



### Full Length Article

## Phytochemical screening and antibacterial potential of natural dye: *Plumeria rubra* (L.)

Rupali Deshpande and Alka Chaturvedi

P.G.T.D. of Botany, R.T.M. Nagpur University, Nagpur  
deshpanderups@gmail.com

#### ABSTRACT

The dye was extracted from pink flower of *Plumeriarubra* in pure aqueous medium and producing various Green, Ivory and Brown shades on silk cloth.. To know the phytochemical constituents, flower dye is chromatographed by TLC and tested against some Bacterial strains for antibacterial activity. The dye is extracted in distilled water as well as in Alcohol and were separated the chemical constituents of dye by TLC. The Antibacterial activity of dye was tested by disk diffusion method against some Bacterial strains. The present chemical constituents of dye were Flavonoid, Anthraquinone and Anthracene as well as Anthocyanidin compounds. The antibacterial assay showed that both the extract inhibit growth of bacterial strains. The distilled water extract of the flower was more active against all bacterial strains. Above present chemical constituents of flower dye could be responsible for antibacterial property. Present investigation is an effort to make such kind of textile dye which is useful to make Ayurvedic Vastra to fight against skin disease.

**Key Words:** Antibacterial, Chromatograph, Dye, Phytochemical, Potential Skin Disease

#### INTRODUCTION

*Plumeriarubra* L. a member of family Apocyanaceae is a common ornamental plant. Distributed throughout India and cultivated near temples and gardens. A deciduous fleshy stemmed tree grows up to 15 meters in height. Leaves simple, arranged in a whorl, with prominent veins, crowded at the end of branches. Flowers reddish pink, with fragrance. Fruits follicles (Singh *et al.*, 2006, ) The Pink flowers of *Plumeria* is due to phenolic compound and is found to be a good source of natural dye for cloth (Wankar). According to Ayurveda; Root is bitter, carminative, and thermogenic. Leaves are useful in inflammation, rheumatism, antibacterial, antifungal, bronchitis and antipyretic. Extract of leaves of *Plumeria rubra* (L.) showed significant antibacterial activity against *Streptococcus. Epidermidis* and *Escherischia strains* (Singh Baghel *et al.*, 2010). In present investigation preliminary phytochemical analysis and chromatography of flower dye was done for

Phenolic groups (Florence *et al.*, 2014). The dye was applied on silk cloth for soothing shades and tested against some bacterial strains for antibacterial activity (Gaikwad, 2013).

#### MATERIALS AND METHODS

##### Extraction of Dye

Flower collected from local area of Nagpur city dried in shade and extraction was done by Soxhlet apparatus for phytochemical screening and antibacterial activity. The dye is extracted in distilled water as well as in alcohol (ethanol & methanol). For dyeing purpose dried powder was boiled for one hour in water and filtered dye was used with different mordants for dyeing silk cloth.

##### Thin layer Chromatography

Silica Gel GF and G were used for TLC and separated the chemical constituents of dye (Wanger and Blatt 1996, Harborne 1998). Different solvent system is used for different phenolic compound as follows.

- Solvent system for Flavonoids :-Butanol : Acetic acid: Water(4:1:5).
- Solvent system for Anthraquinone and Anthracene:- Ethyl acetate : Methanol: Water(100:13.5:10).
- Solvent system for Anthocyanidin :- Butanol:AceticAcid:Water (4:1:5).
- Solvent system for Coumarin:- Ethyl Acetate: FormicAcid: AceticAcid:water (100:11:11:26).

#### Antibacterial activity

The Antibacterial activity of dye was tested by disk diffusion method (Bauer *et al.* 1966) against Bacterial strains *Bacillus cerus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, *Salmonella typhimurium*, *E. coli*. (All the strains are collected from NCL – NCIM Lab. Pune, Maharashtra) Himedia's Nutrient Agar Media is used for culturing Bateria.Himedia's Antibiotic Hexadisk HX055 used standard (*Streptomycin (S,S3)*, *Polymyxin*, *Ampilicine*, *Tetracycline*, *Ciprofloxacin*).

## RESULTS AND DISCUSSIONS

### Natural dye

Flower of *Plumeria* was found to be a good source of natural dye for producing various green, ivory and brown shades on silk cloth. Aqueous medium was suitable for extraction of dye from the flower. Different mordant like Alum, Chrome, Copper sulphate were used to get different shades on cloth. Dye was chromatographed to found various phenolic compounds and tested against some

human pathogenic bacteria strains to find out its antimicrobial activity.

### Thin Layer Chromatography

The flower dye was run in BAW medium to separate flavonoids , total three bands were observed for flavonoids , which were confirmed by comparing with standard Rf value and absorbance spectra (Harbone, 1998) bands of Anthraquinone and Anthracene were observed on TLC plate (Wanger and Baldt 1996). Two bands of Anthocyanidin and single blue band of Coumarin with Rf 85 were observed on plate (Harbone, 1998) (Table 1).

