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In-Vitro Antibacterial Activity and Phytochemical Analysis of Methanolic Stem Extract of *Caesalpinia Pulcherrima*

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ABSTRACT

The medicinal plants are widely used by the medical practitioners for curing various diseases in their day to day practice. *Caesalpinia pulcherrima* (Family: *Fabaceae*) is one of the most valuable medicinal shrub seen throughout tropical India. The antibacterial activities of the hot and cold methanol extracts of the stems of *Caesalpinia pulcherrima* was evaluated on four bacterial strains like *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Proteus vulgaris*. The invitro antibacterial activity of hot and cold methanol extracts was performed by cup plate agar diffusion method at different concentration of 10mg/ml to 50mg/ml using ciprofloxacin (ciprozol-500) in dimethyl sulphoxide as a standard drug for the comparison of antibacterial activity. From the experiment done the hot methanol extract of *Caesalpinia pulcherrima* did produce considerable antibacterial activity than the cold maceration extract was observed. The maximum antibacterial activity of hot and cold methanol extracts was exhibited against *Bacillus subtilis* when compared with standard drug. In addition the preliminary phytochemicals screening of the hot and cold methanol extracts of *C.pulcherrima* stems revealed the presence of alkaloids, carbohydrates, glycosides, saponins, tannins and steroids. The results obtained in the present study suggest that *Caesalpinia pulcherrima* stems can be used in treating diseases caused by the test organisms.

Keywords: *Caesalpinia pulcherrima*, hot and cold methanolic extracts, ciprofloxacin, antibacterial activity.

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INTRODUCTION

Medicinal plants are used locally in the treatment of infections caused by bacteria, fungi, viruses and parasites. For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency¹. Bacterial resistance to currently used antibiotics is becoming a concern to public health². The development of bacterial super resistant strains is resulting in currently used antibiotic agents failing to end many bacterial infections. For this reason the search is ongoing for new antimicrobial agents, either by the design and synthesis of new agents, or through the search of natural sources for as yet undiscovered antimicrobial agents³. Plant derived medications in particular have seen a revival of interest due to a perception that there is a lower incidence of adverse reactions to plant preparations compared to synthetic pharmaceuticals⁴. Coupled with the reduced costs of plant preparations, this makes the search for natural therapeutics an attractive option.

Caesalpinia pulcherrima (Family: *Fabaceae*) is one of the most valuable medicinal shrub seen throughout the world including West Indies and tropical America. *Caesalpinia pulcherrima* is used as a traditional medicine, widely in India, South America and Taiwan⁵. The plant is used as emmenagogue, purgative, stimulant, abortifacient, also used in bronchitis, asthma and malarial fever. It is also known to possess anti-inflammatory, antimicrobial, antioxidant, antipyretic and several other pharmacological properties. The seeds are said to cure bad cough, breathing difficulty and chest pain⁶. The Phytochemicals present in the leaves of *C.pulcherrima* are hydrocyanic acid, tannins and benzoic acid. Stems showed the presence of peltogynoids, bhonducellin and 6-methoxypulcherrimin. The bark contains terpenoid that gives antimicrobial and cytotoxic activities⁷. The objective of this research is to investigate the preliminary phytochemical constituents and antibacterial activity of the hot and cold methanol extract of *Caesalpinia pulcherrima* stems and to test the better extract for significant antibacterial activity at different concentrations.

MATERIALS AND METHODS:

Collection and Authentication of the plant material:

The stems of *Caesalpinia pulcherrima* were collected in the month of January-2013, from the campus of SIMS College of Pharmacy, Andhra Pradesh, India. The plant was identified and authenticated by Dr. S.M.Khasim, Botanist of Acharya Nagarjuna University, Andhra Pradesh, India.

Preparation of extracts:

All the chemicals, including methanol, dimethylsulphoxide (DMSO), and standard antibiotic ciprofloxacin were purchased from National Scientific Products Pvt Ltd., Guntur, Andhra Pradesh, India, and all the solvents used were of analytical grade.

Methanol Purification:

A mixture of 500ml methanol, 25ml furfural and 60ml of 10% NaOH solution was refluxed in a round bottom flask fitted with a double surface condenser for 6-12 hrs. A resin was formed which carried down all the acetone present. The methanol was then fractionated through an efficient column. The first 5 ml which might contain a trace of formaldehyde was rejected⁸.

