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Comparitive *in vitro*, *in vivo* anti-arthritic and anti-inflammatory activities of *Plumeria pudica* Jacq., Enum. Syst. Pl. 13 1760 and *Plumeria rubra* L. Sp. Pl. 209 1753 in albino rats

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ABSTRACT

The present work aimed to estimate the possible anti-arthritic and anti-inflammatory effects of ethanolic flower extracts of *Plumeria pudica* and *Plumeria rubra*. *In vitro* anti-arthritic activities of flower extracts were investigated through protein denaturation and egg albumin methods. The anti-inflammatory activity of flower extracts (150 and 300 mg/kg) was elucidated by carrageenan-induced paw edema and formaldehyde-induced arthritis, in rats. Results indicated that significant anti-arthritic activity and anti-inflammatory were observed by ethanolic flower extract of *P. pudica* compared to ethanolic flower extract of *P. rubra* and the results are comparable with that of standard Diclofenac sodium. The results provided an important basis for developing the flower extracts as a novel anti-inflammatory agent or substitute and for treating arthritis.

Keywords: Anti-inflammatory, anti-arthritic, bovine serum albumin, carrageenan, formaldehyde, *Plumeria pudica*, *Plumeria rubra*.

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INTRODUCTION

Plumeria pudica Jacq. and *Plumeria rubra* L. belongs to Apocynaceae (Oleander family). *Plumeria pudica* is a small tree with fiddle-shaped leaves native to Panama, Colombia and Venezuela. Common names include Bridal bouquet, white frangipani, fiddle leaf plumeria, wild plumeria, Thai champa in Urdu and Naag champa in Bengali. Whereas *P. rubra* is a deciduous plant species native to Mexico, Colombia and Venezuela. Common names include Frangipani and Temple tree, Deva ganneru in Telugu, Golenchi/Golachin in Hindi, and Nela sampangi in Tamil. These two plants are attributed with medicinal properties for the treatment of purgative, remedy for diarrhea, cure of itch, inflammation, bronchitis, cough and asthma, and syphilis; its latex is used to treat ulcers and darter (skin diseases).

Inflammation is the immune system's reaction to harmful stimuli including bacteria, damaged cells, poisonous chemicals, or irradiation (Chen et al., 2018) and it works by eliminating injurious stimuli and initiating the healing process (Hannoodee and Nasuruddin, 2020). As a result, inflammation is a necessary protection mechanism for good health. Typically, cellular and molecular events and interactions effectively mitigate imminent injury or infection during acute inflammatory responses (Choudhary et al., 2014). The restoration of tissue homeostasis and the resolution of acute inflammation are both aided by this mitigation procedure. Uncontrolled acute inflammation, on the other hand, may become chronic, leading to several chronic inflammatory diseases (Zhou et al., 2016).

Arthritis is characterised as an acute or chronic joint inflammation that often coexists with pain and structural damage. It comes from the Greek term for "joint illness" (Ma et al., 2009). Rheumatoid arthritis (RA) is a symmetrical, chronic inflammatory autoimmune condition that affects small joints first, then larger joints, the skin, eyes, heart, kidneys, and lungs. Joint bone and cartilage are often damaged, and tendons and ligaments become

weak (Lee et al., 2017).

Plumeria is a perineal flowering plant that is worn as a decorative plant. It is a Mexican and American native. India is commonly used in places of worship and burial grounds (Rastogi, and Mehrotra, 1990). The pungent, bitter, laxative, and heating root bark. It is a carminative that is used to treat leprosy (Sharma and Kumar, 2012; Vijayalakshmi et al., 2014).

So far much pharmacological work has not been reported on the Anti-arthritic and anti-inflammatory activities on the flower extracts of *P. pudica* and *P. rubra*. Therefore, the present study aims to investigate the anti-arthritic and anti-inflammatory activities of ethanolic flower extracts of *P. pudica* and *P. rubra*.

