Research Article

Anti-oxidant and Anti-inflammatory activity of Plumeria rubra (Flowers)

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ABSTRACT

Medicinal plants cure many diseases associated with inflammation like cardiovascular diseases, diabetes, cancer and rheumatoid arthritis. In recent years, natural compounds such as phenolic acids, flavonoids, terpenoids and alkaloids present in various plants, act as potential anti diabetic and anti-inflammatory agents. The main difficulties in using natural products as a source for pharmaceutical lead compounds involve separating the compounds from the crude extracts. Search for anti-inflammatory and anti-oxidant plant drugs are still intensifying. Plumeria rubra (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 and anti-inflammatory activities of the ethanolic extracts of Plumeria rubra flowers. Fresh flowers of P.rubra were extracted with ethanol and evaluated for antioxidant activities by DPPH, ABTS assay, anti inflammatory activities by human blood cell (HRBC) membrane stabilization method and Inhibition of albumin denaturation method.

Keywords: Antioxidant, Anti-inflammatory, Total antioxidant activity etc

INTRODUCTION

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 supplements. Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infections, burns, 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]. Antioxidants have gained importance in the current scenario for their ability to trap the free radicals produced during degenerative diseases[5].

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]. Plumeria rubra one of the species of the genus Plumeria, 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 like inflammation, rheumatism, ulcers, leprosy, asthma, diabetes, constipation, fever etc[9].

This world gifted with medicinal plants for various diseases and these are found to be grown in different climatic conditions.

Many rural areas depend on medicinal plants for their drug source as well as livelihood. The chemical compound present in all medicinal plants confesses their therapeutic potency.

At present there are so many artificial anti-inflammatory drugs were available in market with some side effects. So it is essential to search for anti-inflammatory drugs from natural source and with less side effects.

Recent research suggests that inflammation is the main gateway for many diseases like diabetes rheumatoid arthritis, cardiovascular diseases and cancer etc.

MATERIALS AND METHODS

Collection of Plant materials

Fresh flowers of Plumeria rubra were collected from Roever Engineering College garden, Perambalur district, TamilNadu, India, during the month of August and Identified by Head, PG & Research Department of Botany, Periyar E.V.R. College, Trichy, Tamil Nadu.

Flower Extraction

2 kg of fresh flowers were soaked with 90% ethanol at room temperature (25 ^{o}C - 30 ^{o}C). After 72 hrs the ethanolic extract was filtered. This extract was concentrated in vacuum and the dry powder obtained was dissolved in DMSO to get required concentrations and were used for screening anti oxidant and anti-inflammatory activities.

In Vitro Antioxidant Activity

DPPH Assay Method

The DPPH free radical is reduced to a corresponding hydrazine when it reacts with hydrogen donors. The DPPH radical is purple in colour and upon reaction with hydrogen donor changes to yellow colour. It is a decoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in ethanol or methanol and the decrease in absorbance was measured at 490nm.

Reagents

A. 2,2-Diphenyl 1-picryl hydrazyl solution (DPPH, 100 μM)

22 mg of DPPH was accurately weighed and dissolved in 100ml of methanol. From this stock solution, 18ml was taken and diluted to 100ml using methanol to obtain 100μM DPPH solution.

B. Preparation of test solutions

21 mg of the solid obtained from ethanolic extract was dissolved in distilled DMSO to obtain a solution of 21mg/ml concentration. This solution was serially diluted to obtain lower concentrations.

C. Preparation of standard solutions

10 mg each of ascorbic acid and rutin were weighed separately and dissolved in 1ml of Dimethyl sulfoxide (DMSO) to get 10mg/ml concentrations. These solutions were serially diluted with DMSO to get lower concentrations.

D. Procedure

The assay was carried out in a 96 well microtitre plate. To 200μl of DPPH solution, 10μl of each of the test sample or the standard solution was added separately in wells of the microtitre plate.

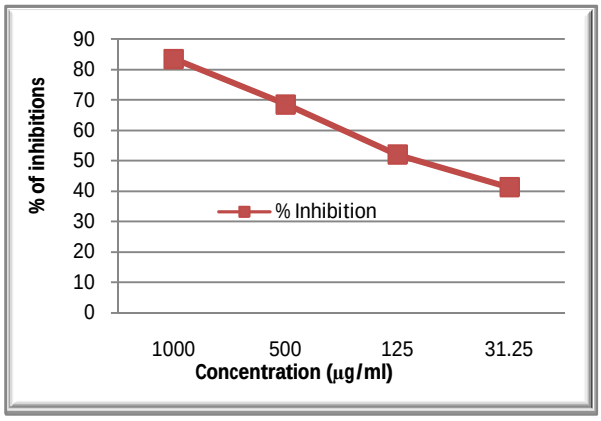
The final concentration of the test and standard solutions used were 1000, 500, 125 and 31.25 μg/ml. The plates were incubated at 37° C for 30 min and the absorbance of each solution was measured at 490 nm, using a microplate reader.

