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Antioxidant and Anti-inflammatory Activities of Flowers of *Plumeria rubra* L. f. *rubra* and *Plumeria rubra* f. *lutea*: A Comparative Study

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ABSTRACT

Plumeria rubra L (Apocynaceae), commonly known as "Frangipani" is an important medicinal plant, widely distributed throughout Southern India. In traditional medicinal system different parts of this plant have been mentioned to be useful in a wide variety of diseases. The present study is aimed to comparatively evaluate the antioxidant, free radical scavenging and anti-inflammatory activities of the methanolic flower extracts of two cultivars of *P. rubra* (*Plumeria rubra* f. *rubra* and *Plumeria rubra* f. *lutea*). Shade dried flowers of both the cultivars of *P.rubra* were extracted with methanol and evaluated for antioxidant and free radical scavenging activities by DPPH, nitric oxide, H_2O_2 scavenging assays, reducing power assay, total phenol and flavanoid content assays. The anti-inflammatory effects were investigated by carrageenan-induced rat paw oedema model at dose levels of 250 and 500 mg/kg b.w., using Diclofenac (50 mg/kg b.w) as standard. Phytochemical analysis demonstrated that the methanolic fractions of flowers of *P. rubra* were rich in flavonoids and phenols and also exhibited a strong reducing power and free radical scavenging ability. The results obtained in all the assays were highly significant and comparable to that of standard, ascorbic acid. Both the extracts have also shown a significant reduction of inflammation in a dose dependent manner. However, MEPR exhibited greater antioxidant (except for its reducing ability) and anti-inflammatory activities at a given dose when compared to that of MEPL. The flowers of *P. rubra,* in particular *P. rubra* f. *rubra* and *P. rubra* f. *lutea* can serve as valuable sources of antioxidant and anti-inflammatory phytonutrients.

Key Words: Antioxidant, Anti-inflammatory, Free radical, MEPR, MEPL, *Plumeria*

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INTRODUCTION

Free radicals are chemical species associated with odd or unpaired electrons. They are formed as by-products when cells use oxygen to generate energy. Free radicals include superoxide anion radical ($O_2^{\cdot}$), peroxyl radical ($ROO^{\cdot}$), reactive hydroxyl radicals ($OH^{\cdot}$), nitric oxide ($NO^{\cdot}$) etc. that result from the cellular redox process. They are very unstable and try to bind to other molecules to increase their stability. Once formed these highly reactive radicals while circulating in the body can start a dangerous chain reaction by stealing electrons from other molecules. Their chief danger comes from the damage they can do when they react with important cellular components such as DNA, or the cell membrane [1]. Cells may function poorly or die when attacked by free radicals. They are thought to play a major part in the ageing process, in some autoimmune diseases, in inflammation and in the development of cancer [2]. To prevent free radical damage the body has a defense system of *antioxidants*.

Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. They act as scavengers by preventing and repairing the damages caused by free radicals, and therefore can enhance the immune defense and lower the risk of cancer and degenerative diseases. They are either naturally produced in situ, or externally supplied through foods and/or supplements.

Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. One purpose of inflammation is to protect the site of an injury. An uncontrolled and persistent inflammation may act as an etiologic factor for many of the chronic illnesses [3]. Chronic inflammation exerts its cellular side effects mainly through excessive production of free radicals and depletion of antioxidants [4].

Antioxidants have gained importance in the current scenario for their ability to trap the free radicals produced during degenerative diseases [5]. Natural antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, flavanoids and phytoestrogens are considered superior to synthetic components (BHT, BHA, EDTA, TBHQ etc.) as they are safe, non-toxic and produce a prominent action. Their use is mainly centered on prevention and maintenance of health. Recently, an intensive search for novel types of antioxidants has been carried out from numerous plant materials and they have proven to show significant free radical scavenging activity or antioxidant activity [6-8].

