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Research Article

SCREENING OF PHYTOCHEMICAL AND PHARMACOLOGICAL ACTIVITIES OF LEAVES OF MEDICINAL PLANT *PLUMERIA RUBRA*

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ABSTRACT

An experiment was conducte to assess the phytochemical and pharmacological activities of *Plumeria rubra* plants at Pharmacy discipline of Khulna University, Bangladeh during the month of January, 2011. For these purposes the concentrated ethanolic extract of the leaves were used. The crude extract was tested for the presence of different chemical groups and reducing sugar, gum, alkaloid, steroid and tannins were identified from the extract. The biological interest of these compounds, coupled with the use of this plant in traditional medicine prompted us to check *Plumeria rubra* for antioxidant, antimicrobial and analgesic activity. The antioxidant test was performed that showed antioxidant property and IC_{50} of the sample was 39 µg/mL. The antimicrobial activity of the ethanolic extract of *Plumeria rubra* was investigated by disc diffusion method, which showed activity against *Salmonella typhi.* Moreover, The extract of leaves produced significant writhing inhibition in acetic acid induced writhing in mice at the oral dose of 500 mg/kg body weight (P<0.05), which was comparable to the standard drug Diclofenac sodium at the dose of 25 mg/kg of body weight.

Keywords: *Plumeria rubra*, antioxidant, antimicrobial, analgesic activity, mice.

INTRODUCTION

The medicinal plants have been used in traditional medicine for hundreds of years with reputation as efficacious remedies although there may not sufficient scientific data to substantiate their efficacy. Large numbers of these plants are importance in modern pharmaceutical industry. They serve as therapeutic agents and raw materials for the manufacturer of traditional and modern medicines. These plants are rich sources of bioactive compounds and thus serve as important raw material for drug production. It has now been established that the plants synthesize and accumulate some secondary metabolites like alkaloids, glycosides, tannins, volatile oils etc that may possess a great potential for biological activity and can be a curative agent in therapeutic purposes. The therapeutic use of plants continued with the progress of civilization and development of human knowledge. Scientists endeavored to isolate different constituents from plants and put them into biological system and perform pharmacological tests to identify and isolate therapeutically active compound, which have been used to prepare modern medicine.

Some manufacturing companies are involved in producing the synthetic drugs, but the synthetic drugs over long use have started manifesting serious side effect, so even in the developed countries the interest of natural drugs and natural foods is reviving, so it is important to pay an attention to our herbs and medicinal plants. However, it is important that these should be collected in the right time, right season and right stage of their growth for finding right constitution of therapeutic purpose also age of the plant is very important.

As Bangladesh is a country of low economic growth, that is why scientific exploration and standardization of potential crude drugs is an urgent need to revolutionize our drug sector. Diverse bioactive metabolites like steroids, terpenoids, flavonoids, alkaloids, glycosides, etc in plants have formed the therapeutic basis

of herbal medication. Thus emphasis is given on the biological screening of medicinal plants for further exploration of their active constituents. Besides this for the current use, the country imports a large quantity of pharmaceutical raw materials including medicinal plants and semi processed plant products to produce drugs and medicines. This huge foreign exchange can be saved if the manufacturers to satisfy their needs utilize the indigenous medicinal plants or their semi-processed products.

Plumeria rubra is a very common evergreen tree in Bangladesh. The plant traditionally use for treatment of diarrhea, dysentery and typhoid Upon significant literature survey it has found that very few information was found on the species and there are no significant research work has been performed on the species to judge its use in pharmaceutical industry. That is why the present research was performed to investigate the scientific basis of the traditional uses of the plant *Plumeria rubra* and in the same time find out the chemical group present in the active plant parts.

MATERIALS AND METHODS

The experiment was conducted in the laboratory of pharmacy discipline, school of life science, Khulna University, Bangladesh. The experimental plant was collected from koyra region of Khulna district, Bangladesh during the month of January, 2011 at morning. Fresh leaves were collected from the healthy leaves and any type of adulteration was strictly maintained. The plants were mounted on paper and the sample was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (DACB Accession No: 35566).

The samples were separated from undesirable materials of plants and were dried in a shaded condition for three weeks. The dried leaves were ground into a coarse powder and were stored in an airtight container. All the samples were kept in a cool, dark and dry place until analysis were done. Cold extraction method was used for the extraction. About 100 gm grinded powders were soaked in 500 ml of 98% of ethanol in a glass container for 8 days accompanying regular shaking and stirring. The extract was separated from the plant debris through filtration by a piece of clean, white cotton material. Then the filtrate was taken into rotary evaporator to evaporate ethanol.

