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EVALUATION OF ANTIBACTERIAL ACTIVITY OF FRESH AND DRY FLOWER EXTRACTS OF *CAESALPINIA PULCHERRIMA* L.

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ABSTRACT

The aim of present work is to screen out the antibacterial properties of flowers. Since plants are used as therapeutic agents, the present study is designed to evaluate the phytochemical profile and antibacterial activities of fresh and dry flower extracts of *Caesalpinia pulcherrima* L. against selective Gram positive & Gram negative strains *invitro*. The fresh and dry flowers of *Caesalpinia pulcherrima* L. were extracted by using solvents like n-hexane, chloroform, acetone, ethanol, methanol and water. Presence of phytoconstituents such as alkaloids, glycosides, saponins, carbohydrates, proteins, aminoacids, flavonoids, tannins, steroids were observed in fresh and dry flowers of *C. pulcherrima.* The antibacterial activity was studied by using various organisms by means of disc diffusion method. Susceptibility of some Gram positive (*Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis*) and Gram negative (*Escherichia coli, Pseudomonas aeruginosa* & *Klebsiella pneumoniae)* bacteria were tested. The antibacterial activity was determined by measuring the diameter of zone of inhibition (mm) produced after incubation. The phytoconstituents present in fresh and dry flowers of *C. pulcherrima* were found to possess potent antibacterial activity. It was found that the ethanol extract of dry flower of *C.pulcherrima* exhibited maximum activity against *Bacillus subtilis* (25mm) bacteria. Chloroform and ethanol extracts of dry flowers exhibited moderate activity against *Bacillus subtilis* and *Klebsiella pneumonia*e (22mm). The minimum inhibitory concentration ranged between 2.5mg/mL and 15mg/mL depending on microorganism and various extract. The results reported in the present work shows evidence that the extracts of *C.pulcherrima* possess potent antibacterial activity against tested pathogenic organisms.

Keywords: *Caesalpinia pulcherrima* L, antibacterial activity, Gram positive & Gram negative bacteria.

INTRODUCTION

Mankind depends on plant sources mainly for herbal medicines, food, forage, construction of dwellings, making household implements, sleeping mats and for fire and shade. Human beings have been using plants as therapeutic agents for thousands of years and continue to rely on them for health care. According to WHO estimate, around 80% of the World's population use plants or their active principles (Gias Uddin M, 1998) for their primary health care. Traditionally used herbal medicines contain a wide range of ingredients that can be used to treat chronic as well as infectious disease. A vast knowledge of how to use of plants is still of great importance (Diallo D *et al*., 1999). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds (Edeoga HO *et al*., 2005). Pharmacological screening of compounds of natural origin has been source of innumerable therapeutic agents.

Medicinal plants represent a rich source of powerful antimicrobial agents. A wide range of medicinal plant parts (root, stem, flower, fruit, twigs, exudates and modified plant organs) are used for extract as raw drugs and they possess varied medicinal properties. The use of medicinal plants as traditional medicine is well known in rural areas of many developing countries for the treatment

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of common infections (Sandhu DS *et al*., 2005; Gupta MP *et al*., 2005). Existing broad spectrum antibiotics has numerous pharmacological drawbacks but traditional medicine is more effective and impart least side effects as compared to synthetic medicines. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity.

Caesalpinia pulcherrima L. is a perennial large shrub or small tree commonly known as Peacock-flower or Pride of Barbados belongs to the family Fabaceae. It is highly attractive shrub that blooms throughout summer. Large cluster of flowers appear on the tips of every branch. The flowers are bowl shaped and the individual flowers open with a bright yellow edge and turns to orange on second day. The most common colour is red-orange but yellow & pink are also available. There are ten long thread-like bright red stamens that extend away beyond corolla. The fruits, typical legumes are flat and when ripe they split open noisily to expose the little brown beans.

Figure 1: *Caesalpinia pulcherrima* L

C.pulcherrima used in the treatment of ulcers, fever, tumors, asthma and skin diseases. Folkloric claims of *C.pulcherrima* are the stem is used as an abortifacient and emmenagogue, while decoctions of the barks, roots are used as a febrifuge and to treat liver disorders as well as ulcers from mouth and throat.

The crude methanolic extracts of bark of *Caesalpinia pulcherrima* were evaluated for its anti-inflammatory and neuropharmacological activities (Utpal Bose *et al*., 2011). The antiulcer (Harshada Takawale *et al*., 2011) effects of the hydroalcoholic and aqueous extracts of bark of *C. pulcherrima* were investigated by pylorus ligation models for protection against Aspirin induced ulcer method.

