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PRELIMINARY PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY ON BARK EXTRACT OF *CAESALPINIA PULCHERRIMA* (L.)

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ABSTRACT

In the present study aqueous extract of Caesalpinia pulcherrima was investigated for antibacterial activity. The microorganisms employed were E.coli and Staphylococcus aureus. The susceptibility of bacteria strains against the aqueous extract determined using the disc diffusion method. Highest antibacterial activity was observed with aqueous extract of Staphylococcus aureus (7.1mm) respectively while minimum activity was observed against E.coli (6.2mm) at 500µg/ml. It was observed that aqueous extract showed maximum inhibitory activity on Staphylococcus aureus than E.coli. The results were compared to those of amoxicillin used as standard drug. Phytochemical screening revealed the presence alkaloids, carbohydrates, proteins, flavonoids. Phytoconstituents present in the plant extract may be responsible for the antibacterial action.

Key words: *Caesalpinia pulcherrima, antibacterial, disc diffusion method.*

INTRODUCTION

Human beings have used plants for the treatment of diverse ailments for thousands of years (Sofowara, 1982; Hill, 1989). According to the World Health Organization, most populations still rely on traditional medicines for their psychological and physical health requirements (Rahe and Van Stoden, 2000), since they cannot afford the products of Western pharmaceutical industries (Salie *et al*., 1996), together with their side effects and lack of health care facilities (Griggs *et al*., 2001). Rural areas of many developing countries still rely on traditional medicine for their primary health care needs and have found a place in day-to-day life. These medicines are relatively safer and cheaper than synthetic or modern medicine (Iwu*et al*., 1999; Idu *et al*., 2007; Mann *et al*., 2008; Ammara *et al*., 2009). People living in rural areas from their personal experience know that these traditional remedies are valuable source of natural products to maintain human health, but they may not understand the science behind these medicines, but knew that some medicinal plants are highly effective only when used at therapeutic doses (Maheshwari *et al*., 1986; Van Wyk *et al*., 2000).

Caesalpinia pulcherrima is also know as peacock flower is the type of genus fabaceae sub family caesalpiniaceae. It is an ever green shrub growing to 3m tall. It is a striking ornamental plant widely grow in tropical gardens. The leaves are bipinnate, 20-40cm long, bearing 3-10 pairs of pinnae, each with 6-10 pairs of leaflets 15-25mm long and 10-15mm broadwith oblong to ovate shape. In India, it is know by the names Radhachura in Bengal, Kenjige in Kannada, Settimandaram in Malayalam, Krishnacura in Manipuri, Sankasur in Marathi. Leaves contains gallic acid, gum, tannin, resin and benzoic acid. Flavonoid as quercetin and diterpenoids, isovouacaperol, sitosterol also present. *Caesalpinia pulcherrima*is used for a various purpose of herbal medicine. It is used as emmenagogue, purgative, stimulant, and abortificient, also used in bronchitis, asthma, malaria fever, and used against kidney stone.The different parts of this herb have been used in common remedies for treament of a number of disoders includings pyrexia, menoxenia, wheezings. It shows antiviral activity. Leaves used as antipyretic, antimicrobial, antibacterial, antioxidant cytotoxic activity were attributed to total phenolic content of the wood. It is used for the treatment of inflammatory conditions in traditional medicine, also shows antitubercular activity. Leaves shows gastric antiulcer activity. Flowers for analgesic and Anti-inflammatory activities. Estabilishment of the pharmacognostic profile of leaves of *Caesalpinia pulcherrima* will assist in standardization which can guarantee quality, purity and indentification of sample. The aim of the study to investigate the antibacterial activity of *Caesalpinia pulcherrima* against bacterial pathogens.

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MATERIALS AND METHODS

Collection and Identification of plant materials:

Mature barks of *Caesalpinia pulcherrima* were collected from Vadaseri at Thanjavur District in Tamilnadu. The plant was identified and authenticated by the botanist at S.T.E.T womens college of Mannargudi.

Preparation of Bark Aqueous Extract:

Fifty grams of selected fresh bark materials was macerated with 50 ml of sterile distilled water in a grinding machine for about 10-15 min. The macerate was first filtered through double layer muslin cloth then centrifuged at 3500 rpm for 30 min. The supernatant was filtered through .Whatman No1 filter paper and sterilized at 120º C for 30 minutes .The extracts were preserved for future use (Gupta *et al*.,1996).