The anti-oxidant potential of the ethanolic extract of Plumeria rubra was determined on the basis of their scavenging of the stable 2,2-diphenyl-1-picryl hydrazyl (DPPH) free radical. Six ethanolic conc. (1.57, 3.125, 6.25, 12.5, 25, 50, 100, 200 and 400µg/ml) sample was prepared. One blank was taken. Only ethanol was taken as blank. The reading was noted down carefully and plotted as % inhibition versus concentration to determine IC50. Absorbance at 517 nm in UV spectrophotometer was determined after 30 minutes IC50 (Inhibitory concentration 50%) was determined.

The formula used for % inhibition ratio is –

% inhibition = (Blank OD-Sample OD/Blank OD) X 100

Antimicrobial activities were done according to the disc diffusion method. For the test, 100 mg of the crude extract of *Plumeria rubra* was accurately measured by the electronic balance and taken into vial. Then one ml of ethanol was added and triturated in unidirectional manner. Both gram positive and gram negative bacteria were used. The bacteria used for the antimicrobial activity of Ethanolic crude extract of the *Plumeria rubra were Escherichia coli, Salmonella typhi, Salmonella paratyphi, Shigella dysenteriae, Staphylococcus aureus, Streptococcus pyogenes.*

In this method measured amount of the test samples were dissolved in definite volumes of solvent to give solutions of known concentration (µg/ml). Then sterile Matricel (BBL, Cocksville, USA) filter paper discs are impregnated with known amount of test substances using micropipette and dried. Standard antibiotic discs and discs on which the solvent used to dissolve the samples is adsorbed and dried were used as positive and negative control, respectively. These discs are then placed in petridishes (120 mm in diameter) containing a suitable agar medium seeded with the test organisms using sterile transfer loop for anti-microbial evaluation. The plates are then kept at 40°C for facilitating maximum diffusion. The test material diffuses from the discs to the surrounding medium. The plates are then kept in an incubator for 18-24 hour to allow the growth of the microorganisms. The antibacterial activity of the test agent is determined by measuring the diameter of the zone of inhibition in term of millimeter.

Analgesic activity of the ethanolic extract of test plant leaves was tested using the model of acetic acid induced writhing in mice (Ahmed *et al*., 2004; Whitle, 1964). Young Swiss-albino

mice aged 4-5 weeks, average weight18-28gm were used for the experiment. They were kept standard environmental condition for one week for adaptation after their purchase and fed properly. Experimental animals were randomly selected and divided into four treatment denoted as treatment-1, treatment-2, treatment-3, treatment-4 consisting of four mice in each treatment. Each group received a particular treatment i.e. control, positive control (Diclofenac) and the two doses of the extract. Each mouse was weighed and marked properly and the doses of the test samples and control materials were adjusted accordingly.

To prepare suspension of the test samples at the doses of 250 and 500 mg/kg body weight, 250 mg and 500 mg of samples were measured respectively. The extract was triturated in unidirectional manner by the addition of small amount of tween-80. After proper mixing of extract and tween-80 the distilled water was slowly added. The final volume of the suspension was made 10 ml. For the preparation of diclofenac at the dose of 25 mg/kg-body weight, 5 tablets of diclofenac Na was weighed and than 25 mg equivalent powder was dissolved in10 ml distilled water. Test samples, control and diclofenac were given orally by means of a feeding needle. A thirty minutes interval was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical, acetic acid solution (0.7%,10 ml/kg) was administered intraperitoneally to each of the animals of a group. After an interval of five minutes, which was given for absorption of acetic acid, number of squirms (writhing) was counted for 5 minutes. Each mouse of all groups was observed carefully to count the number of writhing that they had made in 15 minutes.

RESULTS AND DISCUSSION

The mental findings from the study showed that the ethanolic extract of the leaves of *Plumeria rubra* has the positive of reducing sugar, steroid, gum, tannin and alkaloid. The plant extract showed negative result o the saponin, flavonoid and glycoside (Table 1).

