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Antibacterial activity of Methanolic Spines extracts Pithecellobium dulce L.

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Abstract

In the present study Methanol extract of Pithecellobium dulce Spines was prepared using the Maceration method. The extracts were evaluated for antibacterial activity against the need to search for new Herbal Drug therapy for Microbial Infections has therefore become a constant, exercise to *Staphylococcus aureus* (Gram +ve) *Escherichia coli* (Gram -ve) *Staphylococcus epidermidis* (Gram +ve) *Bacillus subtilis* (Gram +ve) *Bacillus cereus* (Gram +ve) Disc diffusion method open auspicious antibacterial activity of the extracts prepared in polar solvents methanol Spines extract was found to be most active against Microbial strains. The antibacterial activity of Pithecellobium dulce L.Spines Methanolic Spines extract was found to be effective against all the five tested Bacterial strains. These findings suggest that methanolic Spines extracts of Pithecellobium dulce have the potential as an effective anti-bacterial agent.

Keywords: Pithecellobium dulce, Methanol, Penicillin G, Zone of Inhibition.

1. Introduction

The use of plant products with therapeutic properties to cure and prevent diseases is as ancient as human civilization and is the source of bioactive compounds for drug development by pharmaceutical companies and also the only option for treatment in rural communities. According to the World Health Organization, about 80 % of the people in developing countries rely primarily on medicinal plants for their primary health care. Pithecellobium dulce, a medicinal plant cultivated throughout India is a species of the Fabaceae family. The plant reaches a height of about 10-15 m with a spiny trunk, bipinnate Spines, and greenish-white sessile flowers. The plant is inherent in humid America and India it is known as 'vilayet babul' in Hindi Telugu thuma. In India, the plant is also generally called "Madras Thorn or Jungle Jalebi". The plant has outmoded medicinal importance as its bark has been used for the treatment of dysentery and febrifuge. In addition, the bark of this plant holds anti-venomous activity and is useful against dermatitis and eye tenderness besides. The Spines of Pithecellobium dulce have been used as a folk medicine for the treatment of toothache, leprosy, peptic ulcer, ear

ache, and as anti-diabetic. It is testified that roots of Pithecellobium dulce possess estrogenic activity and the fruits exhibit anti-inflammatory activity. While employed in the synthesis and adjustment of bioactive molecules for the development of drug candidates we thought that investigation of Pithecellobium dulce root extract may offer a potent antibacterial agent. The present study aimed to investigate the antibacterial potential of Pithecellobium dulce Spine extract against Five Gram-positive and Gram-negative bacterial strains. The results revealed that polar root extracts of Pithecellobium dulce have promising antibacterial potential. (Jurenka JS et al 2009)

2. Materials and Methods

2.1.Collection of Plant Materials

The Spines of *Pithecellobium dulce L.* in April at the coastal region of Andhra Pradesh. I collected materials washed and dried them in the shade. The dried materials are powdered by using a mixer. The course powdered drug materials are subjected to solvent extraction by maceration processing. (Gupta SC et al 2013)

2.2.Identification and Authentication of Plants Materials

The Spines of Pithecellobium dulce L. identified and authenticated by Mrs.Y.Prameela, Department of Botany, SIMS College of Pharmacy, Guntur, and the specimens **Regd. No's 1469** was preserved in the herbarium for further identification.

2.3.Herbal Drug Extraction by Maceration Method.

Weigh The 300gr of driedSpines of Pithecellobium dulce *L* materials were ground into a coarse powder and macerated separately with 500ml of Methanol for six days at 35^{o}c. The respective Plant extracts were collected and subjected to dryness by using the Rotavapor apparatus under reduced pressure at a temperature of 40^{0}C. Afterward, each plant part was concentrated to dryness afford three samples (MEPD) the dried Herbal Extracts are preserved in a desiccator for further subjected to Experimental Pharmacological invitro and Invivo studies.(Marchiani Aet al 2013)

2.4.Qualitative Phytochemical Analysis

The Qualitative Phytochemical Analysis of dried Methanolic extracts and organic solvents (toluene, ethyl acetate & n-butanol) fractions of F1 to F32 were screened for the presence of alkaloids, tannins, terpenoids, glycosides, flavonoids, saponins, anthraquinones, and steroids. Chemical tests were carried out using standard procedures to identify the active constituents of Methanolic extracts and Isolated Fractions. (Aggarwal BB et al 2013)

3. Invitro Studies

3.1.Free Radical Scavenging Activity of *Pithecellobium dulce L.*

3.2.Preparation of DPPH Stock Solution: DPPH 3 mg was dissolved in 100ml to form 0.1 mM solution.

