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ANTIMICROBIAL ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACT OF *CAESALPINIA PULCHERRIMA* FLOWERS

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ABSTRACT

Caesalpinia pulcherrima (Linn.) Popularly known as Peacock flower in India belongs to family Caesalpiniace. The objective of the present work was to evaluate the in-vitro antimicrobial potency of the ethanolic and aqueous extract of *Caesalpinia pulcherrima* (Linn.) flower (CPF) using agar plate disk diffusion method against *Escherichia coli, bacillus subtilis and streptococcus aureus*. Cephalosporin (5µg/ml) use as a Standard drug. Results were subjected to minimum inhibitory concentration assay by two fold dilution method. Ethanolic extract of *Caesalpinia pulcherrima* was more effective against *Escherichia coli, bacillus subtilis* than aqueous extract and cephalosporin. While in case of *streptococcus aureus* aqueous extract is more effective than ethanolic extract and cephalosporin. Thus, the present study demonstrate that the traditional claim of *Caesalpinia pulcherrima* (Linn.) flower as an antimicrobial has been confirmed as the aqueous and ethanolic extracts displayed activity against the different microorganism used in study.

INTRODUCTION: Natural products perform various functions, and many of them have interesting and useful biological activities [1]. Despite of tremendous progress in human medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health. There impact is particularly large in developing countries due to relative unavailability of medicines and the emergence of widespread drug resistance [2].

During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics [3] has lead to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures, which overcome the above disadvantages [4] Current research on natural molecule and products primarily focuses on plants since they can be sourced more easily and be selected based on their ethno-medicinal uses [5].

With the rising prevalence of microorganism showing resistance to antibiotics, there is an urgency to develop new antimicrobial compounds. Since antiquity, plants have been used to treat common infectious diseases.

The healing potential of many plants have been utilized by Indian traditional medicines like Siddha, Ayurvedha and Unani. Being nontoxic and easily affordable, there has been a resurgence in the consumption and demand for medicinal plants [6].

Caesalpinia pulcherrima (Linn.) Swartz (Leguminosae) commonly known as red bird of paradise (Peacock flower) is a medicinal herb used in the treatment of various diseases [7]. The different parts of this herb have been used in common remedies for treatment of a number of disorders including pyrexia, menoxenia, wheezing, bronchitis and malarial infection [8] The plant is rich in many pharmaceutical active ingredients like flavonoids, aritonoids, glycosides and sterols [9].

MATERIAL AND METHOD:

Plant: The fresh flowers of *Caesalpinia pulcherrima* (Linn.) were collected in the month of September 2010 from its natural habitat at mudkhed village in Nanded region, Maharashtra, India. The plant was authenticated by Dr. A. Chaturvedi of Botany Department; RTM Nagpur University, Nagpur India. A voucher specimen (No: 9439) was deposited at Herbarium, Department of Botany, RTM Nagpur University Nagpur.

Material: Ethanolic and Water extracts of *Caesalpinia pulcherrima* (Linn.) flower, Cephalosporin, *Escherichia coli*, *Bacillus subtilis, staphylococcus aureus*.

Preparation of Extracts of *Caesalpinia pulcherrima (Linn.)* flower: The *Caesalpinia pulcherrima (Linn)* Flowers were dried under shade and undergone crushing in electric blender to form powdered and subjected to successively extraction by Pet. ether and ethanol using Soxhlet's extractor. The percent yield of ethanolic extract was 24.8% w/w and petroleum ether (60 Grade) extract yield 6.1% w/w. Both the extracts were concentrated by evaporation at room temperature and were used for pharmacological studies. Powdered material of *Caesalpinia pulcherrima* (Linn.) flower was kept for maceration with 1000 ml of distilled water for 7 days. The Aqueous extract was double filtered by using muslin cloth and Whatman no. 1 filter paper and concentrated by evaporation on water bath. The extract was dried and used as a powder. The percentage yield of extract was found to be 13.56 percent.

Preparation of extract/drug stock solution: The stock solution of *Caesalpinia pulcherrima* flower extract was prepared on each occasion by careful weighing and dissolving in suitable volume of Dimethylsulphoxide (DMSO) to get a concentration of 100 mg/ml. A tablet of cephalosporin was dissolved in appropriate volume of water to get 5 mg/ml of stock solution.

Culture media: The media employed for the study was: Nutrient agar.

Test microorganisms: Clinical isolates of *Escherichia coli*, *Bacillus subtilis, staphylococcus aureus* were obtained from the Department of Pharmaceutical Microbiology, University of Nigeria, Nsukka.

Sterilization of materials: The petri dishes and pipettes packed into metal canisters were appropriately sterilized in the hot air oven at 170°C for 1 h at each occasion. Solution of the extract and culture media were autoclaved at 121°C for 15 min.