MATERIALS AND METHODS

Plant collection

Plumeria pudica and *Plumeria rubra* flowers were collected in Mamidada, East Godavari District, Andhra Pradesh. These plants have been checked by Dr. T. Raghuram, Taxonomist at Maharani College in Pedapuram.

Preparation of extract

P. pudica and *P. rubra* flowers were collected and dried in the shade for about 3 weeks and mixed into powder. A powder of 500 g of each species was macerated for 3 days in 2 L of ethanol. Then the material was filtered and the filtrate was gathered and produced using the distillation process. In a china dish, the concentrated extract was kept in and stored for 10 days in vacuum desiccators to dry. The evaporation procedure is done by using a rotary evaporator at a temperature of 20°C with a pressure of 64 mbar.

Chemicals and instruments used

BSA (Bovine serum albumin), Diclofenac sodium, 1N HCl, egg, carrageenan, indomethacin, formaldehyde, sodium dihydrogen phosphate, Plethysmometer, UV spectroscopy.

Preliminary phytochemical screening

To assess the existence of different secondary metabolites such as phenols, tannins, alkaloids, flavonoids, glycosides, saponins, proteins, steroids and triterpenoids. (Hamid and Aiyelaagbe, 2011).

Quantitative phytochemical testing

10 mg of individual extracts is dissolved in 10 mL of methanol to make 1000 g/ml aliquots of extract.

Phenolic contents estimation

Using the Folin-Ciocalteu method (Stratil et al., 2006), the phenolic content of ethanolic flower extract of *P. pudica* and *P. rubra* (1 mg/ml, aliquots) was determined. 0.5 ml extract was combined with 3 ml Folin-Ciocalteu reagent (1:10 v/v) and allowed to stand for 5 min. In the mixture tube, 4 ml of sodium carbonate solution (20% w/v) was applied. For color growth, the tubes were held at 30°C for 15 min. A spectrophotometer was used to calculate the absorbance at 765 nm. The Gallic acid equivalent mg/100 mg dry weight of extract was measured using the calibration curve and the results were expressed as Gallic acid equivalent mg/100mg dry weight of the extract.

Flavanoids content Estimation:

The total flavonoid content of *P. pudica* and *P. rubra* ethanolic flower extract (1 mg/ml, aliquots) was determined using the aluminum chloride method (Chang et al., 2002). 0.6 ml extract, 1.8 ml methanol, 0.1 ml 10 percent aluminum chloride, 0.1 ml 1 M sodium acetate, and 3 ml distilled water were added to 0.6 ml extract, 1.8 ml methanol, 0.1 ml 10 percent aluminum chloride, 0.1 ml 1M sodium acetate, and 3 ml distilled water were added and left at 30°C. After 30 min at 415 nm, the absorbance was assessed individually. Total flavonoid was calculated using standard quercetin in methanol and expressed as quercetin equivalent mg/100 mg dry weight of the extract.

Alkaloid content estimation

The alkaloid content of the extract was calculated using the Fazel et al. process, in which an ethanolic flower extract of *P. pudica* and *P. rubra* (1 mg/ml, aliquots) was dissolved in 2N Hydrochloric acid and filtered. 0.1 N NaoH was applied to the filtrate, 1 ml was transferred to a separating funnel, and 5 ml of bromo cresol green solution and 5 ml of phosphate buffer were added. Chloroform was used to remove the mixture after shaking it. At 470 nm, the absorbance was measured. The concentration of alkaloid content in atropine equivalents was determined using the unit's mg/100mg dry weight of extract (Shamsa et al., 2008), and the alkaloid content was calculated from the calibration curve using a normal atropine calibration curve.

In vitro anti-arthritic and anti-inflammatory tests

Method for protein denaturation

Protein denaturation is the main documented cause of inflammation. Drugs such as phenylbutazone, salicylic acid, flufenamic acid, etc. For inflammation that demonstrated dose-dependent potential for thermally mediated denaturation, acid, etc. was used. For the production of new medicines, any new agents/drugs that prevent denaturation will be worthwhile (Sangeetha et al., 2013).