Preliminary phytochemical screening:

Phytochemical analysis of the extract was conducted following the procedure of Indian pharmacopeia, (1985). By this analysis, the presence of several phytochemical like alkaloids, flavonoids, tannins, saponins, esters,resins,sugars and glycosides were tested.

Anti bacterial Assay:

Test organisms:

Microorganisms *Escherichia coli* and *Staphylococcus aureus,* and pure slant cultures were obtained from National Collection of Industrial Microorganism (NCIM) Pune, India.

Selection of antibiotics:

Broad spectrum antibiotic, *amoxicillin* was standard drug.

Determination of antimicrobial activity:

Antimicrobial activity was performed by disc diffusion method.

Preparation of microorganisms for experiment:

The pure cultures of *Escherichia coli* and *Staphylococcus aureus,* were sub-cultured in nutrient broth. And the inoculated broth tubes were incubated at 37 C for 24 hours. After completion of incubation period, when growth was observed the tubes were kept into 2-8 C until use.

Disc diffusion method:

Microbial Inoculum preparation:

The young microbial inoculums culture was prepared and used in the entire research period the nutrient broth (NB) were prepared and poured into tubes and sterilized. The pure microbial cultures were collected from the institute and inoculated in the tubes using inoculated in the tubes inoculation needles or loops, then these tubes were incubated at different temperature and time duration at 37 C for 24-48 hours bacteria.

Media preparation:

Composition of nutrient agar medium:

Peptone	: 5 gm
Beaf extract	: 3 g
Nacl	: 5g
Agar	: 15g
Distilled water	: 1000 ml

And it was weighed in a one liter conical flask containing 1000 ml distilled water were plugged with cotton and sterilized in an autoclave at 15 lbs pressure (121 C) for about 15 minutes.

Anti bacterial assay:

The three different concentration (125, 250, 500 µg/ml) of the bark extracts were tested for antibacterial activity using agar disc diffusion assay according to the method of (Bauer *et al*., 1966). The strains of microorganisms obtained were inoculated in conical flasks containing 100 ml of nutrient broth. These conical flasks were incubated at 37 C for 24 h and were referred to as seeded broth. Media were prepared using Muller Hinton Agar (Himedia), poured on petri dishes and inoculated with the test organisms from the seeded broth using cotton swabs. The plates were incubated overnight at 37 C. Antibacterial activity was assigned by measuring the inhibition zone formed around the discs. The experiment was done three times and the mean values were presented. Amoxycillin (10µg/ml) was used as standard.

Statistical Analysis:

Results are expressed as Mean ± S.D

RESULTS

Preliminary Phytochemical screening:

The preliminary phytochemical screening of aqueous extracts of *Caesalpinia pulcherrima* showed the presence of flavonoids, alkaloids, protein, carbohydrates and absence of tannins, saponins, phenolic compounds are presented in **Table 1.**

The results obtained from preliminary phytochemical screening are comparable with the results reported earlier (Bandaranayake, 1995). Preliminary phytochemical screening of *Luminetzer aracemosa* revealed the presence of secondary metabolites such as alkaloids, flavonoids and steroids (Abeysinghe *et al*., 2010).

Antibacterial Assay:

The bark extract of *Caesalpinia pulcherrima* shown to possess antibacterial activity. The antibacterial activity of aqueous extract of *Caesalpinia pulcherrima* was inspected against the selected experimental pathogens such as *Escherichia coli* and *Staphylococcus aureus* by disc diffusion method. The tested microorganism shows varying degrees of antibacterial activity in examined plant extract.

The antibacterial activity of aqueous extract of *Caesalpinia pulcherrima* at different concentrations (125, 250, 500 µg/ml) was studied and compared with standard amoxicillin (10 µg/ml) using disc diffusion method. The results showed that there has been an increasing effect on bacterial growth inhibition with increasing concentration of the extract. And the extract showed good inhibitory activity on almost all the bacteria tested. It has been found that among all the tested organisms, the gram positive bacterial strain, *Staphylococcus aureus,* was found to be more susceptible to the plant extract by showing inhibition zone ranging from 3.23 ±0.25 - 7.13 ± 0.15mm and the gram negative bacterial strain *E.coli* was less susceptible with the inhibition zone ranging from 2.16 ± 0.20 - 6.2 ± 0.2mm (**Table 2, Plate I & II**).