Hot continuous methanol extraction:

The shade dried coarse powder of the stem (750gm) was packed well in soxhlet apparatus and was subjected with Methanol by continuous hot extraction for about 48hrs. The extract was filtered through whatmann filter paper and concentrated on a water bath. Obtained extract (Dark Reddish Brown) was weighed and % yield was found to be 19.41%.

Cold methanol extraction:

The shade dried coarse powder of the stem (750gm) was kept in stoppered flask and were macerated with Methanol for 24-48 hrs with frequent stirring. Then the extract was filtered through whatmann filter paper and concentrated under air. Obtained extract (dark reddish brown) was weighed and % yield was found to be 11.33%.

The above prepared hot and cold methanol extract was stored in desiccators for further phytochemical and antibacterial investigations. The dried extract was tested for its phytoconstituents by standard methods⁹. The both methanolic extracts were diluted with Dimethylsulphoxide (DMSO) to the concentration of 10mg/ml, 20mg/ml, 30mg/ml, 40mg/ml and 50mg/ml.

Bacterial strains:

The antibacterial activity of the crude extract was screened against gram positive bacteria and gram negative bacteria obtained from the laboratory stock of the Department of Pharmaceutical Microbiology, SIMS College of Pharmacy, AP, India. The Microorganisms like *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Proteus vulgaris* were used for testing.

Media Preparation and Antibacterial evaluation¹⁰.

Cup plate agar diffusion method was used for the antibacterial study. Working stock was prepared as 1ml of each bacterial strain was initially inoculated in 100 ml of sterile nutrient broth and incubated for $37^{\circ}\pm 1^{\circ}\text{C}$ for 24 hr respectively. The 0.2ml of the each test organisms from the working stock were seeded into 100ml sterile nutrient agar medium and cooled to 48°C to 50°C in a sterile Petridish respectively. When the nutrient agar medium solidifies, four holes of uniform diameter (7 mm) were made using sterile aluminium borer. Then, 0.2 ml of each hot, cold methanol extracts, standard solution (10, 20,30,40,50 mg/ml) respectively and vehicle control (DMSO) were placed in each hole separately under aseptic condition. The plates were then maintained at room temperature for 2 hr to allow the diffusion of the solution into the medium. All the bacterial plates were then incubated at $37^{\circ}\pm 1^{\circ}\text{C}$ for 18 hr and the zone of inhibition was measured [cm,

including the diameter of the bore (7 mm)] and the results were given in Table 1 and Figure 1.

Phytochemical Analysis:

Both hot and cold methanol extracts obtained by the powdered stems of the *Caesalpinia pulcherrima* were subjected to various photochemical tests for alkaloids, carbohydrates, flavonoids, glycosides, lignin, saponins, terpenes, tannins, steroids and reducing sugar.

RESULTS AND DISCUSSION:

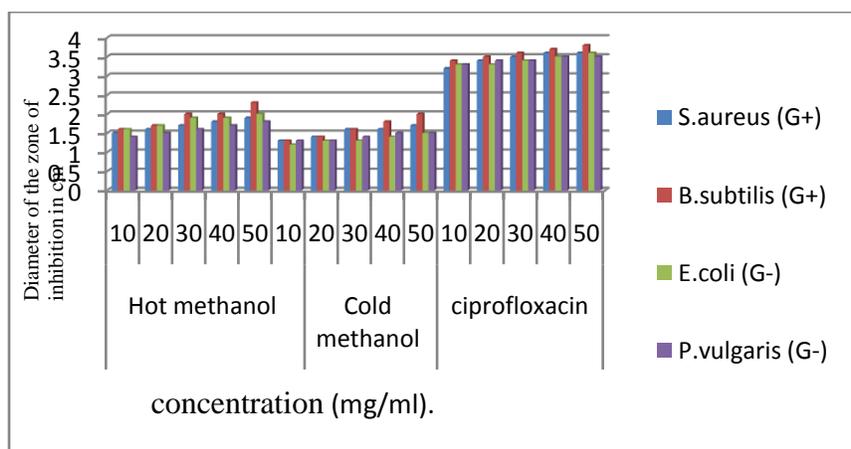
Results of the Antibacterial screening of Hot and Cold Methanol extracts of *Caesalpinia pulcherrima* stem revealed significant antibacterial activity against all tested bacterial strains. The maximum antibacterial activity of Hot and Cold methanol extracts was exhibited against *Bacillus subtilis* when compared with standard drug. The hot methanol extract of *C.pulcherrima* stem showed more significant activity against all tested bacterial organisms than that of the cold methanol extracts. The highest zone was against the *Bacillus subtilis*, while the least zone was against *Proteus vulgaris*.

