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ANTIBACTERIAL ACTIVITY OF PLUMERIA OBTUSA (LINN)

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Abstract—**Herbal medicines have great importance in treatment of diseases since ancient times. *Plumeria Obtusa* which is a herbal medicinal plant and is used for gastroprotective activity, Anti-mutagenic activity, Anti-bacterial activity and anti-inflammatory activity due to the presence of iridoid like 6''-O-acetylplumieride p-E-coumarate and 6"-0-acetylplumieride -p-Z-coumarate. Iridoids are basically secondary metabolites present in various plants, especially in species belonging to the Apocynaceae, Lamiaceae, Rubiaceae, Scrophulariaceae and Verbenaceae families. The paper's aim is to summarize the antibacterial activity and that has been demonstrated. *Plumeria Obtusa* is an important source of many pharmacologically and medicinally important chemicals such as plumeride, isoplumeride, fluvoplumericin, irriod glycoside and other various minor secondary metabolites. The study of antibacterial activity with different extracts obtained from different parts of the plant, which show that the compounds have beneficial effects against a number of diseases. As the scenario is now changing through out the world towards the use of non toxic plant products, development of modern drugs from *Plumeria Obtusa* should be emphasized. This research on *Plumeria Obtusa* focuses over antibacterial activity with a scope of development in future.**

Keywords—**Antibacterial ativity, iridoids, Plumeria Obtusa**

I. INTRODUCTION

Ayurveda, the ancient Indian therapeutic measure is renowned as one of the major systems of alternative and complementary medicine. As other ayurvedic medicine, greater parts of its medicaments are based on native herbals and the thorough knowledge about the medicinal plant is must for everyone is working in the field of Ayurveda for identifying and selecting the appropriate plant for a specific disease. In last few years, the interest in medicinal plants has increased in a great extent. Apart from this people from the western countries have also taken this issue so seriously by conducting various researches on plant based medicines.

Plumeria obtusa, commonly called as white frangipani, is a small, rounded tree of the dogbane family that typically grows to 10-15' tall but less frequently to 25' tall. It is main origin is the Bahamas and the Greater Antilles, but has been introduced into a number of tropical areas around the world. *Plumeria obtusa* is a species of *Plumeria,* native to Greater Antilles, northern Central America & southern Mexico. It is a large shrub trees or small tree growing to a height of 8 metres (26 ft.). It is also found in West Bengal, Kolkata & various other places in India. It is grown for decorating purpose in gardens. 5-Petals with white flowers (to 1 3/4" diameter) with nice fragrance Flowers bloom in clusters from spring till their fall, with heaviest bloom often occurring in month of July and August. In Hawaii, the flowers are commonly used to make perfumes[1]

Various types of plumeria obtuse found various parts of world.

Fig 1. P. obtusa found in Singapore

Fig 2. P. obtusa found in Greater Antilles

Fig 2. P. obtusa found in Brisbane, Australia

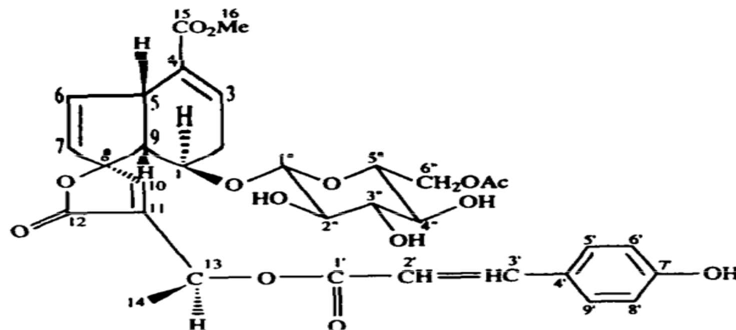
Traditional Uses;
In India, frangipani is a symbol of immortality because of its ability to produce leaves & flowers even after it has been lifted out of soil.
In Vietnam, frangipani is used for its healing qualities, the bark; mashed in alcohol, prevents skin inflammation.
Also used to treat indigestion &high blood pressure.
Roots have purgative effect on animals.
Milk like sap, though poisonous, serves as a balm for skin diseases.
White flowers are used in traditional medicine to cure high blood pressure, cough, haemophilia, dysentery & fever hyper proliferative tissue with gastroprotective activity, Anti-mutagenic activity, Anti-bacterial activity and anti-inflammatory activity due to the presence of iridoid.[17].