The genus *Plumeria* (family: Apocynaceae) originated from Central America and consists of about eight species of which many are widely distributed in tropical countries. *Plumeria rubra Linn*., one of the species of the genus is an important medicinal plant, widely distributed throughout Southern India. *Plumeria rubra* L. f. *rubra* [pink flowers] and *Plumeria rubra* f. *lutea* [white flowers] are the two cultivars of *P. rubra*, famous for their attractiveness and fragrant flowers. In traditional medicinal system different parts of this plant have been mentioned to be useful in a wide variety of diseases like inflammation, rheumatism, ulcers, leprosy, asthma, diabetes, constipation, fever etc [9]. Studies on the extracts revealed the presence of various

phytochemical constituents [10,11] and they were reported to possess anticancer [12,13], antimicrobial [14-16], anthelmintic [17], antioxidant and hypolipidemic activities [18-20]. However, till date there are no reports on the comparative pharmacological evaluation of the flower extracts of different cultivars of *P. rubra*. Therefore in continuation of our work on *Plumeria* [21-23], we here in report the preliminary result of studies on acute toxicity, antioxidant and anti-inflammatory effects of the methanolic extracts of two cultivars of *P. rubra* viz., *Plumeria rubra* f. *rubra* and *Plumeria rubra* f. *lutea* (MEPR & MEPL) on experimental models.

MATERIALS AND METHODS

Collection and Authentication of Plant Materials

The flowers of the plants *plumeria rubra* f. *Rubra* and *plumeria rubra* f. *Lutea* (family: apocynaceae) were collected from Hyderabad, Andhra Pradesh, India. The plant material was taxonomically identified by Dr. Vastavaya S. Raju, Head, Department of botany, Kakatiya University, Warangal. The voucher specimen (no: KS 04/11) has been deposited in our laboratory for future reference.

Preparation of crude extract

The flowers of both the cultivars were dried under shade and then powdered with a mechanical grinder and stored in airtight container. The dried powder material of the flowers was defatted with n-hexane and allowed to dry. The marc thus obtained was then extracted with methanol in a soxhlet apparatus. The solvent was completely removed under reduced pressure and a semisolid mass was obtained (MEPR and MEPL, yield 11.6% & 9.6%). The dried MEPR & MEPL was dissolved in methanol and used for the present study.

Chemicals

DPPH [1, 1-diphenyl-2-picryl hydrazyl] was obtained from Sigma Aldrich, Mumbai. Ascorbic acid and $AlCl_3$ were purchased from Finar chemicals, Ahmedabad. EDTA and Folin-Cio caltaeu reagent were purchased from Merck Specialties, Mumbai. Sodium nitropruside and potassium dihydrogen-ortho-phosphate were obtained from Molychem, Mumbai. N-1-Napthyl ethylene diamine dihydrochloride were purchased from LOBA chemie Pvt. Ltd. Mumbai. Gallic acid was purchased from Quali Kems Fine Chem Pvt. Ltd. Mumbai. Diclofenac, Carrageenan and Sulfanilamide were obtained from HiMedia Lab Pvt Ltd. Mumbai. All chemicals and solvents used were of analytical grade, available commercially.

Preliminary Phytochemical Analysis

The dried extracts of MEPR & MEPL were used to analyze qualitatively various phytoconstituents like alkaloids, proteins, steroids, saponins, flavanoids, phenolic compounds and tannins, by employing standard screening tests [24].

Animals

Male Wistar albino rats (180-200g body weight) and Swiss albino mice, aged 3 months (25 ± 5g body weight) were used for the present study. They were procured from Mahaveer enterprises, Medipalli, Hyderabad, India. The animals were housed in poly acrylic cages (38 cm × 23 cm × 10 cm) at an ambient temperature of 18± 2°C with 12-h-light/12-h-dark cycle. They had free access to standard feed pellet diet, under good management conditions and water *ad libitum*. The maintenance and the handling of animals were performed according to the rules and regulations of Institutional Animal Ethical Committee (VCOP/2011/10/4/14).