Absorbance of each concentration of ehanolic extracts of *Plumeria rubra* was measured at 517 nm. The ethanolic extract of Plumeria rubra exhibited a significant dose dependent inhibition of DPPH activity, with a 50% inhibition (IC50) at a concentration of 39 µg/mL. The IC50 value of the extract was found to be comparable to reference standard ascorbic acid (IC50:14.00 µg/mL). The UV reading of ascorbic acid and blank solution were shown in Table 2. Absorbance decreased with increasing the concentration of leaf extract. The absorbance varied from 0.64 to 0.29. Percent of inhibition gradually increased with the concentration (Figure 1 and 2).

The antibacterial activity of the test agent was determined by measuring the diameter of zone of inhibition in term of millimeter with a calibrated scale. Table 3 showed the results of antimicrobial activity. The antimicrobial activity of *Plumeria rubra* was assessed against a panel of 6 pathogenic bacterial strains (both gram positive and gram negative) at the dose of 250 and 500 µg/disc, and the results were compared with the activity of the positive control, kanamycin (30 µg/disc). At 250 µg/disc the extract showed activity against *Salmonella typhi* (10 mm) and 500 µg/disc the extract showed activity against *Salmonella typhi* (12 mm).

The ethanolic extract of *Plumeria rubra* exhibited effect in acetic acid induced writhing of white albino mice. The extract produced 36.54% and 57.70% writhing inhibition at the doses of 250 and 500 mg/kg-body weight respectively (Table 4 and 5).

The results indicated that the diclofenac sodium is significantly different than control. Though the dose of 250 mg/kg of Plumeria *rubra* extract showed insignificant different than control however, 500 mg/kg of leaf extract were significantly different than control (Table 5).

The leaf extracts of *Plumeria rubra* showed that the plant contain different types of phytochemicals. These chemicals are important for the pharmaceutical use. The presence of different chemical groups that were identified was reducing sugar, gum, alkaloid, steroid and tannins. Phytochemical studies of *Acalypha hisida* leaf found the presence of flavonoids, saponins, glycosides, reducing sugar and steroids (Bokshi et al., 2012) that is similar to the findings of the present. Kardono *et al*., (1990) isolated six cytotoxic constituents from the bark of *Plumeria rubra*.

In the TLC-based qualitative antioxidant assay using DPPH assay, *Plumeria rubra* showed the free radical scavenging properties indicated by the presence of moderate yellow spot on a purple background on the TLC plate. The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple color. When DPPH accepts an electron donated by an

antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. The ethanolic extract of *Plumeria rubra* exhibited a significant dose dependent inhibition of DPPH activity, with a 50% inhibition (IC_{50}) at a concentration of 39 µg/mL. The IC_{50} value of the extract was found to be comparable to reference standard ascorbic acid (IC_{50}: 14.00 µg/mL). The reducing ability of a compound generally depends on the presence of reductants (Duh, 1999), which have been exhibited antioxidative potential by breaking the free radical chain, donating a hydrogen atom (Gordon, 1990).

The antimicrobial activity of *Plumeria rubra* was tested by using the disc diffusion method. The antimicrobial activity was assessed against a panel of 6 pathogenic bacterial strains (both gram positive and gram negative) at the dose of 250 and 500 µg/disc, and the results were compared with the activity of the positive control, kanamycin (30 µg/disc). The extract was found active against *Salmonella typhi*. The zone of inhibition varies within the ranges 10 – 12 mm at the dose of 250 and 500 µg/disc respectively. The highest zone of inhibition was found against *Salmonella typhi* (12 mm) at 500 µg/disc. From the above discussion, it was proved that *Plumeria rubra* had a moderate antimicrobial activity against *Salmonella typhi*. Bokshi (2012) mentioned that *Acalypha hispida* leaves extract exhibited significant antibacterial activity against *Salmonella typhe* and moderate activity against *Enterococcus coli*, *Streptococcus saprophyticus* and *Streptococcus agalactin*.