Procedure

Test solution (0.5 ML): Consist of 0.45 ml (5% w/v aqueous solution) of bovine serum albumin and 0.05 ml (50 mg/ml, 100 mg/ml, 300 mg/ml and 500 mg/ml) of test samples of varying concentrations.

Test control solution (0.5 ML): Consists of 0.45ml (5% w/v aqueous solution and 0.05 ml distilled water of bovine serum albumin.

Product monitoring solution (0.5ML): Consist of 0.45ml of distilled water and 0.05ml of various concentrations of test samples (50 mg/ml, 100 mg/ml, 300 mg/ml, and 500 mg/ml).

Standard (0.5 ML) solution: It consists of 0.45 ml of bovine serum

albumin (5% w/v aqueous solution) and 0.05 ml of sodium diclofenac at various concentrations (50 mg/ml, 100 mg/ml, 300 mg/ml and 500 mg/ml). All of the above solutions using 1N HCL acid have been modified to pH 6.3. The samples are incubated for 20 min at 37°C, and the temperature was raised to hold the samples for 3 min at 57°C. After cooling 2.5 ml of phosphate buffer to the above solution. The absorbance was measured using the UV Visible spectrophotometer at 255 nm.

The percentage of protein denaturation inhibition was determined as follows:

% Inhibition of protein denaturation= 100-[{O.D of test solution- O.D of product control)/O.D of test control} × 100]

The control represents 100% protein denaturation. The results were compared with Diclofenac sodium.

Egg albumin denaturation method

The yolk and the white part are removed from the fresh egg. From above, 2.8 ml pH 6.4 PBS and 2ml of varying concentrations (50,100,300,500mg/ml) were taken and combined with 0.2 ml of albumin, which constitutes around 5ml of the reaction mixture. The following incubations were incubated in BOD for 15 min at 37 ± 2°C and increased to 70°C and heated for 5 min. The UV visible spectrophotometer allows it to cool and its absorbance was measured using distilled water as pure as blank at 660nm. Diclofenac sodium standard solution (10 and 25 mg/ml) is used as a reference drug (Fernandes et al., 2017).

The inhibition is determined as follows:

$$\text{Percent inhibition} = \frac{Absorbance\ (control) - Absorbance\ (treated)}{Absorbance\ (treated)} \times 100$$

Assessment of *in-vivo* anti-inflammatory activity

Carrageenan induced paw edema in rats

Grouping: Albino rats of 150 to 250 g weight were divided into 4 groups of 2 rats each. Each has 2 animals housed in a labeled cage. A period of time was given to animals to adapt to the new environment provided with food and water ad libitum (Adeyemi et al., 2002).

Group 1: 0.1 ml of 1%w/v suspension of carrageenan was given to animals.
Group 2: Standard reference drug Diclofenac sodium has been administered to animals.
Group 3 and 4: Ethanolic extract of *P. pudica* (150 mg/kg and 300 mg/kg) was administered to animals
Group 5 and 6: *P. rubra* ethanolic extract (150 mg/kg and 300 mg/kg) was administered to animals.

Principle:
Inflammation is the result of infection, injury, irritation or foreign substance reactions. It is part of the host defense mechanism, by aging is also considered to be an inflammatory response when it becomes uncontrolled.

With the assistance of irritants, an inflammatory reaction is readily produced in animals in the form of paw edema. Carrageenan, formalin, bradykinin, histamine, 5- hydroxytryptamine, mustard or egg white substances. They develop acute paw edema with an injection in a few minutes when injected into the dorsum of the foot of rats. Carrageenan is a sulphated polysaccharide derived from seaweed (rhodophyceae) and causes inflammation and edema by inducing the release of histamine, 5HT, bradykinin and prostaglandins.