The antimicrobial activity (Jigna Parekh and Sumitra V. Chanda, 2007) of aqueous and methanolic extracts of aerial parts of *C. pulcherrima* was evaluated. It was reported that *C.pulcherrima* can be used in treating diseases caused by the tested organisms. Methanol extract of *Caesalpinia pulcherrima*, was evaluated for its hypolipidemic activity (Christine L. Chichioco-Hernandez and Finella Marie G. Leonido, 2011) in diet-induced lipidemia in mice.

In the present work, the authors have carried out the photochemical investigation and antibacterial activity on the red-orange color fresh and dry flowers of *Caesalpinia pulcherrima* L. in view of its reputed medicinal use in folklore.

MATERIALS AND METHODS

Plant Material

The fresh flowers of *Caesalpinia pulcherrima* were collected from in and around Guntur, Andhra Pradesh, India. The plant was identified and authenticated by Dr. S.M.Khasim MSc., PhD., Department of Botany, Acharya Nagarjuna University, Guntur, Andhra Pradesh. The flowers were collected and healthy flowers were shade dried and then powdered using electric blender to get a coarse powder.

Extracts from Dried Flowers

The n-Hexane, chloroform, acetone, methanol, ethanol extracts of dried flowers of *C.pulcherrima* were prepared by taking 50g of dried flowers powder in separate containers and to this 200mL of each solvent was added and kept in a shaker for 24 h. The extracts were filtered through 5 layers of muslin cloth and collected. The extraction process was repeated twice. Then the collected filtrates were pooled (S. Maneemegalai and T. Naveen, 2010).

Extract From Fresh Flowers

The n-Hexane, chloroform, acetone, methanol, ethanol extracts from fresh flowers of *C.pulcherrima* were prepared by taking 50g of fresh flowers and ground with 200 mL of each solvent separately and kept in a shaker for 24 h and then filtered through 5 layers of muslin cloth and the extracts were collected. The extraction process was repeated twice.

Aqueous Extracts of Dry & Fresh Flowers

The aqueous extract of dry flowers of *C.pulcherrima* was prepared by taking 50 g of dried flowers powder in separate container and boiled with 200mL of water for 2 h at mild temperature and kept for 24 h, then filtered through 5 layers of muslin cloth and extract was collected. The extraction process was repeated twice.

The aqueous extract of fresh flowers of *C.pulcherrima* was prepared by taking 50g of fresh flowers in mortar and ground with pistle using 200 mL water and kept in a shaker for 24 h and then filtered through 5 layers

of muslin cloth and the extract was collected. The extraction process was repeated twice.

The collected extracts were pooled, concentrated and dried at mild temperature. The dried extracts were used for the evaluation of phytochemical screening and antibacterial activity against selected bacterial strains.

Phytochemical Screening

The phytochemical screening of leaves of *Caesalpinia pulcherrima* were reported for the presence of the components such as carbohydrates, saponins, flavonoids, alkaloids, anthocyanin and betacyanin, quinones, cardiac glycosides, terpenoids, (triterpenoids), phenols and tannins, coumarins, acids, proteins and aminoacids (Sivasankari K *et al*., 2010). The phytochemical screening of flowers of *C. pulcherrima* was carried out by standard methods (Evans WC *et al*., 2002; Kokate CK *et al*., 2005). Alkaloids (Mayer's test), glycosides (Legal's test), saponins (froth formation test), carbohydrates (Molisch's test), proteins (Xanthoproteic test), aminoacids (Ninhydrin test), Flavonoids (Lead acetate test), steroids (Salkowski test), tannins (Ferric chloride test), volatile oils (hydro-distillation method) were analyzed.

Experimental Method

Agar Disc Diffusion

The susceptibility of a micro-organism to antibiotics and other chemo therapeutic agents can be determined by paper disc plate technique. The present study was designed to determine the sensitivity pattern of various compounds of *Caesalpinia pulcherrima* utilizing a broad spectrum of pathogenic and non-pathogenic bacteria; Gram positive (*Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis)* and Gram negative (*Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae)* bacteria. The organisms were collected from Department of Microbiology, Acharya Nagarjuna University, Guntur. The stock cultures were maintained in Mueller-Hinton agar medium slope.

