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Antibacterial activity of *Plumeria rubra* Linn. plant extract

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ABSTRACT

A large number of medicinal plants are claimed to be useful in skin disease in all traditional system of medicine and folklore. While these plant remedies are being used orally and by local application. Looking to the scope of herbal drug and increasing demand especially in disease of liver, cancer, diabetes, hypertension, renal disease, inflammation, infectious diseases and skin diseases etc. Plumeria rubra Linn. has been selected because of its easy of availability, therapeutic value and degree of research work which is not done. Plumeria rubra Linn. is a deciduous tree with thick, widely distributed in common rather moist garden, in lawns and in open plantation tree is unusual in appearance. Plant loses leaves for a short time during the winter. Slowly grows up to height 25 feet with 35 spread. About eight species of Plumeria rubra (L.) occurs in India. The ascending branches leave simple Alternate, spiral, elliptic or ovate shape, base tapering (narrow attenuate) or oblique, margins entire or undulate, apex acuminate or acute or obtuse. Pink or red color flowers and flowering period is August to October. According to Ayurveda; root is bitter, carminative, thermogenic, etc. Leaves are useful in inflammation, rheumatism, antibacterial, antifungal, bronchitis, Antipyretic etc. The in vitro antibacterial activity of ethanolic, chloroform, ethyl acetate and aqueous extract of leaves of Plumeria rubra (L.) has been evaluated using disc diffusion method against S. epidermidis and Escherischia coli of bacterial strains. Comparatively, extract showed significant antibacterial activity with specific standard (Ciprofloxacin).

Key Words: *Plumeria rubra* Linn., Antibacterial Activity.

INTRODUCTION

A large number of medicinal plants are claimed to be useful in skin disease in all traditional system of medicine and folklore. While these plant remedies are being used orally and by local application since ancient, the mechanism whereby such effects elicited has not been looked into. These effects have been brought about by their inherent antibacterial activity by different plants.

There are many natural products, which are used as potential antimicrobial agents. Looking to the scope of herbal drug and increasing demand especially in disease of liver, cancer, diabetes, hypertension, renal disease, inflammation, infectious diseases and skin diseases etc. The selection of the plant *Plumeria rubra* Linn. is made on the basis of its Easy of availability, Therapeutic value and degree of research work which is not done[1].

As part of research for new biological active compounds from higher plants, the crude organic extracts of *Plumeria rubra* Linn. as screened. The purpose of this study is to investigate Indian plants potential antibiotic activity by preliminary bio-screening. That is why to evaluate the anti-bacterial potential of different extracts of *Plumeria rubra* (L.) was carried out. *Plumeria rubra* Linn. is a deciduous tree with thick, widely distributed in common rather moist garden, in lawns and in open plantation tree is unusual in appearance. Plant loses leaves for a short time during the winter. It grows up to height 25 feet with 35 spread. Plant growth rate is slow. About eight species of *Plumeria rubra* (L.) occurs in India.

The ascending branches leave simple Alternate, spiral, petiole undissected, elliptic or ovate shape, base tapering (narrow attenuate) or oblique, margins entire or undulate, apex acuminate or acute or obtuse. Pink or red color flowers, spreading cymes fruits elongated.[2] Flowering period is August to October. According to Ayurveda; root is bitter, carminative, thermogenic, laxative, leprosy etc. Leaves are useful in inflammation, rheumatism, antibacterial, bronchitis, cholera, cold and cough, Antipyretic, antifungal, stimulant etc.[3]

About Plant *Plumeria rubra* Linn.

The species for the proposed study was identified by Dr. A. Bala Subramanyam and this same was authenticated by Dr. P. Jayaraman, Botanist, Plant Anatomy Research Center (PARC), Chennai.

Photograph of *Plumeria rubra* Linn.

Taxonomy of *Plumeria rubra* Linn:

Kingdom- *Plantae*
Class- *Magnoliopsida*
Subclass- *Asteridae*
Order- *Gentianales*
Genus- *Plumeria*
Species- *Rubra*
Family- *Apocynaceae*
Tribe- *Plumeria*
Botanical Name- *Plumeria rubra*

EXPERIMENTAL SECTION

Test Micro-organisms

The various extracts of the powdered leaves of *Plumeria rubra* (L.) were subjected to anti-bacterial studies. The organisms used were:

Table- 1: Details of Bacterial Strains used for determination of antibacterial activity

S. No.	NAME OF STRAINS	SOURCES	NO. OF STRAIN
1	*Staphylococcus epidermidis*	Inst. Of Microbial Technology, Chandigardh	MTCC 3615
2	*Escherischia coli*	Inst. Of Microbial Technology, Chandigardh	MTCC 118

The organisms were maintained on Nutrient Agar slants. These were tested using Nutrient broth. One loopful of the respective cultures (*S. epidermis* and *E. coli*) in slants which were maintained below 4^0 C and incubated at 37^0 C for 24 hours and were observed for the growth of the organism with naked eye for their turbid nature and compared with sterile broth. The presence of turbidity indicates growth and suitability of the culture for further work.