The oil of *P. obtusa* was found to be rich in benzyl salicylate (45.4%) and benzyl benzoate (17.2%), but also minute concentrations of alkanoic acids.[11]

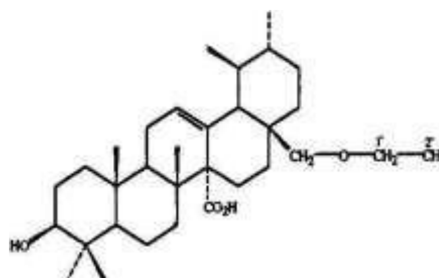
A decoction of leaves of *Plumeria obtusa* is used for treating wounds and skin diseases. latex and bark of *Plumeria obtusa* posses purgative and diuretic properties.[12] Also The stem bark of *Plumeria obtusa* shows antiulcer effect.
In the Sekhukhune District of South Africa, decoction of leaves is taken three times daily for diabetes[13] In Asia, the decoction of leaves is used for treating wounds and skin diseases. Bark and latex are used as diuretic and purgative[14]

Chemical constituents P.obtusa contain pentacyclic triterpenoids namely kaneroside, oleandrin, α-amyrin, neriucoumaric acid, isoneriucoumaric acid, alphitolic acid, oleanonic acid, methyl p-E-coumarate and scopoletin.[2] 6''-Oacetylplumieride.[3],[4]PlumieridePcoumarate.[5],,Obtusilinin ,Oleandrin, Obtusol.[6]Obstusin ,Obstusilic acid, β-Hydroxy-27-[(Z)-p- coumaroyloxy]-urs-12-en-28- oic acid, Obtusinin, Obtusilin (¼ 3b-hydroxy-11-oxours- 12-en-28-oic acid), Obtusinidin, Obtusidin, 27-[p-(E)-Coumaroyloxy] ursolic acid (20Z)-Dammara-,20(22)-dien(3 β,20Z)-dammara-12,20(22)-dien-3-ol, (3β)-Olean-12-ene-3, 27-diol, 27-Hydroxyolean-12-en-3,Urs-12-en-3-one,(3β)-27-[(Z)-Feruloyloxy]-3- hydroxyurs-12-en-28-oic acid.[7]. Uvaol (¼(3b)-3,28-dihydroxyurs-12-ene).[7] iridoids 6''-O-acetylplumieride p-E-coumarate .[9]

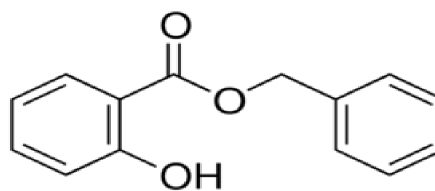
Structure of some of the important chemical constituents.



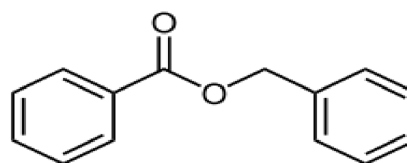
2',3'E(6''-O-acetylplumieride p-E-coumarate)



obtusilinic acid



Benzyl Salicylate



Benzyl Benzoate

II. EXPERIMENTAL WORK

Material and Methods:

2.1 Collection Of Plants

The leaves of Plumeria obtusa were collected from I.I.S.T. campus and surrounding areas during February 2019.

A proper herbarium sheet containing stem, leaf, flower was prepared and plant was given authentication by Dr.K.C.BHATT, NBPGR, PUSA.

They were cleaned (washed) with water to remove solid particles, dust material and other debris. Then they were dried under sunlight for few days.

The dried leaves were then grounded to a coarse powder in a grinder and stored in a well closed air –tight container.

2.2 Selection Of Solvent

Solvent used- Methanol, Chloroform, Distilled water, Benzene, Petroleum ether, Acetic acid, Acetone.

Preliminary screening

Tests for alkaloids, glycosides, flavonoids, saponin glycosides, tannins were done and based on that,

methanol was selected as solvent as maximum constituents were found to be present in it.

2.3 Plant Extract

The Soxhlet apparatus was washed and dried properly.

37 gm. of the coarse dried powder was fitted in the soxhlet apparatus in a thimble and subjected to extraction by methanol.

The extraction was stopped after completion of 12 to 15 cycles in 24 hours.

The solvent was then evaporated by heating the extract obtained, in water bath.

A semi-solid, dark, viscous extract of the crude drugs so obtained was vacuum dried and then used for antibacterial studies.

Wt. of the extract was found to be 4.23 gm.

Further dilutions were made by dissolving required amount of extract in DMSO (di methyl sulphoxide).

2.4Bacteria Used For The Determination Of Antibacterial Activity of Plant Extract

The bacterial strains used for the present investigations were clinical isolates obtained from Department of Pharmaceutical Technology, Jadavpur university and Institute of Microbial Technology (IMT), Chandigarh.