To determine the susceptibility patterns of these organisms against compounds of the *C. pulcherrima*, overnight grown cultures of selected organisms in Mueller Hinton broth served as inoculums. The antibacterial activity was done according to Kirby-Bauer's method (Bauer AW *et al*, 1966) with some slight modifications. The extracts were dissolved in dimethylsulfoxide (DMSO) to a final concentration of $20mgmL^{-1}$. Pure DMSO was used as negative control. The activity was compared with standard drugs Ampicillin, Amoxycillin, Ciprofloxacin at the concentration of 10µg per disc. Mueller Hinton (Himedia) agar was used as bacteriological media. It was prepared according to manufacturer's instructions, sterilized by autoclaving. The sterile medium 20 mL was dispensed per petri dish and allowed to solidify. The set plates were labeled and inoculated with the test organism by using cotton swab rolled in the suspension of overnight grown cultures to streak the surface of plate in a form that lawn growth can be observed after incubation. The paper discs (5mm) were prepared with Whatmann filter paper according to the standard protocol. The sterilized discs were placed on the inoculated plates equidistantly. 20µl of each extract was taken from stock solution ($20mgmL^{-1}$) with micropipette and added to the discs. Each disc received 400µg of crude extract. After proper diffusion of extract into the media, the plates were incubated for 24hrs at 37^{o}c in aerophilic conditions. Zone of inhibition was measured in mm with a ruler. The study was performed in triplicate and the mean values were presented.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration values were determined by broth dilution assay. Varying concentrations of the extracts (80mg/mL, 40mg/mL, 20mg/mL, 15mg/mL, 10mg/mL, 5mg/mL & 2.5mg/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^{0}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 (Atata *et al*., 2003).

RESULTS AND DISCUSSION

The present study carried out on the flower of *Caesalpinia pulcherrima* extracts revealed the possession of medicinal activities. The n-hexane extract of fresh flowers of *C.pulcherrima* does not possess any phytoconstituents. Acetone, methanol & aqueous extracts possess tannins whereas acetone & ethanol extracts possess steroids. The dry flowers possess alkaloids in n-hexane, acetone, ethanol extracts. Glycosides are present in chloroform & aqueous extracts whereas n-hexane, acetone, methanol & aqueous extracts possess tannins. The presence of phytoconstituents was reported in Table-1.

History of use of herbal medicine in the treatment of diseases can be identified with the history of medicine and with the history of civilization itself. All parts of plants were used in Ayurveda, Unani and Homeopathic systems of medicine for the treatment of various human diseases. However, studies with reference to their specific antibacterial activity had been done to negligible extent.

The screening of flowers of *Caesalpinia pulcherrima* L. for antibacterial activity was carried out by disc diffusion method. The selection of this plant is based on its use in folk medicine. In the present study, six bacterial strains were used which are responsible for various minor or major infections in humans. They are *Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae*.

All the extracts of fresh and dry flowers of *C. pulcherrima* had shown potent antibacterial activity. The extracts of dry flowers of *C. pulcherrima* showed higher activity compared to the fresh flowers. The ethanol extract of dry flowers exhibited maximum activity against *Bacillus subtilis*. Ethanol and chloroform extracts of dry flowers were found to have higher inhibitory activity against *K.Pneumoniae* and *B. subtilis* respectively. Ethanol extract of dry flowers exhibited higher inhibitory activity against all the tested organisms except *E.faecalis*. Aqueous extract of fresh flowers was found to have maximum activity compared to aqueous extract of dry flowers against all the organisms except *E. faecalis* and *E. coli*. Methanolic extract of dry flowers possess higher inhibitory activity against *E.faecalis* and moderate activity on *S. aureus* and *E. coli*. Acetone extract exhibited higher activity against *B. subtilis* and moderate activity against *S. aureus*. N-Hexane extracts of fresh and dry flowers exhibited moderate to least activity against all the tested microorganisms. Ampicillin and Amoxycillin at a concentration of 10µg per disc exhibited resistance against *E. coli*. It was reported that the ethanol and aqueous extracts of flowers of *C. Pulcherrima* possess antimicrobial activity against *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus* (Pushpendra S. Dhaked et al., 2011). The results of antibacterial activity of crude extracts of *C. pulcherrima* were summarized in Table-2.

The minimum inhibitory concentration ranged between 2.5mg/mL and 15mg/mL depending on microorganism. All the extracts inhibited the microorganisms at low concentrations. Chloroform and ethanol extracts of dry flowers inhibited *Bacillus subtilis* at a concentration of 2.5mg/mL. The minimum inhibitory concentrations of various extracts were summarized in Table-3.

