EXTRACTION AND EVALUATION OF PHARMACOLOGICAL ACTIVITY OF SAPONIN EXTRACT OF *PLUMERIA RUBRA* LEAVES

Kumar Ajit[1], Chanda Indranil[1] Singh Arti*[2] Kopal[2]

[1] Dept of Pharm. Sci., S.B.S. (PG) IBSR, Dehradun, Uttarakhand, India
[2]Drug Design and Medicinal Chemistry Research Laboratory, College of Pharmacy, IFTM, Moradabad-244001, Uttar Pradesh, India. Tel (+91)0591 2360817; Fax (+91)0591 2360818; E- mail: arti18singh123@rediffmail.com

Summary

The aim of the present studies is to evaluate anthelmintic and anti inflammatory activity of saponin extract of *Plumeria rubra* leaves. The result of the maximum dose of 200mg/kg *P. rubra* extract exhibited a significant reduction in the volume of inflammation. The anthelmintic effect of *P. rubra* extract of 25mg/ml concentration is comparable with that of the effect produced by reference standards piperazine citrate.
The present results revealed that active constituents present in *Plumeria rubra* leaves were alkaloids, carbohydrates, glycosides, steroids, saponin, proteins and free amino acids.

Keywords: Anthelmintic activity, Anti inflammatory activity, Saponin extract of *Plumeria rubra.*

Introduction

Plant and plant derived products have found to exhibit a wide variety of activity ranging from analgesic to antineoplastic. Plants possess these wide ranges of activity on the basis of various phyto-constituents as lipids, steroid, glycosides, proteins, saponins etc. The plant *Plumeria rubra* is a genus of lacticiferous trees and shrubs, a native of tropical America [1-3]. About eight species of genus Plumeria are reported in India [4]. Many species of Plumeria yield essential oils. *Plumeria rubra*, tree is upto 9m tall, stem contains a white, poisonous milky sap, and leaves are usually glossy green, 8-12 cm long. Traditionally, various plant parts are used for different medicinal properties as the fruits are reported to be used as abortifacient, the decoction from stem bark exhibits purgative, emmengogue, febrifuge, antitubercular, antifungal and stimulant action, the latex of plant has rubefaciant and purgative properties and the root is cathartic in activity[5]. The *P. rubra* has found to contain alkaloids, glycosides, phenolic compounds, tannins, steroids and saponins. Saponins are also present in the leaf extract of *P. rubra* and no activity has been reported on their medicinal uses. Hence, our study is aimed to evaluate the pharmacological activity of saponins present in *P. Rubra.*

Experimental Section

Plant materials collection and extraction

The leaves of *P. rubra* were collected from forests of Dehradun and authenticated by Dr G.R.S. Bisht, S.B.S. (PG) Institute of Biomedical Sciences and Research, Balawala, Dehradun, U.K., India. The fresh leaves were dried at room temperature, 25-30^{0}C, for 7-10 days. The dried leaves were crushed and weighed. Powder of these dried leaves was subjected to successive extraction in petroleum ether, chloroform, ethyl acetate, methanol and water. 5 gm of powdered leaf was taken in 5 different conical flasks and 50 ml solvent viz. petroleum ether, chloroform, ethyl acetate, methanol and water, was added in each conical flask and left for 16 hrs. After 16 hrs sample was filtered and filtrate was used for different phytochemical tests [6].

Preparation of Saponin extract of *Plumeria rubra*(SEPR)

400 gm of powdered leaves were taken and defatted by N-hexane, after filtering the residue was dried and extracted with methanol (60%) and filtered. This methanolic extract was concentrated in rota vapour at 40^{0}C and dried (Extract A).
The extract A was dissolved in 30 ml water and extracted three times with an equal volume of n-butanol. The n-butanol fraction was dried (Extract B). The extract B was suspended in a relatively small volume of methanol and allowed to settle, the undissolved part separated and dissolved part (Extract C) was added to a large volume of ethyl acetate. The precipitate formed was separated by centrifugation at 2000 rpm for 20 min and dried (Extract D). This extract D contained all the saponins present and was referred to as Saponin extract of *Plumeria rubra* (SEPR).

