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COMPARATIVE ANALYSIS OF PHYTOCHEMICAL COMPOUNDS AND ANTIBACTERIAL ACTIVITY OF CERTAIN UNDERUTILIZED WILD LEGUMES

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ABSTRACT: The current study was performed to study the antibacterial effects of solvent (ethanol, methanol, chloroform, hexane, acetone, petroleum ether) and aqueous extracts of lima bean, horse gram, and jack bean. The phytochemical studies performed on the extracts revealed the presence of alkaloids, steroids, triterpenoids, flavonoids, tannins, phenols, glycosides, and saponins. The antibacterial activity of these extracts was examined using agar well diffusion method. The extracts were studied for activity against pathogenic bacteria, namely *P. syringae*, *E. coli*, *B. subtilis*, *E. faecalis*, *S. aureus*, *S. typhi*, *B. cereus*, *P. aeruginosa.* The activity of the extracts against bacterial proliferation was estimated by measuring the zone of inhibition. The highest antibacterial activity among all the extracts tested and all the seeds involved in this study, the ethanol extract of horse gram has exhibited the maximum antibacterial effect against *E. coli* (12.0 ± 0.29), *E. fecalis* (11.0 ± 0.29) and *B. substilis* (10.0 ± 0.34). The results indicate that all three seeds in the discussion here show high potential for commercialization as antibacterial agents, due to their rich phytochemical contents.

Keywords: Horse gram, Lima bean, Jack bean, Antibacterial activity, Phytochemical screening

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INTRODUCTION: In recent years, the growing demand for herbal products has led to the exploration and exploitation of plant-based traditional medicinal plant materials across the globe. Plant compounds are of interest as a source of safer and more effective substitutes to synthetically produced bioactive chemical agents. Phytochemical research progress has been aided enormously by the development of rapid and accurate methods for screening plants for particular chemical compounds.

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The secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents [1]. Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of the bacterial infections [2].

Phytochemicals present in plants are active as chemotherapeutic, bacteriostatic and antimicrobial agents [3]. Furthermore, they are becoming models for the synthesis of new drugs with better therapeutic, chemical (or) physical properties than the original compounds [4].

Many such plant materials are being currently used for treating bacterial infections [2]. There are a few reports on the use of plants in traditional healing by tribal people and or indigenous communities [5-9].

The development of drug resistance in human pathogens against commonly used antibiotics has necessitated the search for new and alternative antimicrobial substances from other sources, including plants. Screening of medicinal plants for antimicrobial activities and phytochemical is thus important for finding potential new compounds for therapeutic and protective uses.

In this context, the present study is initiated to investigate the phytochemical constituents and *in-vitro* evaluation of the antibacterial activity of the seed extracts of certain wild and less common legumes such as *Canavalia ensiformis, M. uniflorum* and *Phaseolus lunatus* against pathogenic microorganisms.

MATERIALS AND METHODS:

Samples: The seeds of *Canavalia ensiformis* (Jack bean), *Phaseolus lunatus* (lima bean) and *Macrotyloma uniflorum* (horse gram) were collected from the ecological region of Madurai, Tamil Nadu, India **Fig. 1**. After drying in the sun, the pods were thrashed to separate mature seeds. After thorough cleaning and removal of broken seeds and foreign materials, the seeds were stored in plastic containers at room temperature (25 °C) until further use.

LIMA BEAN **JACK BEAN** **HORSE GRAM**

FIG. 1: UNDERUTILIZED WILD LEGUMES

Preparation of Raw Seed Samples: Dry, mature seeds (10 g each) were powdered in a Wiley Mill to 60-mesh size with suitable precaution to avoid contamination of samples. The powdered samples were stored in plastic containers at room temperature (25 °C) until further use.

Solvent Extraction: Solvent systems used for the extractions were acetone, ethanol, chloroform, petroleum ether, hexane, methanol, and water. Soxhlet extraction procedures were adapted for the extraction. 10g grams of each powered samples were packed in a muslin cloth and used for extraction by Soxhlet apparatus at a temperature below the boiling temperature of each solvent. After 48 h, the extracts were filtered using Whatman filter paper no: 1. the solvent was evaporated, and the residue was dissolved in sterile Dimethylsulfoxide (DMSO-9:1) in 50 mg/ml (w/v) concentration.

The extract was filtered using 0.22 micro filters (Type GV- Millipore) and stored at 4° C for further antibacterial studies.