### Antibacterial Activity

The results indicate that all the test extracts shows good inhibitory activity against all bacterial strains. Minimum inhibitory concentrations (mg/ml) of dye found during the investigation. 0.62 mg/ml dye concentration was sufficient to study the antimicrobial activity of the dye. Distilled Water extract of dye has shown maximum zone of inhibition against *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Table 2). From all bacterial strains *Bacillus cereus* is an endemic, gram positive harmful to humans and cause food borne illness *B. cereus* is responsible for a minority of food borne illnesses (2–5%), causing severe nausea, vomiting and diarrhea(Naranjo 2011) . According to the Federal government, *E. coli*, *Salmonella*, *Staphylococcus aureus* strains are also responsible for food poisoning (www.fda.gov).

**Table: 1The Retention Factor (Rf) for each phenolic compound.**

Sr.No.	Name of Phenolic	Name of Compound with value Retention Factors in Parenthesis.
1	Flavonoid	Myricetin(43), Isoviteixin (55), Isorhamnetin (74)
2	Anthraquinone and Anthracene	Emodic acid(18), Rhein (45), Physicon (73), Crysophanol (82), Emoidin (90)
3	Anthocyanidin	Petunidin (52) , Glycosides of flavonoids
4	Coumarin	Single blue Band of Coumarin with Rf 85.

(Standard Rf value : J.B. Harborne 1998, Wanger and Baldt 1996)

**Table 2: Antimicrobial Activity (zone of Inhibition) obtained with the different extracts of the *Plumeria rubra* dye.**

Bacteria	NCIM no.	Zone of Inhibition in mm			
		Standard HexaDisk	Ethanol extract	Methanol Extract	Distilled Water extract
<i>Bacillus cerus</i>	Microbiology.Lab.	20	06	06	06
<i>Staphylococcus aureus</i>	2583	26	09	06	15
<i>Pseudomonas aeruginosa</i>	2200	26	08	10	20
<i>Klebsiella aerogenes</i>	2283	29	07	07	--
<i>Salmonella typhimurium</i>	2501	23	06	08	08
<i>Escherichia. coli.</i>	Microbiology.Lab.	19	05	06	06

*Staphylococcus aureus* is a facultative anaerobic Gram-positive coccid bacterium. It is frequently found as part of the normal skin flora on the skin and nasal passages (Kluytmans *et al.*, 1997). *Pseudomonas aeruginosa*, a Gram-negative bacteria is found mostly in water reservoirs in the environment, causes severe nosocomial and community acquired infections at various body sites including the urinary tract, surgical or burn wounds, the cornea and the lower respiratory tract (Gerd *et al.*, 2008). Whereas according to Meybeck *et al.*, 2004 *Pseudomonas aeruginosa* is an opportunistic pathogen that can cause acute or chronic pneumonia. *Klebsiella aerogenes* epidermal bacteria which causes infection on wound surface. A survey carried out by E Price *et al.*, 1970, it became epidemic in a neurosurgical intensive care ward and found on the fingertips of nurses and volunteers.

As mentioned above the result indicated that *Plumeria* flower dye showed largest inhibition zone 20 mm against *Pseudomonas aeruginosa* in distilled water and lowest inhibition zone 05mm against *E.coli*. It shows satisfactory zone against *Staphylococcus aureus* and *Salmonella*.

At the point of discussion, it is noticeable that we can use flower dye against skin infection causing bacteria by using dye in ointment or to make colourful and protective handgloves for nurses and volunteers in Hospital or use colourful and protective bed sheets instead of white in

hospital ward. One can use a dyed cloth for daily purpose wearing for soothing effect, fashion as well as protection from bacteria at minute level.

#### Conclusion

In present study it is concluded that flower dye which was found a good source of natural dye for different shades on Silk cloth contain a phenolic compounds like Flavonoid, Anthocyanidin, Coumarin, Anthracene and Anthraquinone. These classes of natural products are becoming the subject of anti-infective research, and above phenolic compounds have isolated and identified the structures possessing antifungal, antiviral and antibacterial activity (Cushnie T.P. Tim *et al.* 2005). As these groups are present in ample amount in flower dye, the dye showed good antibacterial activity against studied human pathogenic bacteria strain.

Various microorganisms which are present in our surrounding deposited and multiply on our body and textile material contact with our skin have a chance to cause some skin disease. Present investigation is a try to make such kind of textile dye which is useful to make Ayurvedic Vastram which fight against skin disease. (In the form of daily wearing/ handgloves, bed sheets, OT wearing in hospital by dyed cotton cloths).

As *Plumeria* flower dye showed good antibacterial activity, here is a chance that the dyed cloth may be use as a Ayurvedic Vastram as a supplementary treatment with ointment which fight against skin disease.

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