The preliminary phytochemical studies revealed the presence of alkaloids, carbohydrates, glycosides, saponins, tannins, and steroids. The inhibitory effects of this medicinal plant on the test microorganisms may therefore, be due to the presence of the above phytochemical components. This piece of work is preliminary screening to suggest that stem of *Caesalpinia pulcherrima* has antibacterial activity. The present study of invitro antibacterial evaluation of *Caesalpinia pulcherrima* forms a primary platform for further phytochemical and antimicrobial studies.

Table 1: Evaluation of antibacterial activity of the methanolic stem extract of *Caesalpinia pulcherrima*

Extracts tested	Conc. (mg/ml)	Diameter of the zone of inhibition in (cm)			
		<i>S.aureus</i> (G+)	<i>B.subtilis</i> (G+)	<i>E.coli</i> (G-)	<i>P.vulgaris</i> (G-)
Hot methanol extract	10	1.5	1.6	1.6	1.4
	20	1.6	1.7	1.7	1.5
	30	1.7	2.0	1.9	1.6
	40	1.8	2.0	1.9	1.7
	50	1.9	2.3	2.0	1.8
Cold methanol Extract	10	1.3	1.3	1.2	1.3
	20	1.4	1.4	1.3	1.3
	30	1.6	1.6	1.3	1.4
	40	1.6	1.8	1.4	1.5
	50	1.7	2.0	1.5	1.5
Ciprofloxacin	10	3.2	3.4	3.3	3.3
	20	3.4	3.5	3.3	3.4
	30	3.5	3.6	3.4	3.4
	40	3.6	3.7	3.5	3.5
	50	3.6	3.8	3.6	3.5

(G+) gram positive bacteria, (G-) gram negative bacteria

**Figure 1: Antibacterial activity of the hot and cold methanol extracts of *Caesalpinia pulcherrima* stem.****CONCLUSIONS:**

In conclusion, the results obtained in this study suggests that the hot methanol extract of *Caesalpinia pulcherrima* stem showed more significant activity against all tested bacterial organisms than that of the cold methanol extracts. These promissory extracts open the possibility of finding new clinically effective antibacterial compounds. Further work is necessary to earmark the particular compound responsible for antibacterial activity and also to determine their full spectrum of efficacy.

REFERENCES:

1. Kirtikar KR, Basu BD. Indian Medicinal Plant, International Book Distributors and Publishers, Dehradun: 1984; 848-50.
2. Monroe S, Polk R. Antimicrobial use and bacterial resistance, Current Opinion in Microbiology. 2000; 3: 496-501.
3. Bhavnani SM, Ballow CH. New agents for Gram-positive bacteria, Current Opinion in Microbiology 2000; 3: 528-534.
4. Chiariandy CM, Seaforth CE, Phelps RH, Pollard GV, Khambay BP. Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties, Journal of Ethnopharmacology 1999; 64: 265-270.
5. Schiebinger Londa L. Plants and Empire: Colonial Bio-prospecting in the Atlantic World. Cambridge, Mass: Harvard University Press ISBN 0-674-01487-1.
6. Nasimul KM, Ali IA, Amtul MS. An Antimicrobial Terpenoids from *Caesalpinia pulcherrima* Swartz; its characterization, antimicrobial and cytotoxic activities. Asian J Plant Science 2003; 2: 1162-65.
7. David DM, Geoffery AC. Diterpenoids from *Caesalpinia pulcherrima*. Phytochemistry 1986; 25: 167-170.
8. Vogel AI. Practical Organic Chemistry. UK: Longmann Group; 1989: 104-05 and 395-467.
9. Rene RP, Pierre GD. *Caesalpinia pulcherrima* leaves. Journal of Crude Drug Research 1967; 7: 134-37.
10. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC. Colour Atlas and Textbook of Diagnostic Microbiology. 5th ed., New Delhi: Lippincott; 1997.