Preparation of test solution and Standard Antibacterial Agent

The test solution of each extract was prepared by dissolving the dry extracts of powdered leaves of *Plumeria rubra* (L.) in respective solvent of each extract and used as control. Concentration of the test extracts was 3 mg/ml and the Standard Anti-bacterial antibiotic Ciprofloxacin (Dr. Reddy's Lab.) 1mg/ml concentration.

Preparation of Inoculum:-

The Bacteria preserve were cultured and sub-cultured pure colonies. The procedure is below:-

(a) One loopful (2 mm) of each bacterial suspension was inoculated in 5 ml of nutrient broth and all test tubes were incubated at 37^0 C for 24 hours.

(b) The overnight grown nutrient broth culture of each test organism was used for streaking over nutrient agar plates and subjected to incubation at 37^0 C for 24 hours and same process was repeated until pure isolated colonies were obtained.

(c) From these isolated colonies fresh sterile nutrient broth and media were re-incubated at 37^0 C for 24 hours. These nutrient broth cultures served as inoculums for determination of anti-bacterial activities of the extract.[4-5]

Determination of Minimum inhibition Concentration (MIC)

Disc diffusion Techniques:-

(a) Stock solution of various leaves extracts of *Plumeria rubra* (L) of 3 mg/ml were prepared with sterile Dimethyl Sulphoxide (DMSO) and measured volume of stock solutions were dispensed in the conical flask to prepare concentration of 250, 500, 750, 1000 and 1500 μg/ml of extracts.

(b) The sterile Petri plates were poured with molten agar medium and allowed to be solidified.

(c) The suspension of test organism or culture were flooded on the solidified nutrient agar medium and kept for 30 minutes in same position for proper inoculation.

(d) Location for each test extract conc. was marked at the back of Agar containing petriplate.

(e) The whatmann filter paper was dipped in appropriate stock extract concentration and placed on the respective place as the marked area on the back of the petriplate.

(f) The plates were incubated at 37^0 C for 24 hours.

(g) The results were analyzed on the basis of zone of inhibition observed.[6-9]

Table- 2: Determination of minimum inhibitory concentration (MIC) of the various leaves extracts of Plumeria rubra (L).

S.No.	Test Compounds.	Name of the Bacteria	Growth media containing different concentration of the extract in µg/ml				
			250 µg/ml	500 µg/ml	750 µg/ml	1000 µg/ml	1500 µg/ml
A	Ethanolic Extract	*S. epidermidis*	+	+	±	--	--
		Escherischia coli	+	+	±	±	--
B	Chloroform Extract	*S. epidermidis*	+	+	+	±	--
		Escherischia coli	+	+	+	±	--
C	Ethyl acetate Extract	*S. epidermidis*	+	+	±	--	--
		Escherischia coli	+	+	±	--	--
D	Aqueous Extract	*S. epidermidis*	+	+	+	±	--
		Escherischia coli	+	+	+	±	--

+ = Growth, ± = Partial inhibition -- = Comp lete Inhibition

Disc diffusion method for determination of antibacterial potency and its comparison