Table 1: DPPH assay Activity of ethanolic extract of flowers of Plumeria rubra.

S.No	Concentrations (μg/ml)	% Inhibition	IC_{50}
1	1000	87.60	94.25 μg/ml
2	500	76.03	
3	125	54.55	
4	31.25	38.02	

Evaluation of total Antioxidant capacity of the Extract

The total antioxidant capacity was determined by phosphormolybdenum method and is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of a green Mo (V) complex which has the maximal absorption at 695nm.



Graph 1: Graphical representation of DPPH activity of ethanolic extract of flowers of Plumeria rubra.

Preparation of Test and Standard Solutions

Weighed accurately 55mg of the sample and the standard, ascorbic acid and dissolved in 5ml of DMSO. The lower dilutions were made serially with DMSO.

Procedure

An aliquot of 0.1ml of the sample solution containing a reducing species in DMSO was combined in an Eppendorff tube with 1ml of reagent solution (0.6mM Sulphuric acid, 28mM sodium phosphate, and 4mM ammonium molybdate).

The tubes were capped and incubated in water bath at 95 °C for 90min. The samples were cooled to room temperature, and the absorbance of each solution was measured at 695nm. The total antioxidant capacity was expressed as mM equivalent of ascorbic acid (Mojca).

Total Antioxidant Activity

252.6μg/ml

ABTS Radical Scavenging Activity

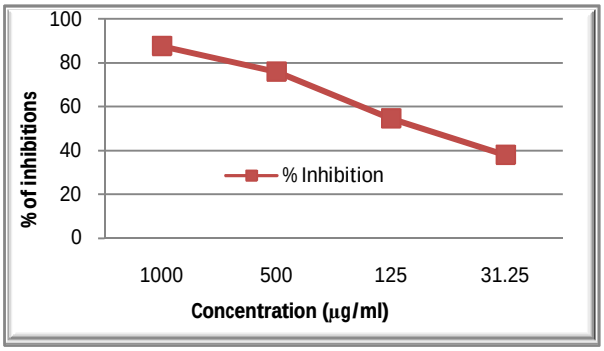
ABTS radical scavenging activity was performed as described by Re with a slight modification. 7.0 mM ABTS in 14.7 mM ammonium peroxo-disulphate was prepared in 5.0 ml distilled water.

The mixture was allowed to stand at room temperature for 24 hours. The resulting blue green ABTS radical solution was further diluted such that its absorbance is 0.70 ± 0.020 at 734 nm. Various concentrations of the sample solution dissolved in ethanol (20.0 μl) were added to 980.0 μl of ABTS radical solution and the mixture was incubated in darkness for 10 min.

The decrease in absorbance was read at 734 nm. A test tube containing 20.0 μl of ethanol and processed as described above served as the control tube. Different concentrations of ascorbic acid were used as reference compound.

Table 2: ABTS assay activity of ethanolic extract of flowers of Plumeria rubra.

S. No	Concentrations (µg/ml)	% Inhibition	IC_{50}
1	1000	83.47	97.22 µg/ml
2	500	68.60	
3	125	52.07	
4	31.25	41.32	



Graph 2: Graphical representation of ABTS radical scavenging activity of ethanolic extract of Plumeria rubra.

Anti-Inflammatory Activity

The human Red Blood Cell (HRBC) Membrane Stabilization Method

The method as prescribed (Gopalkrishnan & Sakat) was adopted with some modifications. The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.5 % citric acid and 0.42 % NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10 % suspension was made. Various concentrations of extracts were prepared in mg/ml using distilled water and to each concentration, 1 ml of phosphate buffer, 2 ml hypo saline and 0.5 ml of HRBC suspension were added. It was incubated at 37 ˚C for 30 minutes and centrifuged at 3,000 rpm for 20 minutes and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (100 Jg/ml) was used as reference standard and a control was prepared by omitting the extracts. The experiments were performed in triplicates and mean values of the three were considered. The percentage (%) of HRBC membrane stabilization or protection calculated using the following formula:

$$\text{Percentage of Protection (\%)} = \frac{(100 - \text{OD of drug treated sample})}{\text{OD of Control}} \times 100$$