Antioxidant assays

DPPH radical scavenging assay:

In the DPPH assay, the antioxidants reduce the stable radical DPPH (2,2-diphenyl-1-picryl hydrazyl) to the yellow colored diphenyl-picrylhydrazine. The radical scavenging activity of MEPR & MEPL was measured in terms of hydrogen donating or radical scavenging ability using DPPH, which offers a convenient and accurate method for titrating the oxidizable groups of natural or synthetic antioxidants [25]. 0.1 mM solution of DPPH in methanol was prepared; 1 ml of the solution was added to 3 ml MEPR & MEPL suspension in methanol at different concentrations (20, 40, 60, 80 and 100µg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm by using a UV-Visible Spectrophotometer and compared with Ascorbic acid as the standard. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The percentage DPPH scavenging effect was calculated using the following equation:

% DPPH Scavenging Activity = $[1 – (Abs_{sample} / Abs_{control})] \times 100$ Where, $Abs_{control}$

is the absorbance of control at 517 nm;

Abs_{sample} is the absorbance of sample extract/standard at 517 nm.

All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the absorbance values of control and samples.

Nitric Oxide radical scavenging assay

Nitric oxide is a free radical which plays an important role in the pathogenesis of pain, inflammation, etc. Scavenging of nitric oxide radical is based on the generation of nitric oxide from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent [26]. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. For the experiment, the reaction mixture (3ml) containing sodium nitroprusside (10mM) in phosphate buffered saline (PBS) and the MEPR & MEPL in different concentrations (20, 40, 60, 80 and 100µg/ml) were incubated at 25

^{0}C for 150 min. Each 30 min, 0.5ml of the sample was removed and 0.5ml of Griess reagent (1% sulphanilamide, 0.1% napthylethylene diamine dihydrochoride in 2% H_3PO_4) was added. The absorbance of the chromophore formed was measured at 546nm. Ascorbic Acid was used as positive control. All the tests were performed in triplicate and the results averaged. The percentage inhibition of nitric oxide generated was measured by comparing the absorbance values of control and test. The nitric oxide radicals scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = ((A_0-A_1) / A_0 \times 100)$$

Where A_0 was the absorbance of the control (without extract)
and A_1 was the absorbance in the presence of the extract

Hydrogen peroxide scavenging assay

The ability of the MEPR and MEPL to scavenge hydrogen peroxide was determined according to the method [27]. Extracts (20-100µg/ml) prepared in methanol were mixed with 0.6 mL of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1M, pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm against blank solution containing the extract without H_2O_2. The percentage of scavenging of hydrogen peroxide of MEPR, MEPL and standard compounds was calculated using the following equation:

$$\% \text{ Inhibition} = ((A_0-A_1) / A_0 \times 100)$$

Where A_0 was the absorbance of the control (without extract)
and A_1 was the absorbance in the presence of the extract

Reductive ability

The reducing capacity of a compound may serve as significant indicator of its potential antioxidant activity. The reducing power of MEPR and MEPL extracts were determined by the method [28]. The capacity of extract to reduce the ferric-ferricyanide complex to the prussian blue ferrous-ferricyanide complex was determined by recording the absorbance at 700 nm after incubation. For this purpose, different concentrations of plant extract (10 mg/mL) in 1 ml of methanol were mixed with phosphate buffer (2.5 mL, 0.2M, pH 6.6) and potassiumferricyanide (2.5 mL, 1%). The mixture was incubated at 50°C for 20 minutes. Aliquots (2.5 mL) of Trichloro acetic acid (TCA, 10%) were added to the mixture, which was then centrifuged at 3000g for 10min. The upper layer (2.5 mL) of solution was mixed with methanol (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%). The absorbance was measured at 700 nm by spectrophotometer. Increased absorbance of the reaction mixture indicates increased reducing ability. Ascorbic acid was used as reference compound. All the tests were performed in triplicate and the results averaged.