Anti inflammatory activity of Saponin extract of *Plumeria rubra*

Healthy albino mice of either sex and of approximately same age, weighing about 25 – 30 gm were used for the study. They were housed in a polypropylene cage maintained under standard conditions (12 hr light/ 12 hr dark cycle, 25 ± 30^{0}C, 36 – 60 % humidity. They were divided into five groups of six animals each. One group served as positive control (received indomethacin 10 mg/kg), one group as negative control (received normal saline 10mg/kg) and rest of the groups received saponin extract of *P. rubra* at doses of 50, 100 and 200 mg/kg body weight, 30 mins prior to the injection of inflammatory agent (0.1ml of 1% w/v suspension of carrageenan), was injected at the subplantar region of the left hind paw of all groups. The paw volume was measured at 1, 2, 3 hrs after carrageenan injection using plethysmograph. The anti inflammatory activity was evaluated by determining the reduction in carrageenan induced hind paw edema [7].

$$\% \text{ inhibition} = \frac{\text{mean paw inflammation of control- mean paw inflammation of paw}}{\text{mean paw inflammation of control}}$$

Carrageenan induced rat paw edema method is used widely as a working model of inflammation in search of new anti inflammatory agents[8], and appeared to be the basis for the discovery of Indomethacin, the anti inflammatory drug[9].

Anthelmintic Activity of Saponin extract of *Plumeria rubra*

Indian adult earthworms (*Pheretima posthuma*) collected from moist soil and washed with normal saline to remove all faecal matter were used for the anthelmintic study. The earthworms of 3-5cm in length and 0.1-0.2 cm in width were used for all the experiment. *Pheritima posthuma* was selected for present study due to its anatomical and physiological resemblance with the intestinal roundworm parasite of humans [10].
Preliminary anthelmintic activity was evaluated for the saponin extract from *P. rubra* at uniform concentration (10mg/ml) of standard and test [11].

Statistical analysis

Results were expressed as the mean value ± standard error of the mean. Treated groups were compared with the controls for statistical significant differences ($P < 0.005$). [12, 13].

Results and Discussion

Phytochemical screening of *Plumeria rubra* leaf extract

The results of phytochemical screening revealed the presence of alkaloids, glycosides, carbohydrates, phenolic compunds, tannins, steroids and saponins. Fixed oils were not detected. (Table 1)

Table 1: Phytochemical investigation of *Plumeria rubra* leaf extract

Phytochemicals	Detected
Alkaloids	++
Glycosides	+
Carbohydrates	+
Phenolic Compounds	+
Tannins	+
Steroids	+
Saponinns	+++
Fixed Oils	-

"+++" Very prominent, "++" Prominent, "+" Present, "-"Absent

Anti inflammatory activity of Saponin extract of *Plumeria rubra*

The percentage inhibition at 50, 100 and 200 mg/kg body weight of the tested mice for the anti inflammatory study gave 47.22%, 62.04% and 76.85% respectively, at 3h post carrageenan administration. The saponin extract of *Plumeria rubra* showed moderate anti inflammatory activity (Table 2). The value was lower than that of the reference drug which gave 83.33% inhibition at 10 mg/kg dose at 3h post carrageenan administration.

Table 2: Percentage inhibition of Saponin extract of *Plumeria rubra* (SEPR) on carrageenan induced rat paw edema

Treatment	Dose (mg/kg)	Mean paw volume in cm (% inhibition)		
		1hr	2hr	3hr
Normal Saline		0.93±0.06 (--)	1.01±0.04 (--)	1.08±0.02 (--)
Indomethacine	10mg/kg	0.22±0.03** (76.34%)	0.19±0.02** (81.19%)	0.18±0.02** (83.33%)
SEPR	50mg/kg	0.69±0.08 (25.81%)	0.63±0.06 (37.62%)	0.57±0.05 (47.22%)
SEPR	100mg/kg	0.62±0.06 (33.33%)	0.44±0.09* (56.44%)	0.41±0.018 (62.04%)
SEPR	200mg/kg	0.53±0.04 (43.01%)	0.29±0.03** (71.29%)	0.25±0.22** (76.85%)

N= 6; *P value> 0.01, ** P value> 0.001.

In the present study maximum inhibition, 76.85%, is produced by SEPR at effective dose of 200mg/kg. Anti inflammatory effect may be due to the active constituents alkaloids, glycosides and saponins found in the *Plumeria rubra* leaf. Further investigation is needed on exact mode of action of individual constituents of *Plumeria rubra* leaves.