The bacterial strains used are:

Name of the Strain	No Of Strain
Pseudomonas aeruginosa	AP585NLF
Pseudomonas aeruginosa	MTCC424
Actinobacter species	AP586
Morganelle morganii	AP590
Salmonella typhi	MTCC733
Proteus vulgaris	AP679NLF
Staphylococcus aureus	ML267
Staphylococcus aureus	MTCC3750
Vibrio cholerae	1023
Shigella boydii	22461
Shigella sonoi	E08869
Shigella flexneri type	36NK381
Shigella somnei	E08869
Klebsiella pneumonia	MTCC109
Enterobacter species	AP596
Escherichia coli	ETEC LT57

2.5 Standard Antibacterial Agent Used For Comparison Of Antibacterial Activity

Amoxycillin reference standard was used as a standard antibacterial agent.

2.6 Media

Nutrient Agar Medium IP 1996

2.7 Dilution Techniques For Antibacterial Activity

2.7.1 Preparation of Plates

Nutrient Agar medium of the given composition was prepared (300 ml).30 ml of the media was dispensed in one 50 ml conical flask and 29 ml of the media was dispensed in a number of 50 ml conical flask, plugged with cotton and sterilized in autoclave at 121 °C, 15 psig for 30 minutes. Stock solution of 10 mg/ml was prepared. The 70 mm petridishes were sterilized in autoclave at the same temperature as above. Measured quantities of the stock solution were poured in molten nutrient agar medium to prepare concentrations of 50, 100, 200, and 400 μg/ml of the extract and then poured aseptically in the sterilized petridishes in front of HEPA filter. The petridishes were marked accordingly. One sterile nutrient agar medium petriplate without the extract served as control.

These plates were refridgerated at 4°C for overnight for uniform diffusion of the *Plumeria obtusa* leaf extract throughout the media. The plates were dried at 25°C by keeping them in the incubator at this temperature for about 2 hour prior to spot inoculation and location for each test organism was marked at the back of the agar containing petridishes.

2.7.2 Preparation of inoculum

One loopful (loop diameter: 3 mm) of an overnight grown nutrient agar medium culture of each test organism served as the inoculum for such antibacterial activity determination.

2.7.3 Minimum Inhibitory Concentration (MIC)

The petridishes containing the medium were marked in a checker board design. Each marked area in the Nutrient Agar Medium plates was inoculated by a sterile nichrome wire loop with one loopful of the prepared inoculum containing the specific bacterial test organism ie; S aureus, P.aeruginosa and S.typhi. Thus each plate was inoculated with all the test organism on the allotted positions and after inoculation all the plates were incubated at 25°C for 24 hrs.

No growth of the organism on the test plate along with the growth on the control plate was taken as an indicator of

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antibacterial activity of the drug. The readings were recorded in a tabular form.

The minimum inhibitory concentration or MIC was indicated by the least concentration of the extract at which no growth was observed.

2.8 Disc Diffusion Method For The Determination Of Antibacterial Potency Of The Extract And Its Comparison With A Standard Antibacterial Agent

The stock solution (each of 10 mg/ml) of both extract and Amoxycillin were prepared. From these stock solutions two sets of five dilutions i.e. reference and extract (10, 20, 30, 40, 50 µg/ml) each of leaf extract (solvent –DMSO) and Amoxycillin (solvent- distilled water) were prepared. Sterile agar medium plates were prepared and incubated at 25°C for 24 hrs. to check the presence of any sort of contamination.

Then each sterilized agar plates were flooded with liquid culture of bacterial strains and then dried for 30 minutes at 25°C after drawing the excess bacterial suspension. The sterile What man filter paper disc (4 mm diameter) were soaked in five different dilutions of the crude extract and placed in appropriate position of the plates marked as quadrant at the back of petridishes.

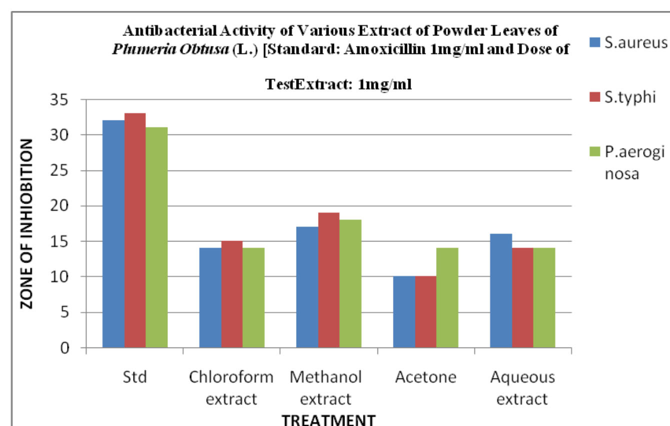
All the flooded plates with the corresponding paper discs soaked with appropriate dilution of the extract were incubated in incubator at 25°C for 24 hrs. Diameter of zone of inhibition were measured in mm and the similar procedure was adopted for Amoxycillin and corresponding zone diameters were measured and compared accordingly.