Assay for total phenolic content

Total phenolic content in the MEPR & MEPL was determined using the Folin-Ciocalteau reagent [29]. Folin-Ciocalteau colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue absorption with a maximum at 760 nm. The intensity of the light absorption at that wave length is proportional to the concentration of phenols. Gallic acid was used as standard for the calibration curve. The total phenolic content was expressed as mg gallic acid equivalents (GAE) per gram of sample (mg/g)

Absorbance = 0.001 x Gallic acid (µg) + 0.0033

Assay for total flavonoid content

Aluminum chloride colorimetric method was used for flavonoids determination [30]. The method is based on the quantification of yellow color produced by the interaction of flavonoids with $AlCl_3$ reagent. 1 ml of MEPR & MEPL from stock solution was mixed with 3 ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1M potassium acetate and 5.6 ml of distilled water. Retained it at room temperature for 30 min; the absorbance of the reaction mixture was measured at 420 nm with UV Visible spectrophotometer. The % of total flavonoid was calculated by plotting calibration curve of standard flavonoid, Quercetin solution (20, 40, 60, 80 and 100 g/ml) in methanol**.** The concentration of flavonoid was expressed in terms of mg/ml.

Acute toxicity study

Acute toxicity studies were carried out using female albino mice as per the OECD guidelines 425 [31]. The animals were fasted for 4 h, but allowed free access to water. Following the period of fasting, the animals are weighed and accordingly dose was calculated. The fasted mice were divided into three groups of five animals each. The group I received MEPR 2000 mg/kg orally, group II received MEPL 2000 mg/kg orally and third group received similar volume of vehicle (5 ml/kg) and served as control. Mortality and signs of toxicity in each group were observed for 14 days.

Anti inflammatory Activity

Acute inflammation model: carrageenan induced rat paw oedema

The rats were divided into six groups (n = 6). The different groups were treated orally with MEPR & MEPL (250 and 500 $mgkg^{-1}$ b.w), Diclofenac (10 $mgkg^{-1}$ b.w), and vehicle control (0.9% NaCl, 5 $mlkg^{-1}$ b.w). The administration of extract and drugs was 30 min prior to injection of 0.1 ml of 1% freshly prepared suspension of carrageenan in normal saline in the right hind paw subplantar of each rat. Before induction of oedema, the dorsiventral thickness of both the paws of each rat was measured using Plethysmometer. The measurements were taken at 1

hour intervals after induction of oedema for up to 6 hours [32,33]. Oedema was monitored as the percentage increase in paw thickness in the carrageenan injected paw. To assess the oedema in control paw (right) saline was injected subcutaneously. The anti-inflammatory effect of MEPR & MEPL was calculated by the following equation:- The percent inhibition of paw thickness is calculated using the formula:

$$\% \text{ inhibition} = 100 \times [1 - (Yt / Yc)]$$

Yt = Average increase in paw thickness in groups tested with test compounds. Yc= Average increase in paw thickness in control.

Statistical analysis

All of the experiments were carried out in triplicate. The content of total phenols and flavonoids was presented as mean ± SD. All data were computed using Graph Pad Prism (Graph Pad Software, San Diego, CA, USA).

RESULTS AND DISCUSSION

MEPR & MEPL were screened for the following free radical scavenging activities. It was observed that the test compounds scavenged free radicals in a concentration dependent manner in all the models. The antioxidant activity was expressed as IC_{50} (the amount of antioxidant needed to decrease the radical concentration by 50%). The lower the IC_{50} value, the higher is the antioxidant activity of the tested sample.

DPPH radical scavenging assay

Table 1 illustrates a significant decrease in the concentration of DPPH radical due to the scavenging ability of MEPR, MEPL and ascorbic acid. The scavenging effect of MEPR, MEPL and ascorbic acid on the DPPH radical decreased in the order of ascorbic acid > MEPR > MEPL (Fig. 1). DPPH free radical scavenging activity of both MEPR and MEPL increased with an increase in concentration. IC_{50} values were found to be 65.132 µg/mL, 72.184 µg/mL and 51.491µg/mL for MEPR, MEPL and ascorbic acid (standard) respectively.