3.3.Preparation of test Solutions: weigh the methanolic extract 1mg and dissolved in 100 ml of distilled water was prepared.

3.4.Preparation Standard Solutions: 10 mg/ml of Ascorbic acid was weighed separately and dissolved in 0.95 ml of DMSO to get 10.5 mg/ml concentrations. This solution was serially diluted with DMSO to get lower concentrations.

3.5. Procedure: The DPPH scavenging activity was done using the method. A total of 3 µL of 0.1 mM DPPH solution was added to 10 µL of Methanolic Extracts MEPDand standard (Ascorbic acid) of different test concentrations (100 to 600 µgr/ml) and allowed to react at room temperature. After 30 min, the absorbance values were measured at 517nm and converted into the percentage antioxidant activity using the following equation. Test solution (10 µl) was used as a blank, while DPPH solution plus methanol was used as a negative control. The positive controls were DPPH solution plus each ml of standard (Ascorbic acid). The IC50 values were calculated by linear regression of the plot, the Methanolic extracts of MEPD represent the Percentage of scavenging capacity. Each experiment was carried out and IC50 value (µg/mL) of the Methanolic extracts were reported.(Neelofar K et al 2011)

3.6. Calculation

The percentage of antioxidant activity of Methanolic extracts of MEPD was calculated using the following formula.

$$\text{Percentage of DPPH scavenging} = \frac{\text{absorbance of blank} - \text{absorbance of test}}{\text{absorbance of blank}} 100$$

4. Gram Stating Method

4.1. Procedure

Take a clean, grease-free slide. Prepare the smear of suspension on the clean slide with a loopful of samples. Air dry and heat fix Crystal Violet was poured and kept for about 30 seconds to 1 minute and rinsed with water. Flood the gram's iodine for 1 minute and wash with water. Then, wash with 95% alcohol or acetone for about 10-20 seconds and rinse with water. Add safranin for about 1 minute and wash with water. Air dry, Blot dry, and Observe under the Microscope.

4.1.Invitro Anti-Microbial Activity of Methanol Extract of *Pithecellobium dulce L*

4.2. Selected Microorganisms In the present study, the following four Gram-positive and one Gram-negative bacteria were selected.

Table No.01 Selected Bacterial Strains for Treatment of MEPD

S.No	Gram Stain	Microorganism strains	Type	Causes
01.	Gram (+ve)	*Staphylococcus aureus*	ATCC 25923	Wound infection, Pneumonia
02.	Gram(-ve)	*Escherichia coli*	ATCC 45866	Bloody Diarrhoea, Urinary Tract Infections.
03.	Gram (+ve)	*Staphylococcus epidermidis*	ATCC 25688	Infection of prosthetic medical device
04.	Gram (+ve)	*Bacillus subtilis*	ATCC 45878	Food Poisoning
05.	Gram (+ve)	*Bacillus cereus*	ATCC 11778	Food Poisoning, Vomiting, Diarrhoea,

The above bacterial strains were used in this study. All the bacterial strains were grown and maintained on nutrient agar slants. The inoculum size of each test strain was 108 bacteria/ml for disc diffusion assay which was standardized by adjusting the optical density of the bacterial suspension to a turbidity corresponding to spectrophotometric

Absorbance = 0.08 (OD_{620} = 0.08) at 620 nm.

4.3.Preparation of Test Sample

Weigh accurately 100 to 1000 mg of Methanolic Extract and dissolved it in 100 ml of distilled water and pipetting out 10 mg/mL.

4.4.Preparation Standard sample

Weigh accurately 100 to 1000 mg of Penicillin G and dissolved in 100 ml of distilled water and pipetting out 10 mg/mL.

4.5.Inoculum preparation

A fresh microbial suspension was prepared by subculturing the bacterial colonies into the nutrient broth medium (Hi Media pH 7.4) and incubated at 37°C to maintain the uniform growth rate of each organism. The bacterial suspension of approximately 1x108 CFU/ml, which is equivalent to 0.5 Me Farland turbidity standard to density (Perilla et al,, 2003) was used throughout the experimentation.

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4.7.Procedure for Liquid Agar Media

Be sure to accurately weigh the chemical components of the broth that contains nutrients and place them in the beaker that holds 500ml distillate water. Begin to heat the contents gently by stirring lightly to dislodge the ingredients. You can add more distillation water to increase 1 liter of volume. Determine your broth's pH using a pH meter. Then, alter your pH until 7.0 with the addition of drops of either NaH or HC1 solution. Place the basket in the pressure cooker/autoclave and sterilize at 121°C for 30 mins once the temperature is cool, you can get the broth tubes out. Use the broth tube as required, or store it at room temperature to allow for later use. (Liang G et al 2008)

5. Results

Table No.02: Percentage yield and Physical characteristics of Methanolic Extract.

