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Investigating the Phytochemical Composition and Antimicrobial Activity of Leaves of Plumeria Rubra Linn

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ABSTRACT

From the studies that carried out under this title it is clear that plant may have good phytoconstituents like flavonoids, carbohydrates, phenols, tannins. The present study reveals the in- vitro antimicrobial activity of ethanol and ethyl acetate extract of Plumeria rubra leaves has been evaluated using Disc- diffusion method against Staphylococcus aureus with best inhibition zone (11.22 mm) ethanol extract, Bacillus subtilis (16.22 mm) ethyl acetate extract, Escherichia coli and Pseudomonas aeruginosa of bacterial strains and Aspergillus flavus and Candida albicans of fungal strains showed near about same antifungal activity at the given highest concentration of 8000µg/ml with specific standard Ciprofloxacin and Fluconazole respectively.

Keywords: *Plumeria Rubra, Apocynaceae, Phytochemical Screening, Antibacterial, Antifungal, Leaves Extract.*

1. INTRODUCTION

Ornamental and flower crops are not only grown for the display of aesthetic features, but also have some nutritive and medicinal properties as Plumeria rubra Linn. Nature has provided a complete store house of remedies to cure all ailments of mankind[1]. There is growing focus on the importance of medicinal plants in the traditional healthcare system[2]. Lal Champa is generally a small deciduous tree growing to about 25ft high with 35 spread. The ascending branches and ascending leaves are simple alternate, spiral, petiole undissected, elliptic or ovate shape, base tapering or oblique, margins entire or undulate, apex acuminate or acute or obtuse[3].The plant is reported to contain cardiac glycosides[4-5], iridoids, fulvoplumierin, allamcin, plumericin, plumeridoids, Scopoletin, ursolic acid, oleanolic acid, β-sitosterol and a mixture of common sterols[6].

2. MATERIALS AND METHOD

Plant Material

The fresh leaves of Plumeria rubra Linn. was collected from local areas of Hanumangarh, Rajasthan in the month of July. The sample was authenticated by Botanist Dr. Namarta Gupta under voucher no.SD-2011-02, Botany Department, Punjab Agricultural University, Ludhiana (Punjab).After authentication the leaves were washed, cleaned, dried under shade and then coarsely powdered and stored in well closed container for further use.

Extraction of the Plant Materials

The coarsely powdered Plumeria rubra Linn leaves was extracted with petroleum ether (60- 80ºC), benzene, ethyl acetate, ethanol and water by using Successive solvent extraction method with Soxhlet apparatus[7-8].

Afterwards the extracts were filtered off through Whatman filter paper No. 1 and the solvent was removed under vacuum at 30ºC until dry mass were obtained. These dried extracts were preserved in vacuum desiccators.

Phytochemical Screening

The small quantities of all extracts obtained by successive solvent extraction were dissolved in respective solvents and were screened for different classes of phytoconstituents such as Alkaloids, glycosides, Saponins, tannins, flavonoids, phenolic compounds, terpenoids, carbohydrates, fats, lipids, etc., present in the leaf extracts[9-10].

Test for Alkaloids (Meyer's Test)

0.5g of dried powdered sample was boiled with 20 ml of water and then filtered. A drop of Meyer's reagent was added to a small quantity of filtrate and observed for a creamy or white colour precipitate.

Test for Saponins

2 g of powdered sample was boiled with 20 ml of water in a water bath. Then 10 ml of filtrate was mixed with 5 ml of water and shaken vigorously to get a stable persistent froth.

Test for Flavonoids

A 4ml extract solution was combined with 5ml of 95% ethanol solution. The solution was warmed and 0.5g magnesium metal was added. 5-6 drops of concentrated hydrochloric acid was added to this solution. The red colour developed within a minute confirms the presence of flavonoids.

Test for Phenols/ Tannins

To 2-3 ml of aqueous and alcoholic extract, added few drops of 5% ferric chloride solution. Appearance of deep blue black colour indicates positive test for Phenols/ Tannins.

Test for Carbohydrates

1 ml Fehling's A and 1 ml of Fehling's B solutions were combined and boiled for 1 minute. Equal volume of extract solution was added and heated over water bath. First yellow, then a brick red precipitate was observed.

Test for Glycosides

To 5 ml of extract was dissolved in 1 ml of Pyridine. Then 1 ml of sodium hydroxide and sodium nitroprusside was added alternatively. Appearance of blood red colour shows presence of Glycosides.