Preparation of culture media: All culture media were formulated according to manufacturers' specification. Basically for nutrient agar, this involves appropriate weighing of nutrient agar, distributing into bijou bottles (in 50 ml) and then sterilization using autoclave at 121°C, 151 b/sq. inch for 15 min; then allowed to cool to 45°C before pouring into the agar plate. The pH of the agar medium was maintained at 7.4.

Maintenance and standardization of test organisms: The organism (*E. coli*, *B. subtilis, S. aureus*) was maintained by weekly sub culturing on nutrient agar slant. Before each experiment, the organism was activated by successive sub culturing and incubation. Standardization of the test microorganism was according to previously reported method [10].

***In vitro* determination of antibacterial activity:** Stock cultures were maintained at 4°C on slants of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of colonies from the stock culture to peptone water and incubated for 4h at 37°C. Antibacterial activity was determined by agar disc diffusion method [11]. The medium was sterilized by autoclaving at 120°C (15 lb/in2). About 30 ml of the medium (nutrient Agar Medium) with the respective strains of bacteria was transferred aseptically into each sterilized Petri plate.

The Plates were left at room temperature for solidification. Each plate, a single well of 6 mm diameter was made using a sterile borer. The extracts were freshly reconstituted with suitable solvents (Dimethyl Sulphoxide) and tested at various concentrations. The samples were placed in 6-mm diameter well. Antibacterial assay plates were incubated at 37±2°C for 24 h, Standard disc (6 mm diameter) with cephalosporin (5µg/ml) was used as a positive control for antibacterial activity Plates were kept in laminar flow for 30 minutes for pre diffusion of extract to occur and then incubated at 37^{o}C for 24 hours. Resulting zone of inhibition was measured using a Hi media zone scale [12].

Determination of Minimum Inhibitory Concentration (MIC): The minimum inhibitory concentration values were determined by broth dilution assay. Varying concentrations of the extracts (100, 50, 25, 12.5, 6.25, 3.125, 1.56 mg/ml) were prepared. 0.1mL of each concentration was added to each 9mL of nutrient broth containing 0.1mL of standardized test organism of bacterial cells.

The tubes were incubated at 37°C for 24h. Positive controls were equally set up by using solvents and test organisms without extracts. The tube with least concentration of extract without growth after incubation was taken and recorded as the minimum inhibitory concentration [13].

RESULTS AND DISCUSSION: In the present study, antimicrobial activity of was evaluated against *E. coli, B. subtilis and S. aureus* was evaluated by agar well diffusion method. Ciprofloxacin was used as standard and distilled water was used as negative control. As revealed from **Table 1**, the Caesalpinia pulcherrima aqueous extract and ethanolic extract were found to be effective against *Escherichia coli, bacillus subtilis and streptococcus aureus* with zone of inhibition. Ethanolic extract of Caesalpinia pulcherrima was more effective against *Escherichia coli, bacillus subtilis* than aqueous extract and cephalosporin. While in case of *streptococcus aureus* aqueous extract is more effective than ethanolic extract and cephalosporin. Minimum inhibitory concentration of the active extracts is shown in Table 1.

TABLE 1: ANTIMICROBIAL ACTIVITY OF *CAESALPINIA PULCHERRIMA (LINN)* FLOWER.

Bacterial species	Zone of Inhibition Mean of Diameter in (cm)			MIC (µg/ml)		
	Cephalosporin (µg/ml)	Aqueous CPF	Ethanolic CPF	Cephalosporin	Aqueous CPF	Ethanolic CPF
Escherichia coli	15	17	19	18.9	16.7	15.5
Bacillus subtilis	13	15	16	22.4	15.7	14.4
Staphylococcus aureus	17	22	20	19.1	14.8	16.3

Results were subjected to minimum inhibitory concentration assay by two fold dilution method**.** Lower Minimum inhibitory concentration of the extract shown more zone of inhibition against the bacterial species. Lower MIC is shown by the ethanolic extract of caesalpinia pulcherrima. Results of these kinds herald an interesting promise of designing a potentially active antibacterial synergized agent of plant origin. There might be several reasons for the lower antibacterial activity shown by the plant extracts as suggested by Parekh and Chanda [14], either the plant part used or the type of extraction might have resulted in the lower activity in this study, or the time of collection of herbal material and climate, which might, inturn, affect the amount of active constituents in the plant material.

The difference in potency shown by the plant extracts may be due to different sensitivity of food associated strains, difference in concentrations, methods of extraction used in the study and the little diffusion properties of these extracts in the agar as suggested by El- Astal *et al* [15]. Although the plant extracts in the present study have shown higher antimicrobial activity, they still might be considered as potential drug agents for curing infectious diseases.

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