from methanolic & aqueous extracts of *Albizia lebbeck*, *Leucenea leucocephala*, *Ceasalpina pulcherima* & *Tecoma stans*.

CONCLUSION: The results of the present study revealed that the methanolic extracts of *Caesalpina pulcherima* have exhibited great antibacterial activity against both gram-positive & gram-negative bacteria tested in this study. Further work may be carried out to determine the active principle from these extracts.

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CONFLICT OF INTEREST: There are no conflicts of interest.

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