Microorganisms used for the Study

The bacterial strains namely Staphylococcus aureus, Bacillus subtilis (positive strains) and Escherichia coli, Pseudomonas aeruginosa (negative strains) including two fungal strains Aspergillus flavus and Candida albicans were collected for their antimicrobial testing from Department of Biotechnology, S.D College of Pharmacy, Barnala (Punjab).

Antimicrobial Assay

Disc diffusion method[11] was used to test the antibacterial activity of the extractives against Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli. Dried and sterilized filter paper discs (6mm diameter) were then impregnated with known amount of the test substance dissolved in ethanol and ethyl acetate separately using micropipette and the residual solvents were completely evaporated. Further antibacterial and antifungal studies were done.

Antibacterial Studies

Preparation of Test Inoculums

The strains Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli were inoculated into conical flask containing 100ml of sterile nutrient broth. These conical flasks were incubated at 37°C+1 for 24 hours. This was referred to as seeded broth.

Standardization of Seeded Broth (Viable Count)

Dilutions

1ml of the 24 hours seeded broth of each strain was diluted with 99ml of sterile water containing 0.05% Tween 80 (8 drops of Tween 80 in 100ml of normal saline). From that, 8000µg, 4000 µg, 2000 µg and 10 µg/ml seeded broth were obtained.

Inoculation on Nutrient Agar Petri Dishes

The dilutions were studied by inoculating 0.2ml of each dilution on to the solidified nutrient Agar medium by spreading method. After incubation at 37°C+1 °C for 24 hours, the numbers of well-formed colonies on the petridishes were counted.

Preparation of Solution of Test Drug

The drug solution was prepared by dissolving in dimethyl sulphoxide (DMSO) in a specific gravity bottle (Stoppard). The DMSO was removed from the refrigerator 1 hr prior to its use and allowed to warm up to room temperature.

The solution of the test drug (70% ethanol and ethyl acetate extract) of leaves of Plumeria rubra at the concentration of 20mg/ml in DMSO standard drug (Streptomycin sulphate) 100µg/ml in DMSO was prepared. Solvent control of DMSO was maintained throughout the experiment.

Preparation of Culture Media

The media used for the growth of Bacterial was Nutrient Agar Medium. The culture media were sterilized by autoclaving at 15 lb/sq. inch, pressure at 120°C for 20 mins. The Nutrient Agar medium was prepared by dissolving 28gms of nutrient Agar (Hi-Media) in 100ml of distilled water.

Determination of Antibacterial Susceptibility of Ethanol Extract and Ethyl Acetate Extract of Plumeria Rubra Leaves Using Disc- Diffusion Method

Disc diffusion method was used to test the antimicrobial activity of the extractives against B. subtilis, E. coli, S. aureus, P. aeruginosa (Table-1). Dried and sterilized filter paper discs (6 mm diameter) were then impregnated with known amount of the test substances dissolved in methanol (30µg/ml) using micropipette and the residual solvents were completely evaporated. Discs containing the test material with different concentrations each were placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard disc of Ciprofloxacin (10 µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively. These plates were then kept at low temperature (4˚C) for 24 hours to allow maximum diffusion of test samples. The plates were then incubated at 37°C for 24 hours to allow maximum growth of the organisms. The test materials having antimicrobial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the disc. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition in millimeter.

Antifungal Studies

The fungal kingdom is large but few of them are known to cause diseases in man. The following strain of fungi has been used for our study:

3. CANDIDA ALBICANS

Preparation of Culture Media: The media used for the growth of fungi was Sabouraud Dextrose Agar (SDA) and testing was done in Sabouraud Dextrose Agar (SDA) subculture and viable counts were carried out by same procedure as done in antibacterial studies. But the temperature was maintained at 28°C. The same solution of the test drugs was used for antifungal studies.

Determination of Antifungal Susceptibility of Ethanol Extract and Ethyl Acetate Extract of Plumeria Rubra Leaves Using Disc- Diffusion Method

Antifungal test was carried out using a disc diffusion method 106 colony forming units/mL of yeast spread on SDA. Extracts were applied to the discs (6 mm in diameter) and allowed to soak in, and were then placed on the inoculated media. Negative controls were prepared using the same solvents as employed to obtain the extracts i.e. ethanol extract and ethyl acetate extract. As positive control, Fluconazole (10µg/ml) for Candida albicans is used. The inoculated plates were incubated at 28°C for 48 h for yeast. Antifungal activity was evaluated by measuring the inhibition zone against test microorganisms.