Recently, much attention has been directed toward plant extracts and biologically active compounds isolated from popular plant species. The use of medicinal plants plays a vital role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms (Munoz-Mingarro *et al*., 2003).

Gram-positive bacteria were more susceptible to the extract than the gram-negative bacteria. Possibly because of the presence of outer membrane that serves as an effective barrier in gram-negative species (Nikaido, 1996; Adesokan *et al*., 2007).In addition, since the zone of inhibition is almost equal to the standard, it shows that the test organisms are sensitive to the plant extract. *S. aureus* was the most susceptible bacterium, an observation that may be attributed to the presence of single membrane of the organism which makes it more accessible to permeation by active principles of the extract of *Boerhaavia diffusa.*

Table No. 1: Qualitative Phytochemical screening of *Caesalpinia pulcherrima*

S.No	Compound tested	Aqueous extract
1	Alakloids	+
2	Carbohydrates	+
3	Tannins	+
4	Proteins	-
5	Flavonoids	+
6	Saponins	-
7	Phenolic Compounds	+

+ indicates presence; - indicates absence

Table No. 2: Antibacterial activity of aqueous bark extract of *Caesalpinia Pulcherrima* against *E. coli* and *Staphylococcus aureus*

Bacterial Strain	Zone of Inhibition (mm)			
	Aqueous bark extract (µg/ml)			Reference drug (Amoxicillin) (µg/ml)
	125	250	500	10
E.coli	2.16 ± 0.20	3.43 ± 0.20	6.2 ± 0.2	6.43 ± 0.30
Staphylococcus aureus	3.23± 0.25	4.5± 0.2	7.13± 0.15	

Values are Mean ± SD of the three triplicates

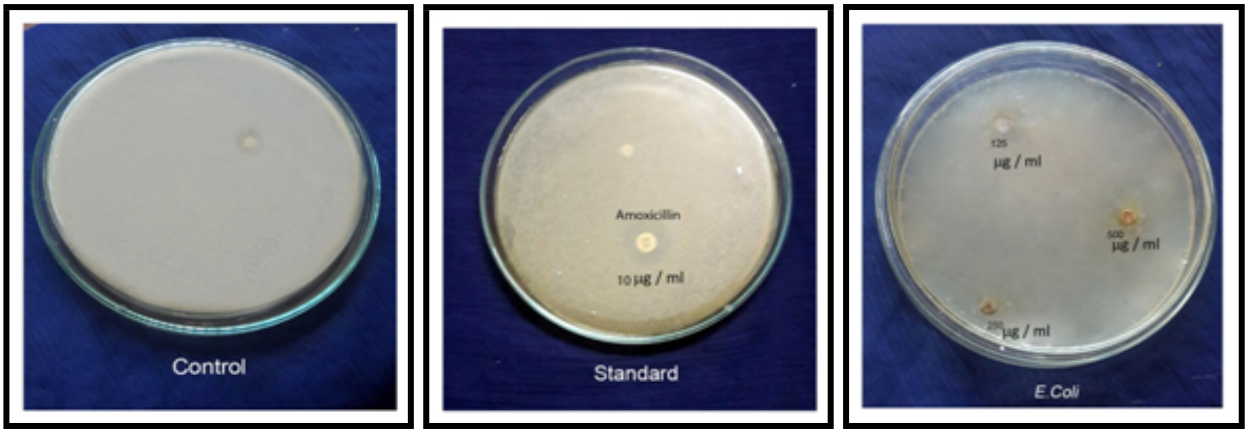

Plate 1: Effects of Antibacterial Activity of *Caesalpinia pulcherrima* against *E.Coli*

Plate 2: Effects of Antibacterial Activity of *Caesalpinia pulcherrima* against *Staphylococcus aureus*

CONCLUSION

The present study has shown that *E.coli* and *Staphylococcus aures* were susceptible to aqueous extracts of *Caesalpinia pulcherrima in vitro* which means the plant extract has antibacterial activity. In conclusion, *Caesalpinia pulcherrima* represent a valuable plant in both ayurvedic and modern drug development areas of its versatile medicinal uses and can be further be explored for the isolation of its bioactive compound.

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