Phytochemical Screening of the Seed Extract: The seed powder extracts were analyzed for the presence of glycosides, tannins, phenols, flavonoids, alkaloids, saponins, steroids, and terpenoids by the methods [10, 11].

Screening of Antibacterial Activity: Microorganisms (*Pseudomonas syringae* (ATCC 7386), *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (MTCC 441), *Enterococcus faecalis* (ATCC29212), *Staphylococcus aureus* (MTCC 29212) *Salmonella typhi*, *Bacillus cereus*, *Pseudomonas aeruginosa.*

The agar well diffusion method, as described [12, 13,] was used to determine the inhibitory effects of the extracts of the seeds against the isolates. The bacterial isolates were first grown in nutrient broth for 18 h at 37 °C, then 0.2 ml of the broth culture of the isolates were aseptically inoculated onto a molten nutrient agar which had been cooled to 45 °C, mixed gently and poured into sterile Petri dishes and allowed to set. The suspension was diluted with sterile distilled water to obtain

approximately 10^5 CFU/ml. These were delivered into wells (8 mm diameter) bored unto the surface of the inoculated nutrient agar plates. The extracts were allowed to diffuse into the medium for 30 min. The plates were incubated at 37 °C for 24 to 48 h. The zone(s) of inhibition was measured in millimeter diameter using meter rule [14].

RESULTS AND DISCUSSION: Phytochemical tests were carried out in the various seed extracts to determine the alkaloids, steroids, triterpenoids, flavonoids, tannins, phenols, glycosides and saponins. The results were as depicted in **Table 1**. Alkaloids were found to be present in ethanol, methanol, hexane, and petroleum ether extracts. The steroids were found in ethanol and methanol extracts, while methanol extracts revealed steroids. Triterpenoids were seen in acetone extracts, and flavanoids tannins and phenols were observed in ethanol and petroleum ether extracts. Glycosides and saponins were found to be present in chloroform, petroleum ether, hexane, and aqueous extracts.

The antibacterial activity of the various seed extracts was observed against eight bacterial pathogenic strains using agar well diffusion method. Zone of inhibition of the individual seed extracts are shown in **Table 2-4,** respectively. The extracts were seen to exhibit different degrees of antibacterial activity ranging from 6 to 12 mm against the studied pathogens. The antibacterial activity of the seed extracts showed good magnitude of inhibition patterns in comparison with standard positive control. All the extracts were shown significant zone of inhibition against the studied pathogens.

Horse gram seed extracts are effective against all the tested strains of pathogens. The highest inhibition was observed against *E.* coli (12.0 ± 0.29 mm) in ethanol extract. While it was less significant against *S. aureus* (6.0 ± 0.28 mm) in acetone extract. Overall, the ethanol extracts of horse gram showed the best range of antibacterial activity against all the tested bacterial pathogens. Lima bean extracts showed inhibition of almost all the bacterial strains tested with the range of inhibition ranging from 6.0 - 11.0 mm.

The highest inhibition was seen in the methanol extract against *E. coli* (11.0 ± 0.25 mm). The lowest inhibition was observed against *P.syringe* with an aqueous extract (6.0 ± 0.04 mm). The methanol extract of lima bean seemed to show the highest pathogen resistance among all the extracts studied. Jack bean extracts, showed inhibitory level with a range of about 6.0 - 11.0 mm inhibition zones. The chloroform extracts were able to significantly effect against *S. typhi* (11.0 ± 0.32). The lowest inhibitory activity (6.0 ± 0.26 mm) was recorded by the hexane extracts against *E. fecalis, S. aureus, P. syringea, B. cereus.* In general, ethanol and methanol extracts showed better inhibitory activity against studied. The results highly coincided with the earlier studies on pathogens the hull extracts of Bengal gram, mung and pigeon pea for their antimicrobial activity against common food pathogens namely *B. cereus, S. aureus, E. coli* and *P. fluorescent* [15].

TABLE 1: PHYTOCHEMICAL ANALYSIS OF COMMON AND WILD LEGUMES

Phytochemical	Acetone			Chloroform			Ethanol			Methanol			Hexane			Petroleum Ether			Water		
	LB	HG	JB	LB	HG	JB	LB	HG	JB	LB	HG	JB	LB	HG	JB	LB	HG	JB	LB	HG	JB
Alkaloids	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
Steroids	-	+	+	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	-	+
Triterpenoids	+	+	+	-	-	-	+	-	-	-	+	-	+	-	-	-	-	-	-	+	+
Flavanoids	-	-	-	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	+	-	-
Tannins	+	-	-	-	-	-	+	+	+	+	+	-	-	+	+	+	+	+	-	-	-
Phenols	+	-	-	-	-	-	+	+	+	+	-	-	-	+	+	+	-	+	-	-	-
Glycosides	+	+	+	+	+	+	-	-	-	-	+	-	-	-	-	+	+	+	-	+	+
Saponins	-	-	-	+	-	-	+	-	-	+	-	-	+	+	+	+	-	-	+	+	+