Table 1. Phytochemical Screening of Fresh& Dry Flower Extracts of *Caesalpinia pulcherrima* L

TEST	n-Hexane Extract		Chloroform Extract		Acetone Extract		Ethanol Extract		Methanol Extract		Aqueous Extract	
	FF	DF	FF	DF	FF	DF	FF	DF	FF	DF	FF	DF
Alkaloids	-	+	-	-	-	+	-	+	-	-	-	-
Glycosides	-	-	-	+	-	-	-	-	-	-	-	+
Saponins	-	-	-	-	-	-	-	-	+	+	-	-
Carbohydrates	-	+	+	+	-	+	+	+	-	-	+	+
Proteins	-	+	-	-	-	-	-	+	-	-	-	+
Aminoacids	-	-	-	-	-	-	+	-	-	-	-	-
Flavonoids	-	+	-	-	-	+	-	+	-	-	-	-
Steroids	-	-	-	-	-	-	+	-	-	-	-	+
Tannins	-	+	-	-	+	+	-	-	+	+	+	+
Volatile oils	-	-	-	-	-	-	-	-	-	-	-	-

+ & - denotes Presence and Absence of phytoconstituents respectively
FF&DF represents Fresh and Dry flowers respectively

Table 2. Antibacterial Activity Of Flower Extracts Of *Caesalpinia Pulcherrima* L (values are mean of three replicates)

Organisms	Zone Of Inhibition in mm															
	Conc 400µg												DMSO (20µl)	Conc 10µg		
	nHE		CE		AE		EE		ME		AqE			AP	AM	CP
	FF	DF	FF	DF	FF	DF	FF	DF	FF	DF	FF	DF				
B.subtilis	11	12	16	**22**	13	21	12	**25**	13	11	14	10	Nil	09	08	34
S.aureus	11	14	19	15	13	17	9	20	13	15	13	08	Nil	09	08	33
E.faecalis	12	11	19	17	12	12	14	14	13	16	08	08	Nil	08	10	32
E.coli	08	10	13	12	13	13	13	20	14	15	15	08	Nil	Nil	Nil	32
P.aeruginosa	11	10	14	19	13	11	12	20	12	14	11	12	Nil	09	12	31
K.pneumoniae	10	9	16	21	15	12	12	**22**	14	13	12	11	Nil	10	13	32

FF&DF represents Fresh and Dry flowers respectively.
nHE = n-Hexane Extract, CE = Chloroform Extract, AE = Acetone Extract, EE = Ethanol Extract, ME = Methanol Extract, AqE = Aqueous Extract, DMSO =Dimethyl sulphoxide, AP=Ampicillin, AM=Amoxycillin, CP=Ciprofloxacin

Table 3. Minimum Inhibitory Concentration Of Flower Extracts Of *Caesalpinia Pulcherrima* L (values are mean of three replicates)

Organisms	Concentration mg/mL											
	n-Hexane Extract		Chloroform Extract		Acetone Extract		Ethanol Extract		Methanol Extract		Aqueous Extract	
	FF	DF	FF	DF	FF	DF	FF	DF	FF	DF	FF	DF
B.subtilis	10	10	10	2.5	15	05	15	2.5	10	10	10	15
S.aureus	15	10	05	05	10	10	15	05	15	05	10	15
E.faecalis	10	10	05	05	15	15	10	10	10	05	15	15
E.coli	15	10	10	10	15	10	15	10	05	10	10	15
P.aeruginosa	10	10	10	05	10	15	15	10	10	10	15	10
K.pneumoniae	10	15	05	05	05	15	15	05	10	15	15	15

CONCLUSION

The results revealed that the crude extracts contain certain constituents like alkaloids, glycosides, tannins & steroids which could make the plant useful in treating different ailments and have potential to provide useful drug for human use. The present study exhibited the antibacterial effect of various extracts of *Caesalpinia pulcherrima*. The inhibitory effect of the extracts justified the medicinal use of *Caesalpinia pulcherrima*. Hence, it is apparent that this plant has been found to possess effective antibacterial substances against a wide range of microorganisms.

Further studies which aimed at the isolation and structure elucidation of antibacterial active constituents from this plant have been initiated. The plant can also be further explored for its activity against wide spectrum of microbes and can be developed into a powerful antibiotic.

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