Pain has been officially defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Pain acts as a warning signal against disturbances of the body and has a proactive function (Tripathi, 1999). Crude ethanolic extracts of *Plumeria rubra* 500 mg/kg orally significantly ($p < 0.05$) inhibited acetic acid-induced writhes in mice. Similarly, diclofenac sodium (100 mg/kg IP) markedly reduced acetic acid-induced writhes in the animals. These observations tend to suggest that crude ethanolic extracts may possess centrally- and peripherally-mediated analgesic properties. The peripheral analgesic effect of the plant's extract may be mediated via inhibition of cyclooxygenases and/or lipoxygenases (and other inflammatory mediators), while the central analgesic action of the extract may be mediated through inhibition of central pain receptors. This hypothesis is in consonance with those of Eddy and Leimback (1953), Koster *et al*. (1959) and Williamson *et al.* (1996) who postulated that acetic acid-induced writhing and hot-plate test methods are useful techniques for the evaluation of peripherally- and centrally-acting analgesic drugs, respectively. Diclofenac, a non-steroidal anti-inflammatory drug (NSAID), is commonly employed in the treatment and/or management of rheumatoid arthritis, osteo-arthritis and ankylosing spondylitis (Siraux, 1977; Brooks *et al*., 1980), and for its anti-inflammatory and analgesic effects. Diclofenac reduces inflammation, swelling and arthritic pain by inhibiting prostaglandins synthesis and/or production (Todd and Sorkin, 1988; Skoutakis *et al*., 1988; Mahgoub, 2002). Diclofenac has also been reported to suppress inflammation induced by various phlogistic agents in experimental animal models (Menasse *et al*., 1978; Al- Tuwaijri and Mustafa, 1992; Mahgoub, 2002). Although the present experimental findings are inconclusive, the results obtained tend to suggest that crude ethanolic extracts of *Plumeria rubra* probably exerts its anti-inflammatory and peripheral antinociceptive effects by inhibiting the release, synthesis and/or production of inflammatory cytokines and mediators, including: prostaglandins, histamine, polypeptide kinins, and so on. In the present study, the reduction of the anti-inflammatory process obtained within the first hour is probably related to reduction in the release of preformed inflammatory agents, rather than to a reduced synthesis of the inflammatory mediators by inhibition of cyclo-oxygenases and/or lipoxygenases (and other inflammatory mediators).

Table 1: Presence of different photochemical identified from the *Plumeria rubra* plant

Name of test	Test solution	Inference
Test for Alkaloids	Mayer's reagent and Dragendroff's reagent	Alkaloid positive
Test for Steroids	Sulfuric acid	Steroid positive
Tests for Saponins	Shaken in a graduated cylinder	Saponins negative
Tests for Reducing sugars	Fehling's A and B solution, Benedict's reagent	Reducing sugars positive
Tests for Tannins	Potassium dichromate solution	Tannins negative
Tests for Gums	Molish reagent and sulfuric acid	Gums positive
Tests for Flavonoids	Ammonia solution, Sodium carbonate solution, Sodium hydroxide solution	a) Flavonoids negative in all solutions
Test for Glycosides	Aquous sodiumhydroxide, Fehling'solution, sulfuric acid	Glycoside negative in all solutions

Table 2: UV reading for ascorbic acid (standard) solution and extract of *Plumeria rubra*

Concentration	1st reading	2nd reading	Average	% inhibition
Blank	0.719	0.719	0.719	
UV reading for ascorbic acid (standard) solution				
1.57 µg/ml	0.713	0.705	0.709	1.39
3.13 µg/ml	0.71	0.63	0.67	6.82
6.25 µg/ml	0.562	0.560	0.564	21.56
12.5 µg/ml	0.391	0.395	0.393	45.34
25 µg/ml	0.22	0.22	0.22	69.40
50 µg/ml	0.093	0.081	0.087	87.90
100 µg/ml	0.053	0.049	0.051	92.91
200 µg/ml	0.044	0.046	0.045	93.74
400 µg/ml	0.047	0.041	0.044	93.88
UV reading for extract of *Plumeria rubra*				
1.57 µg/ml	0.643	0.642	0.643	10.57
3.13µg/ml	0.632	0.681	0.632	12.10
6.25 µg/ml	0.584	0.583	0.584	18.78
12.5 µg/ml	0.521	0.520	0.521	27.54
25 µg/ml	0.393	0.392	0.393	45.34
50 µg/ml	0.338	0.339	0.339	52.85
100 µg/ml	0.315	0.314	0.315	56.19
200 µg/ml	0.296	0.297	0.297	58.69
400 µg/ml	0.285	0.284	0.285	60.36

Table 3: In vitro antimicrobial activity of ethanol extract of *Plumeria rubra* plant using six pathogenic bacteria

Bacterial Strains	Diameter of Zone of Inhibition in mm			
	Blank	Kanamycin (30 µg/disc)	Leaf extract 250µg/disc	Leaf extract 500 µg/disc
Gram(+)ve Bacteria				
Staphylococcus aureus	-	25	-	-
Streptococcus pyogens	-	23	-	-
Gram(-)ve Bacteria				
Salmonella paratyphi	-	20	-	-
Shigella dysenteriae	-	25	-	-
Salmonella typhi	-	25	10	12
Escherechia coli	-	25	-	-