4. RESULTS AND DISCUSSION

Phytochemical analysis of various extract of Plumeria rubra leaves showed the presence of tannins flavonoids, phenols and alkaloids and results were given in the Table – 1. In this, we have done preliminary phytochemical screening and pharmacological studies using microbes to find out the antimicrobial oriented activity of plant extract Plumeria rubra Linn. The antimicrobial activity was determined by disc diffusion method and the size of zone of inhibition (mm) was noted. Ciprofloxacin was used as a standard antibacterial drug while Fluconazole as a standard antifungal drug to evaluate antimicrobial activity of the extract of leaves of Plumeria rubra Linn. We have seen antimicrobial activity studies with the four different concentrations i.e 1000µg/ml, 2000µg/ml, 4000µg/ml and 8000µg/ml. Four bacterial strains i.e Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa and two fungal strains were used i.e. Candida albicans and Aspergillus flavus for this research work. The results of antimicrobial testing have been represented in Table 2-5. It has shown highest antimicrobial activity against Staphylococcus aureus (11.22 mm) as compared to Bacillus subtilis (10.64 mm), Escherichia coli (8.58 mm) and Pseudomonas aeruginosa (7.88 mm). In case of ethyl acetate extract, it showed highest antimicrobial activity against Bacillus subtilis (16.22 mm) as compared to Staphylococcus aureus (15.21 mm), Escherichia coli (8.64 mm) and Pseudomonas aeruginosa (7.39 mm) at the given highest concentration of 8000 µg/ml. In case of antifungal activity, ethyl acetate extract of leaves of Plumeria rubra Linn. Showed good antifungal action as compared to ethanol extract. Candida albicans and Aspergillus flavus both showed near about same antifungal activity for ethanol extract and also for the ethyl acetate extract i.e. for ethanol extract and ethyl acetate extract, Candida albicans (8.87 mm, 9.50 mm) and Aspergillus flavus (8.23 mm, 9.41 mm) at the given highest concentration of 8000µg/ ml respectively.

Table 1: Phytochemical Screening of Plumeria Rubra Leaves Extract

S.No	Qualitative tests	Petroleum ether extract	Benzene extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
1.	Alkaloids	-	-	-	+	-
2.	Carbohydrates	-	-	-	+	+
3.	Proteins	-	-	-	-	+

4.	Amino acids	-	-	-	-	-
5.	Steroids	-	-	-	+	-
6.	Glycosides	-	-	-	-	+
7.	Flavonoids	-	-	+	++	+
8.	Tannins	-	-	-	++	+
9.	Phenols	-	-	-	++	+

Table 2: Antibacterial Study of Ethanol Extract by Disc- Diffusion Method

S. No.	Name of Bacteria	Microbial Activity/ Zone Inhibition (mm)				
		10µg/ml (Std)	1000µg/ml	2000µg/ml	4000µg/ml	8000µg/ml
1.	Bacillus subtilis	25.24	8.21	8.81	9.20	10.64
2.	Staphylococcus aureus	19.78	8.98	9.01	9.11	9.22
3.	Pseudomonas aeruginosa	22.4	7.11	7.19	7.21	7.88
4.	Escherichia coli	19.28	7.12	7.54	8.11	8.58