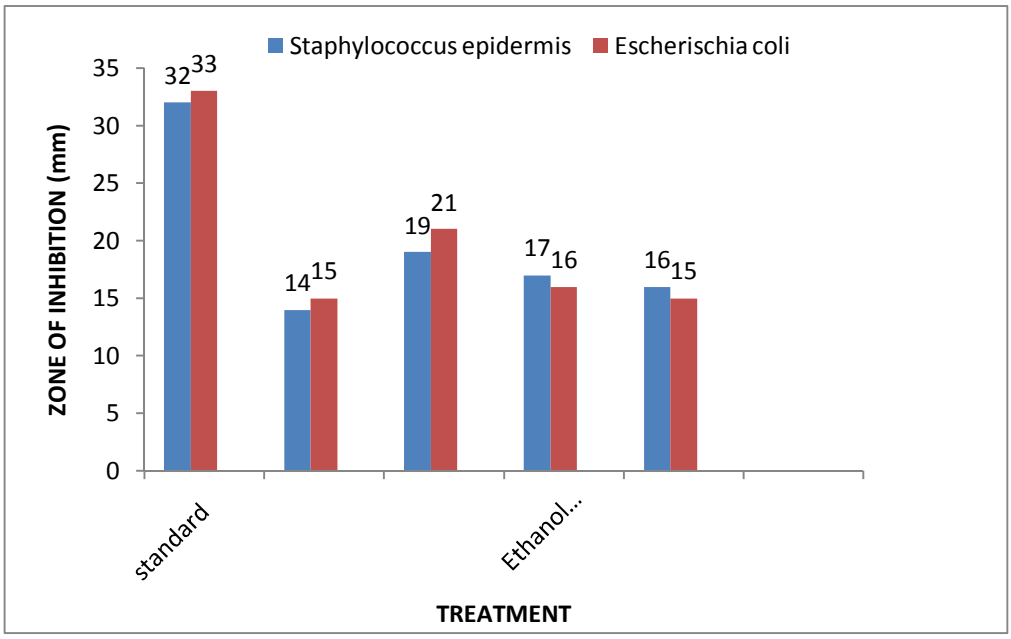
CIPROFLOXACIN:

(a) Sterile Disc of Whatmann filter paper no.1 of 6 mm diameter was prepared.

(b) Nutrient Agar media were prepared, sterilized and poured on to sterile Petri dishes and then kept in the incubator at 37 ^{0}C for 24 hrs.

(c) One set of two dilutions each of extract and ciprofloxacin was prepared and stored in a properly capped volumetric flask.

(d) All plates were flooded with corresponding culture of the test organism under Laminar Airflow and left for 30 minutes.



Graph No. 1: Graphical Representation of Antibacterial Activity of Various Extract of Powder Leaves of *Plumeria rubra* (L.) [Standard: Ciprofloxacin 1mg/ml and Dose of Test Extract: 3mg/ml]

(e) The excess inoculum was discarded using a sterile Pasteur pipette.

(f) Sterile disc were soaked in these dilutions and placed on the corresponding quadrants of the flooded nutrient Agar plates marked at the back with same concentration. This was done both for the test compounds as well as for ciprofloxacin.

(g) The plates were kept overnight in the incubator at 37 ^{0}C. After incubation, the diameter of zone of inhibition around each disc was measured and the results were tabulated for various compounds and ciprofloxacin. [10-11]

Table No. 3: Anti Bacterial Activity of Various Extract of powdered leaves of *Plumeria rubra* (L.)

Test Microorganism	Zone of inhibition (mm)				
	Standard	Chloroform Extract	Ethyl acetate Extract	Ethanol Extract	Aqueous Extract
S. Epidermidis	32	14	19	17	16
E. Coli	33	15	21	16	15

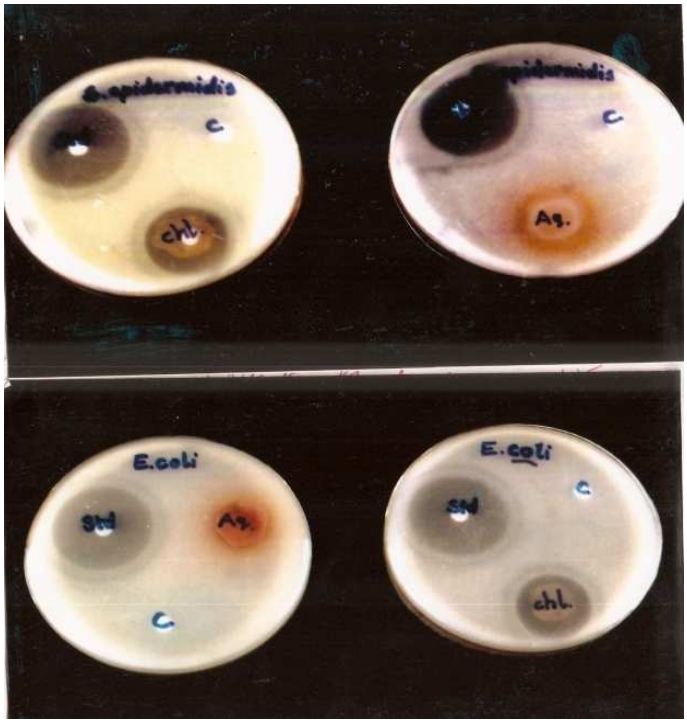


Fig. 1: Antibacterial activities of various extracts of powdered leaves of *Plumeria rubra* Linn.
[Std.: Standard (Ciprofloxacin), C: Control, Chl.: Chloroform extract, Aq.: Aqueous extract]