Plant Name	Plant part	Solvent	Percentage Yield (gr)	Color
Pithecellobium dulce L	Spines	Methanol	30	Light Green

5.1.Qualitative Phytochemical Analysis of MEPD.

Preliminary phytochemical investigation of MEPD showed the presence of alkaloids, Flavonoids, Glycosides, Carbohydrates, Phytosterols, Phenolics and Tannins, Saponins, Proteins &Amino Acids, Fixed oils & Fats, Volatile Oils, and Gums.

Table. No.03: Preliminary Phytochemical analysis of Methanol Extracts of MEPD.

S. No	Chemical Constituents	MEPD
01.	Alkaloids	+
02.	Flavonoids	+
03.	Glycosides	+
04.	Carbohydrates	+
05.	Phytosterols	--
06.	Phenolics and Tannins	+
	Saponins	--
08.	Proteins &Amino Acids	+
09.	Fixed oils & Fats	--
10.	Volatile Oils	--
11.	Test for Gums	+

5.2.Experimental Pharmacological Invitro Studies

1. Antioxidant Effects of Methanolic Extracts of MEPDby DPPH Assay

The capabilities of Methanolic extracts of Spines of *Pithecellobium dulce L*to scavenge DPPH were measured *in-vitro* the Percentage of scavenging activity was showed in the mentioned in below Table No.04

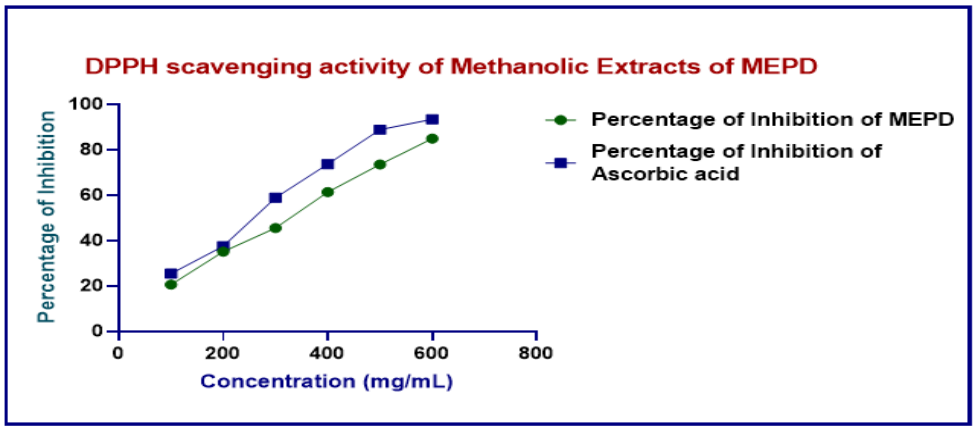
Table No.04: DPPH scavenging activity of Methanolic Extracts of MEPD.

Samples	Conc. (µg/ml)	Absorbance	Percentage of Inhibition	IC50 (µg/ml)
MEPD	100	0.530	20.53±0.21	95
	200	0.433	35.08±0.23	
	300	0.364	45.42±0.25	
	400	0.258	61.31±0.66	
	500	0.177	73.46±0.46	
	600	0.101	84.85±0.11	
Ascorbic acid	100	0.529	25.36±0.36	86
	200	0.417	37.48±0.65	
	300	0.275	58.77±0.24	
	400	0.175	73.62±0.44	
	500	0.077	88.76±0.24	
	600	0.044	93.40±0.28	

Values represent mean± SEM(n-6).

All values are mean ±S.D. (n=10). p < 0.05 all groups are compared with the control group (One-way ANOVA followed by Dunnett s multiple comparison test)

Figure No.01.DPPH Scavenging Activity of Methanolic Spines Extract of MEPD



5.3.Invitro Studies of Antimicrobial Activity

Table No.05 Invitro Antibacterial activity of *S. aureus*, E.coli & *S. epidermidis*

Dose Concentration *(µg/mL)*	Zone of Inhibition (mm)					
	S. aureus		E.coli		*S. epidermidis*	
	MEPD	Penicillin G	MEPD	Penicillin G	MEPD	Penicillin G
0.1	--	1.5	2	4	1	2
0.2	1	2	4	5	2	4
0.3	3	4	6	7	4	5
0.4	4	6	8	10	6	7
0.5	5	7	10	12	8	9
0.6	7	9	12	15	10	12
0.7	9	11	14	15	11	14
0.8	10	12	14	16	13	15
0.9	12	13	15	17	15	16
1	13	14	16	18	15	17

Values represent mean± SEM(n-6).