LB – Lima Bean, HG – Horse Gram, JB – Jack Bean, + Presence, - Absence

TABLE 2: EFFECT OF HORSE GRAM SEED EXTRACT ON THE BACTERIAL CULTURES

Bacterial culture	Zone of inhibition(mm diameter)								
	Seed extract								
	Positive control	Solvent control	Petroleum ether	Hexane	Ethanol	Methanol	Water	Acetone	Chloroform
E. coli	20	-	9.0 ± 0.39	7.0 ± 0.33	12.0 ± 0.29	11.0 ± 0.25	10.0 ± 0.31	7.0 ± 0.29	11.0 ± 0.26

E. fecalis	13	-	6.0 ± 0.38	7.0 ± 0.41	11.0 ± 0.29	9.0 ± 0.29	8.0 ± 0.34	6.0 ± 0.39	9.0 ± 0.28
S. typhi	16	-	8.0 ± 0.33	8.0 ± 0.29	9.0 ± 0.34	8.0 ± 0.27	9.0 ± 0.39	7.0 ± 0.37	9.0 ± 0.28
S. aureus	19	-	10.0 ± 0.24	7.0 ± 0.32	8.0 ± 0.29	9.0 ± 0.27	8.0 ± 0.31	6.0 ± 0.28	8.0 ± 0.38
P. syringe	14	-	7.0 ± 0.25	8.0 ± 0.29	9.0 ± 0.34	10.0 ± 0.25	7.0 ± 0.34	7.0 ± 0.29	9.0 ± 0.37
B. substilis	17	-	8.0 ± 0.23	6.0 ± 0.33	10.0 ± 0.34	9.0 ± 0.29	9.0 ± 0.28	7.0 ± 0.30	8.0 ± 0.39
B. cereus	16	-	9.0 ± 0.37	9.0 ± 0.37	8.0 ± 0.30	11.0 ± 0.25	7.0 ± 0.29	8.0 ± 0.37	7.0 ± 0.37
P. aeruginosa	21	-	8.0 ± 0.33	7.0 ± 0.29	9.0 ± 0.34	8.0 ± 0.25	6.0 ± .39	7.0 ± 0.29	9.0 ± 0.37

Concentration of extract- 100µl/well, (-) - No zone of inhibition observed, Positive controls-chloromphenicol (10 µg/ml), Solvent control - 10% DMSO

TABLE 3: EFFECT OF LIMA BEAN SEED EXTRACT ON THE BACTERIAL CULTURES

Bacterial culture	Zone of inhibition(mm diameter)								
	Seed extract								
	Positive control	Solvent control	Petroleum ether	Hexane	Ethanol	Methanol	Water	Acetone	Chloroform
E. coli	20	-	9.0 ± 0.11	7.0 ± 0.21	9.0 ± 0.05	11.0 ± 0.25	9.0 ± 0.11	8.0 ± 0.02	8.0 ± 0.16
E. fecalis	13	-	7.0 ± 0.05	6.0 ± 0.11	7.0 ± 0.03	10.0 ± 0.01	7.0 ± 0.34	7.0 ± 0.30	9.0 ± 0.20
S. typhi	16	-	8.0 ± 0.21	8.0 ± 0.39	9.0 ± 0.14	9.0 ± 0.07	9.0 ± 0.39	6.0 ± 0.15	9.0 ± 0.28
S. aureus	19	-	9.0 ± 0.02	7.0 ± 0.33	7.0 ± 0.39	9.0 ± 0.24	8.0 ± 0.11	8.0 ± 0.20	7.0 ± 0.02
P. syringe	14	-	8.0 ± 0.25	7.0 ± 0.19	9.0 ± 0.33	8.0 ± 0.27	6.0 ± 0.04	7.0 ± 0.01	7.0 ± 0.10
B. substilis	17	-	6.0 ± 0.13	6.0 ± 0.31	9.0 ± 0.31	9.0 ± 0.33	9.0 ± 0.10	8.0 ± 0.10	6.0 ± 0.39
B. cereus	16	-	7.0 ± 0.10	6.0 ± 0.11	8.0 ± 0.20	7.0 ± 0.24	9.0 ± 0.11	6.0 ± 0.11	6.0 ± 0.11
P. aeruginosa	21	-	7.0 ± 0.33	7.0 ± 0.25	7.0 ± 0.31	8.0 ± 0.05	6.0 ± 0.12	6.0 ± 0.05	7.0 ± 0.33