Table 1: Effect of MEPR & MEPL on DPPH radicals

Concentration (µg/ml)	Percentage Scavenging activity		
	Ascorbic acid	MEPR	MEPL
20	7.17 ± 1.34	13.24 ± 1.43	0.75 ± 0.12
40	37.17 ± 4.12	27.68 ± 2.43	9.99 ± 1.23
60	55.58 ± 4.53	47.56 ± 3.86	30.40 ± 3.23
80	75.42 ± 5.21	69.14 ± 4.32	61.15 ± 4.98
100	90.01 ± 5.81	77.31 ± 4.87	76.74 ± 5.23
IC_{50}(µg/ml)	51.491 ± 4.23	65.132 ± 3.76	72.184 ± 4.57

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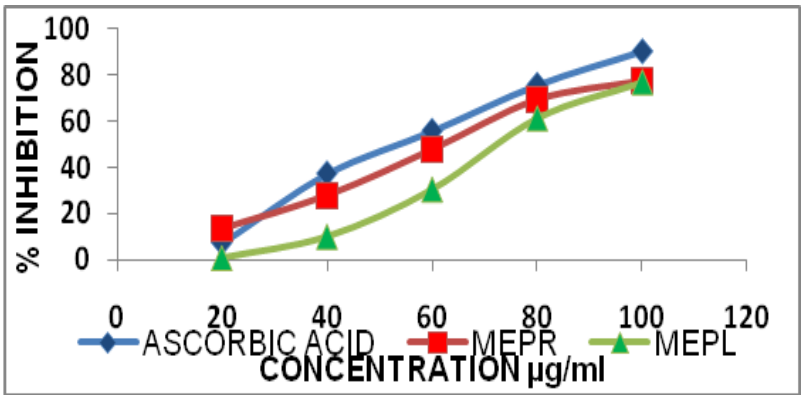

Fig. 1: DPPH radical scavenging Activity

Nitric oxide scavenging assay

MEPR and MEPL effectively reduced the generation of nitric oxide from sodium nitroprusside (Fig. 2). However, MEPR (IC_{50}=44.14 µg/ml) has shown a higher nitric oxide radical scavenging activity than MEPL (IC_{50}=55.85 µg/ml), but less than the standard, ascorbic acid (IC_{50}=40.17µg/ml) (Table 2). This may be due to the antioxidant potential of the active principle of the extracts which compete with oxygen to react with NO− thereby inhibiting the generation of nitrite.

Table 2: Effect of MEPR & MEPL on Nitric oxide radicals

Concentration (µg/ml)	Percentage Scavenging activity		
	Ascorbic acid	MEPR	MEPL
20	32.30 ± 4.55	23.91 ± 2.08	20.81± 2.45
40	50.43 ± 5.25	43.48 ± 3.45	30.43 ± 3.21
60	68.79 ± 5.55	67.11 ± 3.76	57.14 ± 3.87
80	75.96 ± 5.76	72.85 ± 4.65	61.80 ± 4.21
100	83.85 ± 6.32	80.59 ± 5.34	73.60 ± 4.54
IC_{50} (µg/ml)	40.17 ± 4.5	44.14 ± 4.32	55.85 ±4.85

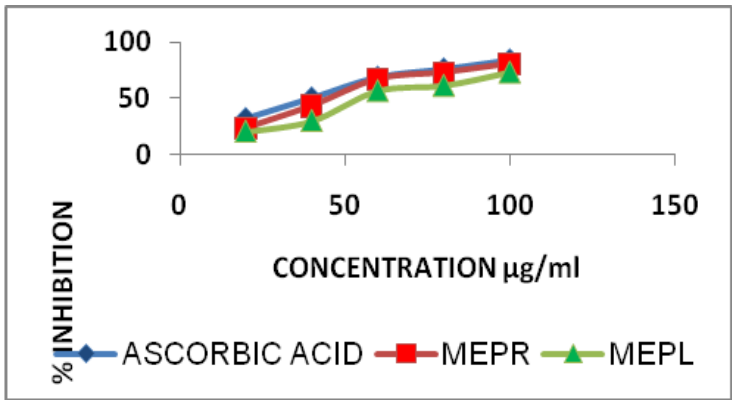

Fig. 2: Nitric oxide scavenging activity

Hydrogen peroxide scavenging assay

MEPR and MEPL (dose of 20, 40, 60, 80 & 100µg/ml) significantly scavenged hydrogen peroxide in a concentration dependent manner (Fig. 3). MEPR extract showed higher hydrogen peroxide scavenging than MEPL. The IC_{50} values for MEPR and MEPL were found to be 49.26 µg/ml and 52.7 µg/ml, respectively while IC_{50} value for that of Ascorbic acid was 43.6 µg/ml as shown in Table 3.