III. RESULT

Table No. 3: Anti Bacterial Activity of Various Extract of powdered leaves of *Plumeria obtusa.* (L.) using S.typhi, S.aureus and P.aeruginosa as test microbe

Test microbe	Zone of inhibition(mm)				
	Std	**Chloroform extract**	**Methanol extract**	**Acetone extract**	**Aqueous extract**
S.aureus	**32**	**14**	**17**	**10**	**16**
S.typhi	**33**	**15**	**19**	**10**	**14**
P.aeruginosa	**31**	**14**	**18**	**14**	**14**

Graphical Representation of Antibacterial Activity of Various Extract of Powder Leaves of *Plumeria obtusa* (L.) [Standard: Ciprofloxacin 1mg/ml and Dose of Test Extract: 1mg/ml]



Amoxicillin

(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 amoxacillin was prepared and stored in a properly capped volumetric flask.

(d) All plates were filled with their corresponding culture of the test organism under Laminar Airflow in aseptic room and left for 30 minutes

(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 amoxicillin

(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 amoxicillin. .[15],[16]

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

S.No.	Test Compounds.	Name of the Bacteria	Growth media containing different concentration of the extract in µg/ml			
			50 µg/ml	100 µg/ml	200 µg/ml	4000 µg/ml
A	Methanolic Extract	S.typhi	+	+	±	--
		P.aurigunosa .	+	+	±	±
		S.aureus	+	+	+	+
B	Chloroform Extract	S.typhi .	+	+	+	±
		P.aureginosa .	+	+	+	±
		S.aureus	-	-	+	+
C	Acetone Extract	S.typhi .	+	+	±	--
		P.aureginosa .	+	+	±	--
		S.aureus	-	-	-	+
D	Aqueous Extract	S.typhi..	+	+	+	±
		P.aureginosa .	+	+	+	±
		S.aureus	+	+	+ .	.+

Fig. 1: Antibacterial activity of methanol ,chloroform and acetone extracts of powdered leaves of *Plumeria obtusa* L.*[Std.: Standard (Amoxicillin), C: Control,] S.*aureus as test microbe

Fig. 2: Antibacterial activity of methanol ,chloroform and acetone extracts of powdered leaves of *Plumeria obtusa* L.*[Std.: Standard (Amoxicillin), C: Control,] S.*typhi as test microbe

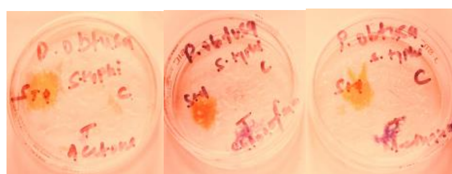
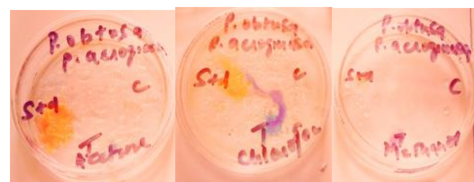


Fig. 3: Antibacterial activity of methanol ,chloroform and acetone extracts of powdered leaves of *Plumeria obtusa* L.*[Std.: Standard (Amoxicillin), C: Control,] P.aeruginosa* as test microbe



IV. DISCUSSION

The results indicate that all the test extracts shows good inhibitory activity against all these bacterial strains. Chloroform extract and Acetone extract of leaves is showing partial antibacterial activity against S.aureus,P.aeruginosa and S.typhi where as complete good antibacterial activity is shown by Methanol extract and Aqueous extract in all the three test microbes ie; S.aureus,P.aeruginosa and S.typhi at 50 µg/ml ,100 µg/ml ,200 µg/ml ,400 µg/ml . Standard Ciprofloxacin is showing complete antibacterial activity against S.aureus and S.typhi and P.aerugunosa .

V. CONCLUSION

The literature survey revealed that this species of *Plumeria* is an important source of many pharmacologically and medicinally important chemicals such as plumeride, isoplumeride, fluvoplumericin, irriod glycoside and other various minor secondary metabolites. Study of antibacterial activity with different extracts obtained from the plant which show that the compounds have beneficial effects against a number of bacterial diseases. As the present scenario is now changing towards the use of non toxic plant products, development of ayurvedic drugs from *Plumeria obtusa* should be emphasized. Clinical trials should be conducted to support its therapeutic use.

VI. ACKNOWLEDGEMENT

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