Table 4: Effects of the crude extract of *Plumeria rubra* at the doses of 250 and 500 mg/kg-body weight on acetic acid induced writhing of mice

Administered (Dose)	Numbering of mice	Weight of mice (gm)	Dose (ml)	Total writhing	Average writhing
Control (Treatment 1)	1	26	0.26	17	13
	2	32	0.32	10	
	3	27	0.27	11	
	4	30	0.30	14	
Diclofenac (25 mg/kg) (Treatment 2)	1	40	0.40	0	1.75
	2	28	0.28	5	
	3	34	0.34	1	
	4	35	0.35	1	
Extract (250 mg/kg) (Treatment 3)	1	31	0.31	14	8.25
	2	34	0.34	8	
	3	34	0.34	8	
	4	29	0.29	3	
Extract (500 mg/kg) (Treatment 4)	1	38	0.38	9	5.5
	2	32	0.32	6	
	3	28	0.28	5	
	4	36	0.36	2	

Table 5: Mean and percent of writhing, percent protection of the crude extract of *Plumeria rubra* on acetic acid induced writhing of mice and its statistical value

Animal group	Mean writhing	Percent of writhing	SD	SE	Percent protection	T-test (value of p)
Control	13	100	2.74	1.58	0	
Diclofenac (25mg/kg)	1.75	13.46	1.92	1.11	86.54	5.83 (p<.001)
Extract (250 mg/kg)	8.25	63.46	3.9	2.25	36.54	1.72 (p<.1)
Extract (500 mg/kg)	5.5	42.31	2.5	1.44	57.70	2.05 (p<.05)

SD = Standard deviation, SE = Standard error.

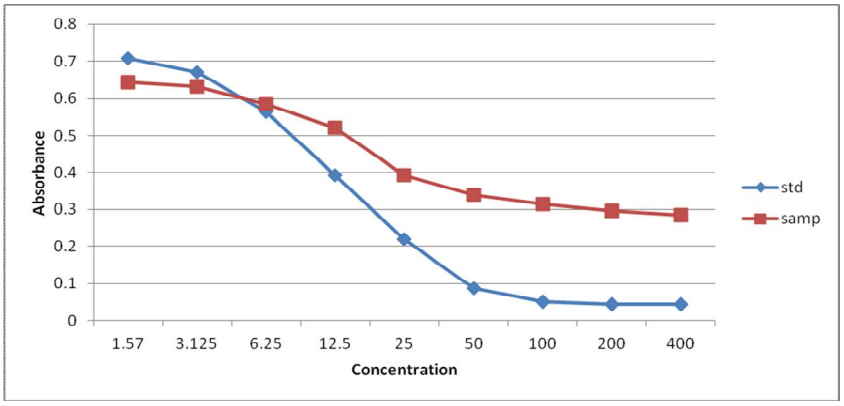

Fig. 1: Absorbance against leaf extracts concentration

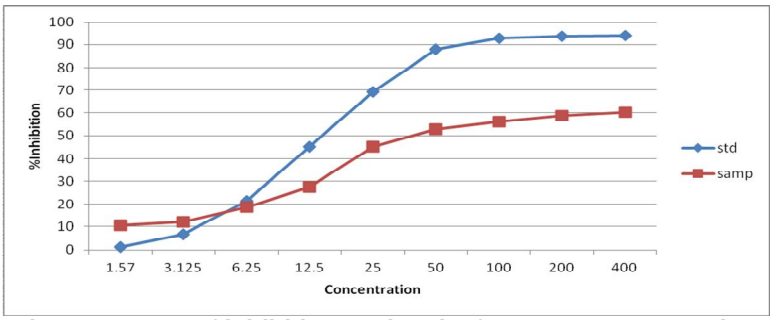

Fig. 2: Percent of inhibition against leaf extracts concentration

CONCLUSION

It can be concluded that the crude extract indicated that the experimental plant contain different types of chemicals including reducing sugar, gum, alkaloid, steroid and tannins. The crude ethanolic extract of *Plumeria rubra* showed antioxidant activity and it may possess moderate antimicrobial activity. Analgesic activity of *Plumeria rubra* was tested by acetic acid induced writhing model in mice. The extract produced significant writhing inhibition at the dose of 500mg/kg-body weight. The crude extract of experimental plant showed analgesic activity.

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