Table 3: Effect of MEPR & MEPL on Hydrogen peroxide

Concentration (µg/ml)	Percentage Scavenging activity		
	Ascorbic acid	MEPR	MEPL
0	0	0	0
20	32.33±1.52	29.33±1.53	25.34±2.51
40	48.35±2.08	39.33±1.15	35.66±1.53
60	69.66±3.05	64.66±0.57	59.33±2.51
80	72.66±2.08	69.33±0.57	65.66±1.52
100	77.66±1.63	74.33±0.57	69.33±1.15
IC_{50} (µg/ml)	43.6±2.19	49.26±0.55	52.7±2.05

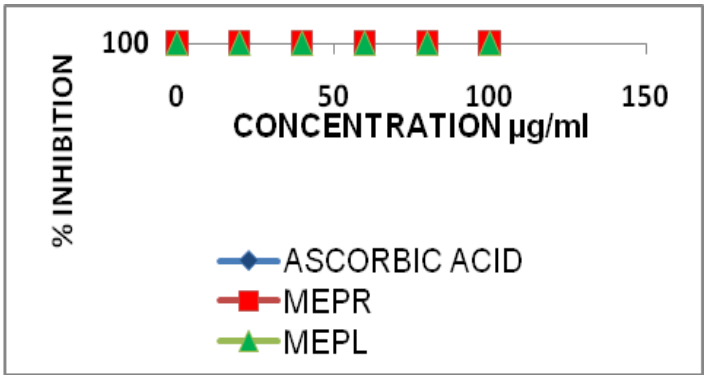

Fig. 3: Hydrogen peroxide scavenging activity

Reducing ability

In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of antioxidant samples. MEPR and MEPL exhibited effective reducing power when compared to the standard (Ascorbic acid) by the potassium ferricyanide reduction method (Table 4). The reducing power increased as the MEPL concentration increased, indicating some compounds in MEPL are electron donors and could react with free radicals to convert them into more stable products and to terminate radical chain reactions when compared with MEPR (Fig. 4).

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Table 4: Reductive ability of MEPR & MEPL

Concentration (µg/ml)	% Inhibition		
	Ascorbic acid (standard)	MEPR	MEPL
0	0	0	0
20	0.146 ± 0.025	0.096 ± 0.005	0.110 ± 0.020
40	0.243 ± 0.015	0.170 ± 0.02	0.203 ± 0.0114
60	0.323 ± 0.011	0.246 ± 0.025	0.273 ± 0.0152
80	0.403 ± 0.015	0.370 ± 0.02	0.410 ± 0.020
100	0.45 ± 0.020	0.366 ± 0.015	0.413 ± 0.0208

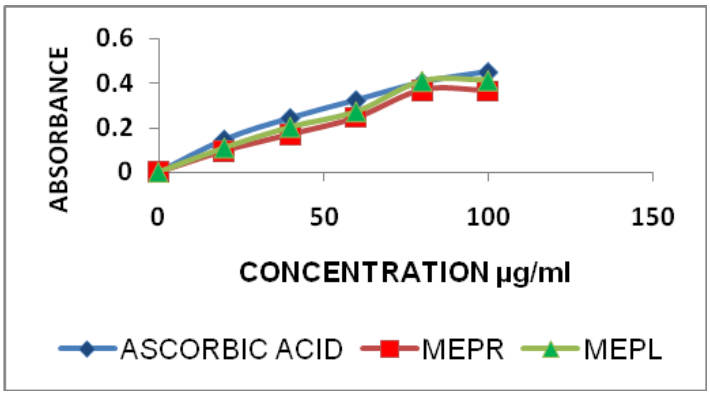

Fig. 4: Reductive ability of MEPR & MEPL of *Plumeria rubra* flowers

Total phenolic and flavonoid content

Total phenol compounds, as determined by Folin Ciocalteu method, are reported as Gallic acid equivalents i.e., the results expressed in terms of µg GAE/g dry sample with reference to standard curve (y = 0.0058x+0.002, r2 = 0.985) (Fig. 5). The total flavonoid contents are reported as mg quercetin equivalent/g of extract powder, with reference to standard curve (y = 0.058x + 0.001, r2 = 0.994) (Fig. 6). It was observed that MEPR exhibited significantly higher total phenol and flavonoid contents than MEPL (Table 5). The high amount of phenols and flavonoids in extracts may explain for their high antioxidant potential.