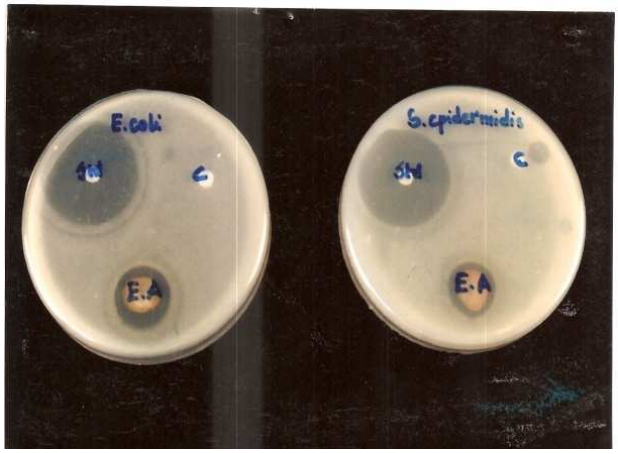


Fig. 2: Antibacterial activity of ethyl acetate extracts of powdered leaves of *Plumeria rubra* L.
[Std.: Standard (Ciprofloxacin), C: Control, E.A.: Ethyl Acetate]

RESULTS AND DISCUSSION

The results indicate that all the test extracts shows good inhibitory activity against all these bacterial strains. Ethanolic extract of leaves is showing partial antibacterial activity against *S.* epidermidis at 750 and 1000 μg/ml and at 1500 μg/ml showing complete antibacterial activity while in *Escherischia coli* at 1500 μg/ml respectively. Chloroform extract of leaves is showing partial antibacterial activity against *S. epidermidis* at 750 and 1000 μg/ml and showing complete antibacterial activity at 1500μg/ml while *Escherischia coli* at 1500 μg/ml respectively. Ethyl acetate extract of leaves is showing partial antibacterial activity against *S. epidermidis* at 1000 μg/ml and showing complete antibacterial activity at 1500 μg/ml while *Escherischia coli* at 1000 μg/ml respectively. Aqueous extract of leaves is showing partial antibacterial activity against *S. epidermidis* at 1000 μg/ml and showing complete antibacterial activity at 1500 μg/ml while *Escherischia coli* at 1500 μg/ml respectively. Standard Ciprofloxacin is showing complete antibacterial activity against *S. epidermidis* and *Escherischia coli* at 500 μg/ml and 750 μg/ml respectively.

REFERENCES

[1] Kirtikar and Basu., The Wealth of India, Vol. 8,165.
[2] Kirtikar and Basu., *Indian medicinal plant*, Vol: 4, 320.
[3] Rastogi B., Srivastava M., *Journal of essential oil:* (60), 677, **2006**.
[4] Adolfo Andrade-Cetto, Michael Heinrich, *Journal of Ethnopharmacology* (99) 325-348, **2005**.
[5] John Kartesz Biota of North America Project, University of North Carolina, **1996**.
[6] Bratman S, Girman A. Mosby's., Handbook of Herbs and Supplements and their Therapeutic Uses. St. Louis, MO: Mosby, Inc.; **2003**.
[7] Bratman S, Girman A. Mosby's., Handbook of Herbs and Supplements and their Therapeutic Uses. St. Louis, MO: Mosby, Inc.; **2003**.
[8] Gaud R.S, Gupta G.D., Practical Microbiology, 2nd edition, Nirali prakashan pg. 40-45, **2004**.
[9] N.K. Jain, Pharmaceutical Microbiology, 1st edition reprint, Vallabh prakashan, pg. 28-46,286-297, **2000**.
[10] Anonymous, Indian pharmacopeia, Government of India, Ministry of Health and Family Welfare, Controller of publication, Delhi,947-949, A-53-54, A124, A70-A71, A-89, A74, A76, A105, **1996**.
[11] Sharma B.K., Instrumental method of Chemical Analysis, 21st edition , Goel Prakashan, Meerut, 96-112, 134-216, 39-133, **2002**.