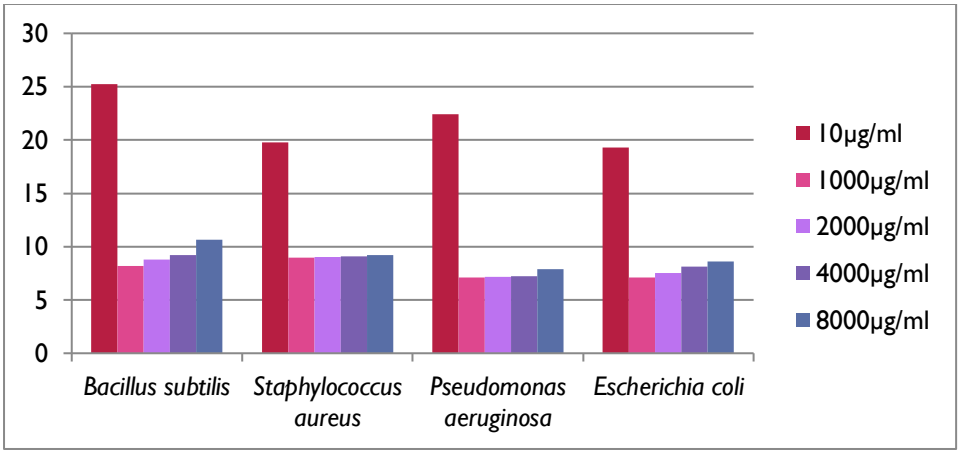

Fig 1: Antibacterial Activity of Ethanol Extract of Plumeria Rubra Leaves

Table 3: Antibacterial Study of Ethyl Acetate Extract by Disc- Diffusion Method

S. No.	Name of Bacteria	Microbial activity/ Zone inhibition (mm)				
		10µg/ml (Std)	1000µg/ml	2000µg/ml	4000µg/ml	8000µg/ml
1.	Bacillus subtilis	23.87	11.65	12.28	13.19	16.22
2.	Staphylococcus aureus	25.35	12.23	13.43	14.61	15.21
3.	Pseudomonas aeruginosa	24.65	6.75	6.78	7.15	7.39
4.	Escherichia coli	31.93	6.58	7.11	8.20	8.64

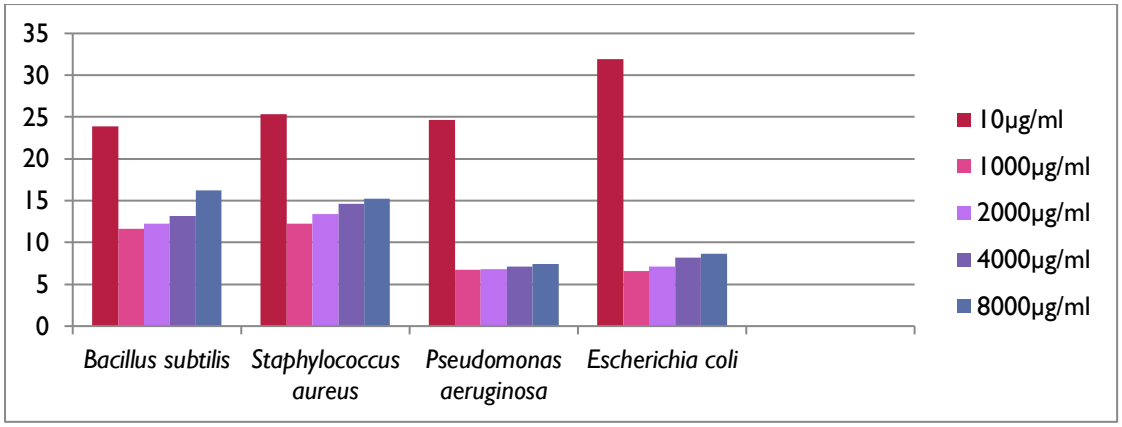

Fig 2: Antibacterial Activity of Ethyl Acetate Extract of Plumeria Rubra Leaves

S. No.	Name of Fungi	Fungal activity/ Zone inhibition (mm)				
		10µg/ml (Std)	1000µg/ml	2000µg/ml	4000µg/ml	8000µg/ml
1.	Candida albicans	14.30	9.50	9.15	8.41	8.23
2.	Aspergillus flavus	13.5	7.1	7.3	8.0	8.23

Table 4: Antifungal Study of Ethanol Extract by Disc- Diffusion Method

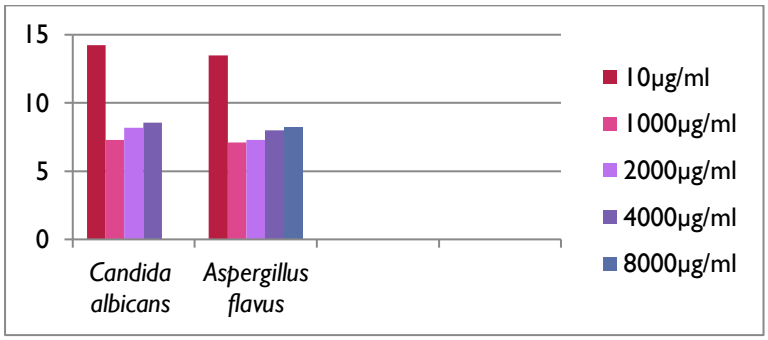

Fig 3: Antifungal Activity of Ethanol Extract of Plumeria Rubra Leaves

Table 5: Antifungal Study of Ethyl Acetate Extract by Disc- Diffusion Method

S. No.	Name of Fungi	Fungal activity/ Zone inhibition (mm)				
		10µg/ml (Std)	1000µg/ml	2000µg/ml	4000µg/ml	8000µg/ml
1.	Candida albicans	14.30	9.50	9.15	8.41	8.23
2.	Aspergillus flavus	13.65	8.14	8.32	9.25	9.41