Table 5: Determination of total phenolic and flavonoid content (conc vs abs)

Concentration (µg/ml)	Total phenolic Absorbance(gallic acid)	Flavonoid Absorbance(quercetin)
20	0.015	0.129
40	0.029	0.243
60	0.049	0.324
80	0.057	0.489
100	0.071	0.592
1mg MEPR	0.027	0.316
1mg MEPL	0.023	0.193

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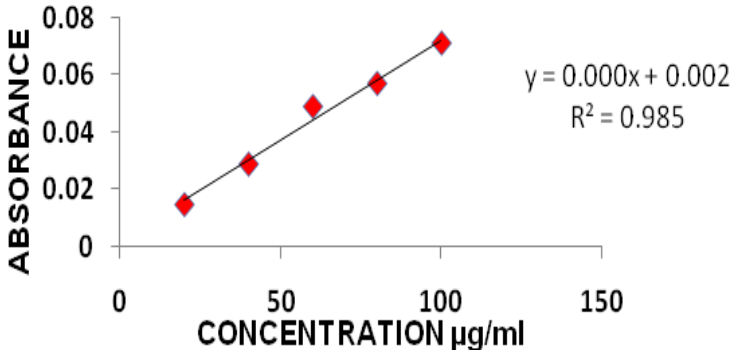

Fig. 5: Standard Graph of Gallic acid.

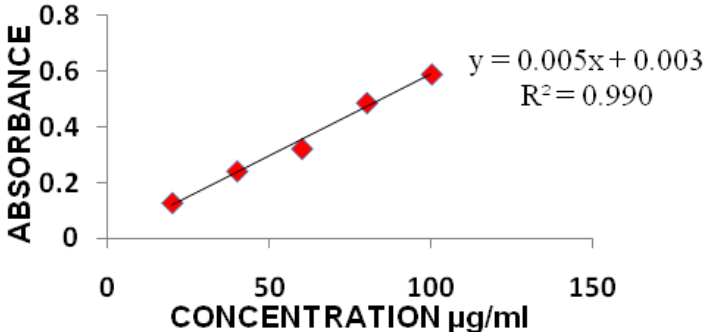

Fig. 6: Standard Graph of Quercetin

Acute toxicity study

No toxicity signs and mortality were observed in animals at 2000mg/kg body weight up to 14 days, which reveals the safety of MEPR and MEPL upto this dose.

Carrageenan induced rat paw oedema

MEPR and MEPL at the doses of 250, 500 mg/kg exhibited significant ($p<0.05$-0.001) percentage inhibition of paw oedema at 4^{th} hour after carrageenan injection ranging from 24.29%, 9.00%, to 30.00%, 15.1% compared to control group (Fig.7). MEPR at 500 mg/ kg dose showed maximum percentage inhibition of maximal paw oedema (30.00%) in comparison with that of the standard, Diclofenac at 50 mg/kg (31.5%) (Table 6 & Table 7).In the present study it was observed that both MEPR and MEPL showed concentration dependent free radical scavenging activity and this antioxidant effect may be responsible for their in-vivo anti-inflammatory activity.