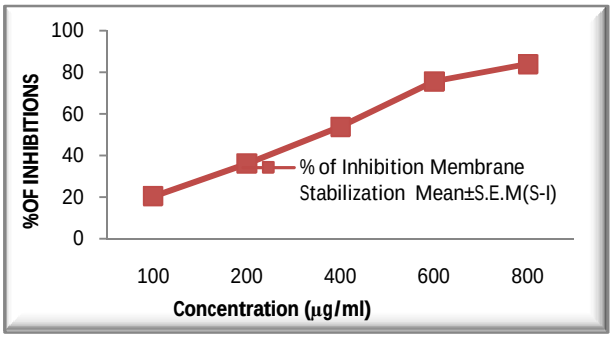
Albumin Denaturation Method

The method as prescribed (Sakat) was followed with modifications. The reaction mixture was consisting of test extracts and 1% solution of bovine albumin fraction pH of the reaction mixture was adjusted using small amount of HCl. The sample extracts were incubated at 37 °C for 20 minutes and then heated to 51 °C for 20 minutes. After cooling the samples the turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was taken as a standard drug. The experiment was performed in triplicates and the mean value of the three was considered. Percent inhibition of protein denaturation was calculated as follows:

$$\text{Percentage of Inhibition (\%)} = \frac{(\text{OD of Control} - \text{OD of Sample})}{\text{OD of Control}} \times 100$$

Table 3: The human red blood cell (HRBC) membrane Stabilization activity of ethanolic extract of flowers of Plumeria rubra

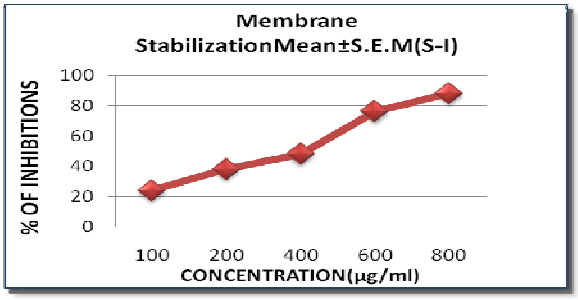
S. No	Concentration (µg/ml)	% of Inhibition
		Membrane Stabilization Mean ± S.E.M. (S-I)
1	100	20.43 ± 0.83
2	200	36.19 ± 1.48
3	400	53.82 ± 1.36
4	600	75.64 ± 1.74
5	800	84.02 ± 1.08



Graph 3: Graphical representation of human red blood cell (HRBC) membrane Stabilization activity of ethanolic extract of flowers of Plumeria rubra.

Table 4: The Inhibition of Albumin Denaturation activity of ethanolic extract of flowers of Plumeria rubra.

z	Concentration (µg/ml)	% of Inhibition
		Membrane Stabilization Mean ± S.E.M (S-I)
1	100	24.15 ± 0.12
2	200	38.59 ± 1.42
3	400	48.16 ± 0.34
4	600	76.52 ± 1.46
5	800	88.12 ± 1.89



Graph 4: Graphical representation of Inhibition of Albumin Denaturation activity of ethanolic extract of flowers of Plumeria rubra.

RESULTS AND DISCUSSION

Isolation of pure, pharmacologically active constituents from plants remains a lengthy and tiresome process. For this reason, it is crucial to have methods available which eradicate unnecessary separation procedures. Chemical screening is thus performed to allow localization and targeted isolation of new or useful constituents with potential activities. Phytochemical analysis of the ethanolic extracts of P. rubra flowers revealed the presence of tannins, alkaloids, flavonoids, saponins, gums and terpenoids. Strong occurrence of tannins in extract has been shown to possess potent anti-inflammatory properties. Analgesic and anti inflammatory effects have been observed in flavonoids.

The results strongly suggest anti-inflammatory effect and anti oxidant effects by percentage of inhibitions which are explained in the Table 1, 2, 3, 4 and also by graphical representation 1, 2, 3 and 4.

CONCLUSION

In conclusion, the present study has demonstrated that both DPPH assay and ABTS have showed a strong antioxidant activity and also the human red blood cell (HRBC) membrane stabilization.

Inhibition of albumin denaturation indicated the 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.

The medical use of Plumeria rubra as a useful remedy in 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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