All values are mean ±S.D. (n=10). p < 0.05 all groups are compared with the control group (One-way ANOVA followed by Dunnett s multiple comparison test)

Table No. 06. Invitro Antibacterial activity of MEPD on *B. subtilis* & *B. cereus*

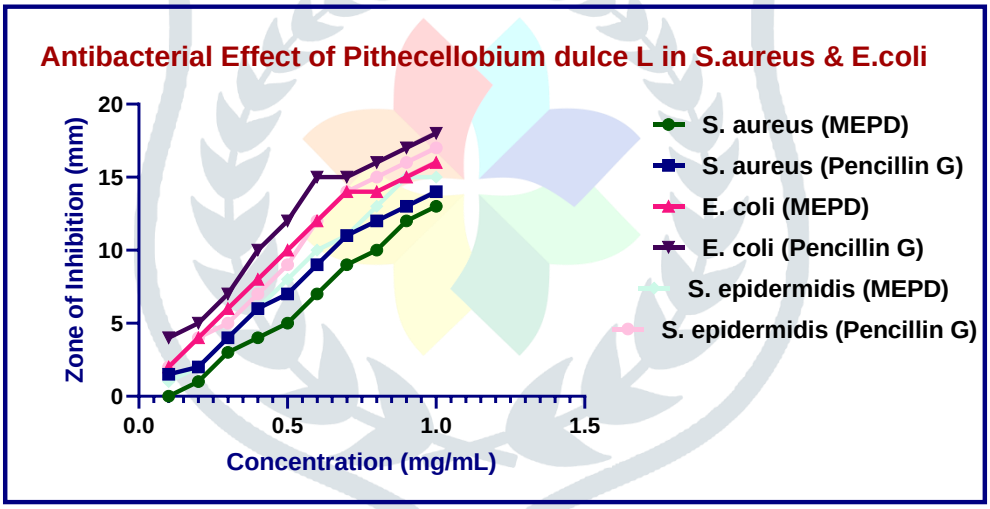
Dose Concentration (µg/mL)	Zone of Inhibition (mm)			
	B. subtilis		*B. cereus*	
	MEPD	Penicillin G	MEPD	Penicillin G
0.1	2	3	1	2
0.2	4	5	2	4
0.3	6	6	3	6
0.4	8	8	4	8
0.5	9	9	5	9
0.6	11	12	6	12
0.7	13	13	8	14
0.8	14	15	9	15
0.9	15	16	10	16
1	16	18	11	16

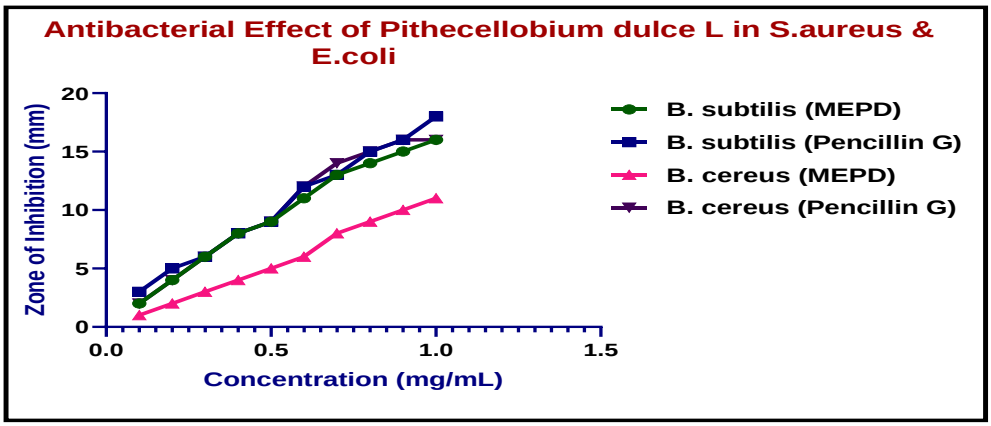
Values represent mean± SEM(n-6).

All values are mean ±S.D. (n=10). $p < 0.05$ all groups are compared with the control group (One-way ANOVA followed by Dunnett s multiple comparison test)

Figure No.02 antibacterial Effect of *Pithecellobium dulci L.* in S. aureus, E.coli & S.epidermidis

Figure No.03 antibacterial Effect of *Pithecellobium dulci L.* in B. subtilis & B. cereus.





Conclusion

In summary, the methanol Spines extract of Pithecellobium dulce L. was found to possess Potent antibacterial activity against Gram-positive and Gram-negative bacterial strains. Therefore, the extract can be processed further for the phytochemical investigation to isolate, characterize and screen the constituents present for antibacterial potential. The isolated constituents may exhibit better antibacterial activity compared to the extract.

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