Concentration of extract- 100µl/well, (-) - No zone of inhibition observed, Positive controls – chloromphenicol (10 µg/ml), Solvent control - 10% DMSO

TABLE 4: EFFECT OF JACK BEAN SEED EXTRACT ON THE BACTERIAL CULTURES

Bacterial culture	Zone of inhibition(mm diameter)								
	Seed extract								
	Positive control	Solvent control	Petroleum ether	Hexane	Ethanol	Methanol	Water	Acetone	Chloroform
E. coli	20	-	9.0 ± 0 .23	7.0 ± 0.27	11.0 ± 0 .25	12.0 ± 0.25	9.0 ± 0.34	8.0 ± 0.33	10.0 ± 0.23
E. fecalis	13	-	8.0 ± 0.27	6.0 ± 0 .26	11.0 ± 0 .25	8.0 ± 0.28	7.0 ± 0 .37	7.0 ± 0.33	9.0 ± 0.27
S. typhi	16	-	8.0 ± 0.33	8.0 ± 0 .28	9.0 ± 0 .29	9.0 ± 0 .29	9.0 ± 0.33	6.0 ± 0.33	11.0 ± 0.32
S. aureus	19	-	10.0 ± 0.36	6.0 ± 0.26	10.0 ± 0 .25	11.0 ± 0 .25	8.0 ± 0 .34	7.0 ± 0.37	10.0 ± 0.31
P. syringe	14	-	8.0 ± 0.28	6.0 ± 0 .26	9.0 ± 0.29	10.0 ± 0.25	8.0 ± 0 .37	7.0 ± 0.33	9.0 ± 0.33
B. substilis	17	-	6.0 ± 0.34	7.0 ± 0 .27	9.0 ± 0 .29	9.0 ± 0.29	9.0 ± 0 .29	8.0 ± 0.26	8.0 ± 0.39
B. cereus	16	-	7.0 ± 0.35	6.0 ± 0 .26	8.0 ± 0 .28	11.0 ± 0.25	10.0 ± 0 .25	6.0 ± 0.35	11.0 ± 0.29
P. aeruginosa	21	-	7.0 ± 0.33	7.0 ± 0 .27	9.0 ± 0 .29	8.0 ± 0 .28	7.0 ± 0.33	6.0 ± 0.28	7.0 ± 0.30

Concentration of extract- 100µl/well, (-) - No zone of inhibition observed, Positive controls – chloromphenicol (10 µg/ml), Solvent control - 10% DMSO

It was inferred that the high polyphenolic contents of the hull extracts were primarily responsible for the antimicrobial activity observed [16, 17]. The seed extracts of the current study also possess the considerable phenolic compound activity, and this may be the cause of their high inhibitory activity as well. Legume seeds contain lectins and protease inhibitors that are reported to have antimicrobial activity. Interactions with seed lectins have been used to obtain structural information about the cell envelop and cell wall polymers of several Gram-negative and Gram-positive microorganisms [18]. The seeds in discussion in the current study are also classified as legumes, and thus, the above statement might also explain the antibacterial activity of the extracts obtained from these legumes.

Previously the methanolic extracts of *E. prostrata* were studied and found to have antibacterial activity tested against *M. luteus, S. aureus, E. coli* and *P. aeruginosa* [19]. The antibacterial and antioxidant activities of the essential oil and methanol extract of endemic *Zataria multiflora* Boiss were studied [20]. They concluded that the essential oil, extracted from the plant mentioned above showed very high antibacterial activity in comparison to the methanol extract. In the current study, the extracts have almost uniformly exhibited inhibition of the pathogens studied.

CONCLUSION: More presumably the peptides and proteins in the seed extracts along with the phenolic compounds might be responsible for their antibacterial activity but further mechanistic studies are required to elucidate the compounds responsible for antimicrobial activity as well as any pharmacological or toxicological properties that such extracts might have. The studies to identify the causative agents for these properties are in progress. The ethanol extracts of all three seeds

show effective prevention of bacterial growth. Thus we conclude that among the 3 seeds studied here, Horse gram seeds exhibit the highest antibacterial activity.

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CONFLICT OF INTEREST: Nil

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