Anthelmintic Activity of Saponin extract of *Plumeria rubra*

Anthelmintic Activity

Saponin extract of *P. rubra* was dissolved and volume is adjusted to 10 ml with saline water. All drugs and extract solutions were freshly prepared before starting the experiment. One group served as positive control (received piperazine citrate 15 mg/ml), one group as negative control (received normal saline) and rest of the groups received saponin extract of *P. rubra* at doses of 2.5, 5, 10, 25 and 50 mg/ml in normal saline. Observations were made for the time taken to paralyze and/or death of individual worm up to three hours of test period. The mean paralysis and/or death time for each group was recorded (Table 3). Paralysis was said to occur when the worm did not revive even in normal saline. Death was concluded when the worm lost their motility following with fading away of their body color [14].

Table 3: Anthelmintic activity of Saponin extract of *Plumeria rubra(*SEPR)

Compound	Concentration (mg/ml)	Time (minute)	
		For paralysis	For death
Control (Normal Saline)	---	--	--
Reference (Piperazine citrate)	15	26.32±0.16	49.62±0.22
SEPR	2.2	121.36±3.32	148.20±4.02
SEPR	5.0	86.18±1.23	112.31±2.10
SEPR	10	61.57±0.81	88.26±1.62
SEPR	25	31.24±0.33	58.16±0.57
SEPR	50	19.13±0.19	38.26±0.15

Results are expressed as Mean ± SEM from six observations.

Conclusion

Results of preliminary phytochemical tests suggest the presence of alkaloids, glycosides, phenolic compounds, tannins and saponins. The saponin extract was used for testing anti inflammatory and anthelmintic activity of *Plumeria rubra* leaves. The maximum anti inflammatory activity found was 76.85% inhibition produced by the saponin extract of *Plumeria rubra* at effective dose of 200mg/kg. This was less than the reference drug, Indomethacin. The anthelmintic effect of SEPR at 25 mg/ml concentration was comparable with that of the effect produced by reference drug, Piperazine citrate. saponin extract of *Plumeria rubra* showed higher anthelmintic activity than Piperazine citrate at 50 mg/ml concentration.

References

1. Defillips RA. Useful Plants of Commonwealth of Dominica West Indies Smithsonian Institution, Washington D.C.
2. U.S. National Tropical Botanical Biordens, Kalahea, Hawaii
3. Nicolson DH, Flora of Dominica Part 2 Decotydoreae, Smithsonian Institution, Washington D.C.
4. Kirtikar KR, Basu BD, Indian Medicinal Plants II, India
5. Jack Scheper, www.floridata.com, Florida, accessed on 25-04-05.
6. Wall ME, Eddy CR, Mcclenna ML, Klump ME, Detection and estimation of steroid and sapogenins in plant tissue, Analytical Chemistry, 1952; 24: 1337-1342.

7. Emir V, Manueli F, Diaz GJ, Gonzalez A, Bermejo J. Antinociceptive, anti inflammatory and antipyretic effects of Lapidin, a bicyclic sesquiterpene. Planta Med., 1992; 60:395-399.
8. Winter CA, Risley EA, NussGW. Carrageenan induced edema in hind paw of rat as assay for anti inflammatory drugs, Exp. Biol. Med., 1962; 111:544-547.
9. Winter CA, Risley EA, NussGW.Anti inflammatory and antipyretic activities of indomethacin, 1-(p- chlorobenzyl) – 5- methoxy – 2- methylindole -3- acetic acid, J. Pharmac. Exp. Ther., 1963; 141:369.
10. Edwards CA. Testing the effects of chemicals on earthworms: The advantages and limitations of field tests. In: Ecotoxicology of Earthworms, Andover: Intercept Ltd; 1992. p. 75-84.
11. Sollmann T. Anthelmintics: Their efficiency as tested on earthworms. J Pharm Exp Ther 1918; 12:129-70.
12. Wayne, W.Daniel. Biostatistics, A foundation for the analysis in the health sciences;7th Edn., John Wiley and sons (Asia) Pvt Ltd, Singapore, 2004,312.
13. Gold stain A., Biostatistics; Macmillan co, New York 1967, 70-72
14. Shivkar YM, Kumar VL, Anthelmintic activity of latex of calotropis procera. Pharma Biol 2003; 41:263-5.