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Table 6: Anti inflammatory activity of MEPR and MEPL by carrageenan induced paw edema in rats

Group	Mean paw volume in ml			
	1h	2 h	3 h	4 h
Control	0.25±0.05	0.35±0.05	0.38±0.04	0.33±0.05
Standard	0.13±0.05^{c}	0.21±0.05^{c}	0.25±0.08^{c}	0.21±0.07^{c}
MEPR 250mg/kg	0.23±0.05^{a}	0.30±0.06^{a}	0.31±0.04^{b}	0.25±0.05^{a}
MEPR 500mg/kg	0.18±0.07^{b}	0.23±0.08^{c}	0.28±0.63^{c}	0.23±0.05^{b}
MEPL 250mg/kg	0.21±0.04	0.31±0.04	0.36±0.40	0.30±0.05
MEPL 500mg/kg	0.21±0.06^{a}	0.30±0.06^{a}	0.32±0.83^{b}	0.28±0.06^{b}

All values were expressed as Mean ± S.D., $^{a}p<0.05$, $^{b}p<0.01$ and $^{c}p<0.001$ in response to controls

Table 7: Percentage inhibition of carrageenan induced paw edema in rats by *Plumeria rubra*

Group	% Inhibition of paw edema			
	1h	2 h	3 h	4 h
Standard	48^{c}	40^{c}	34.2^{c}	31.5^{c}
MEPR 250mg/kg	34.2^{a}	14.2^{a}	18.4^{b}	24.2^{a}
MEPR500mg/kg	28^{b}	34.2^{c}	26.3^{c}	30
MEPL 250mg/kg	16	11.4	5.2	9^{b}
MEPL 500mg/kg	16^{a}	14.2^{a}	15.7^{b}	15.1^{b}

All values were expressed as Mean ± S.D., $^{a}p<0.05$, $^{b}p<0.01$ and $^{c}p<0.001$ in response to controls

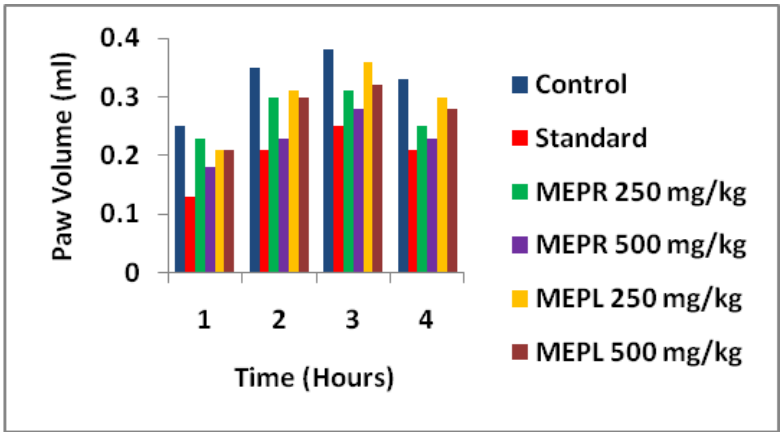

Fig. 7: Anti inflammatory activity of *Plumeria rubra* by carrrageenan induced paw edema in rats.

CONCLUSION

In conclusion, the present study has demonstrated that both MEPR and MEPL have a strong antioxidant activity and a radical-scavenging action in all of the tested methods. This could be well attributed to the phenolic and flavonoid content in the extracts which can scavenge the free radicals. This suggests that *Plumeria rubra* f. *rubra* and *Plumeria rubra* f. *lutea* flowers are a good source of natural antioxidants. In addition, the flower extracts of both the

cultivars of *P. rubra* also exhibited significant anti-inflammatory activity. The present investigations have demonstrated a strong correlation between the anti-inflammatory and antioxidant activities of *P.rubra* flowers. The prevention of oxidative damage to tissue could therefore be one of the mechanisms responsible for the anti-inflammatory effect shown by both the cultivars of this plant. Confirmation of the anti-inflammatory activity in animal model further justifies the traditional use of this plant for inflammatory disorders. The results strongly suggest that radical quenching may be one of the mechanisms responsible for its anti-inflammatory activity. The ethno medical use of *P.rubra* as a useful remedy in inflammatory and arthritic disorders could possibly be because of its excellent anti-inflammatory and antioxidant potential. The isolation, purification and mechanism of action of phenolic and flavonoidal components and other components of *Plumeria rubra* flowers are of interest for further investigation and shall be carried out in future studies.

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