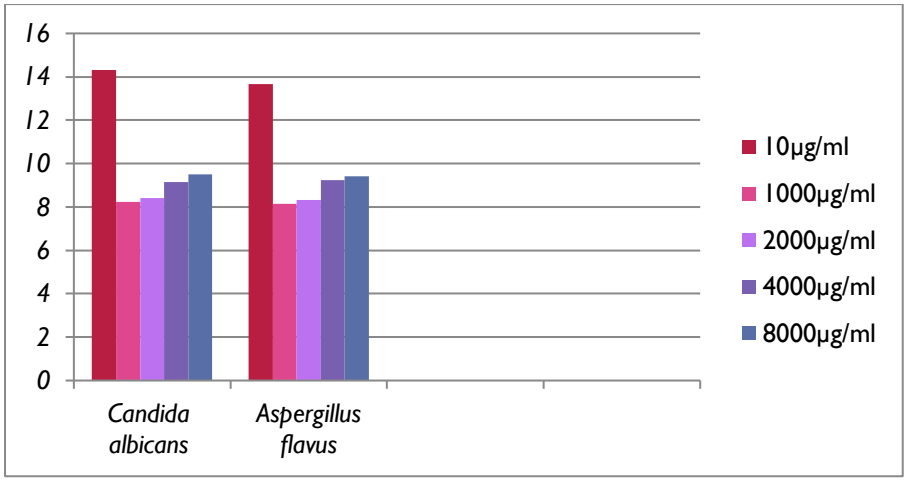

Fig 4: Antifungal Activity of Ethyl Acetate Extract of Plumeria Rubra Leaves

5. CONCLUSION

The preliminary phytochemical screening and Antimicrobial activity of ethanol and ethyl acetate extracts were evaluated and it was evident that these both extracts have good antibacterial and antifungal activity. Ethyl acetate extract has better antibacterial activity ranging from 6.58 mm to16.22 mm as compared to ethanol extract ranging from 7.11 mm to 11.22 mm at different concentrations against all the four strains of bacteria under study. Plumeria rubra Linn has shown highest antimicrobial activity against Staphylococcus aureus (11.22 mm) as compared to Bacillus subtilis (10.64 mm), Escherichia coli (8.58 mm) and Pseudomonas aeruginosa (7.88 mm). In case of ethyl acetate extract, it showed highest antimicrobial activity against Bacillus subtilis (16.22 mm) as compared to Staphylococcus aureus (15.21 mm), Escherichia coli (8.64 mm) and Pseudomonas aeruginosa (7.39 mm) at the given highest concentration of 8000 µg/ml.

In case of antifungal activity, Candida albicans (8.87 mm, 9.50 mm) and Aspergillus flavus (8.23 mm, 9.41 mm) both showed near about same antifungal activity for ethanol as well as ethyl acetate extract respectively. From the above mentioned data we can conclude that plant needed to explore scientifically as more as possible.

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Conflict Of Interest

There was no conflict of interest reported by the authors.

REFERENCES

[1] Kokate C K, Purohit A P, Gokhale S B,1996.Textbook of Pharmacognosy, 20th edition,pp1.

[2] Mukherjee P K, Shau M, Suresh B, 1998. Indian Herbal Medicines, Eastern Pharmacist, 42(8), pp 21-24.

[3] Kritikar and Basu, 1991. Indian Medicinal Plant, 4, pp 320.

[4] Edward F. Gilman and Dennis G. Watson (1993). Plumeria rubra: Frangipani. Fact sheet ST- 490, a series of the environmental Horticulture Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, pp 1-3.

[5] Kardono L B,Tsauri S, Padmawinata K, Pezzuto J M, Kinghorn A D, 1990. Cytotoxic constituents of the bark of Plumeria rubra collected in Indonesia. J Nat Prod, 53 (6): pp1447-55.

[6] Radford D J, Gillies A D, Hinds JA, Duffy P, 1986. Naturally Occurring cardiac Glycosides. Med J Aust; 144 (10): pp 540-44.

[7] Evans W C, 2006. Principles related to the commercial production, quality and standardization of natural products, Pharmacognosy, pp 98.

[8] Aggarwal S.S and Paridhavi M, 2007. Extraction, isolation and analysis of phytopharmaceuticals, Herbal Drug Technology, pp 325-326.

[9] Quality Control methods for medicinal plant materials, 1998. World Health Organization, Geneva.

[10] Biren N Shah and Nayak B S, 2008. Preliminary Phytochemical Investigation, Experimental Pharmacognosy, PV Publishers, pp 190-200.

[11] Bauer AW, Kirby WMM, Sheriss J C and Turck M. 1966. Antibiotic susceptibility testing by standardized single method. American journal of clinical pathology.45, pp 493-496.