Requirements:
Animals: Albino rats (150-250 g)
Drugs: Diclofenac sodium (10mg/kg I.P, 0.1 ml of 1 %w/v suspension of carrageenan. Ethanolic extract of *Plumeria pudica* and *Plumeria rubra* dose (150mg/ml & 300 mg/kg).

Procedure:
1. Weigh the animals and number them (rats). The assay performed as described previously (Ayoola et al., 2009; Shaikh et al., 2016).
2. Carrageenan was used in this study to cause edema. The animals were pretreated with ethanolic extract (150 mg/kg and 300 mg/kg) with 2% acacia positive regulation suspended. Diclofenac sodium (10 mg/kg) I.P negative control group was obtained by animals with a comparable volume of 2% acacia. Subcutaneously on the sub plantar area of the left hind paw of the animals was injected after 30 min 0.1ml of 1% w/v suspension of carrageenan in distilled water. Paw sizes are assessed immediately before and 1 hr after the injection of carrageenan.
3. Calculate % edema inhibition at different times (0hr, 1hr, 2hr, 3hr and 4 hr) intervals.

Percentage inhibition was calculated using the following formula:

$$\text{Percentage inhibition} = \frac{\text{(Vt-Vo) control group- (Vt-Vo) treated}}{\text{(Vt-Vo) control group}} \times 100$$

Where
Vo = before edematogenic agent injection at 0 h
Vt = 1, 2, 3 and 4 h intervals after drug administration.

Formaldehyde induced arthritis in rats

Animals are randomly divided into 6 groups. Using a plethysmometer, the paw diameter of all rats was initially calculated.

Group 1: Obtained animals 0.1 ml of 2% formaldehyde-treated as disease regulated
Group 2 and 3: Animals were high and low dosages administered with *P. pudica* extract (150 and 300 mg/ml).
Group 4 and 5: Animals were treated with a high and low dosage of *P. rubra* extract (150 and 300 mg/ml).
Group 6: As a control, animals receive saline.

Animals were induced by injecting 0.1ml of 2% v/v formaldehyde sub-planarly into the left hind paw of animals after 30 min of oral administration of the crude extract (150 and 300 mg/ml) vehicle to animals, which is called day 1. On day 3, both animals were injected with 0.1ml of 2%v/v formaldehyde into the same hind paw. The animals were given high and low doses of both extracts every day for 10 days. Paw diameter was determined by using a plethysmometer on 0, 2, 4, 6, 8, 10 and 14^{th} day after administration. Values were noted and relevant graphs were plotted and comparative analysis was conducted on both extracts to determine which species extract had better results (Sostres et al., 2010).

Statistical analysis

Data were analyzed by Graph pad Insta version3.0 software and presented as mean ± SEM values. The statistical tests used were one-way analysis of variance (ANOVA) followed by Dunett's multiple comparison test.

RESULTS

Screening of preliminary phytochemical quantitative tests

According to the findings, the extract includes alkaloids, flavonoids, saponins, carbohydrates, proteins, and amino acids, which are the major phytochemical classes of biological activity. The quantitative phytochemical screening results were tabulated (Table 1 and 2).

One of the primary causes of RA due to autoantigens in the denaturation of the proteins. One of the benefits of anti-inflammatory activity is the avoidance of lysosomal membrane lysis. In the analysis, RBC membrane lysis induced by plant extract showed a protective effect against heat and hypotonicity. It can be therefore be inferred that the plant extract can also stabilize the RBC membrane-like lysosomal membrane and cause anti-inflammatory effects there. In some arthritic disorders, elevated levels of trypsin have been implicated. The plant extract has demonstrated substantial inhibition of proteinase activity.

When comparing the ethanolic flower extracts of both species from Table 3, *P. pudica* showed a stronger inhibitory effect compared to *P. rubra* or in vitro bovine serum albumin and egg albumin for the determination of anti-arthritic function. Samples of the using a UV spectrophotometer at 416 and 660 nm respectively, various concentration absorbance's were measured.

Table 1. Quantitative screening of phytochemicals of ethanolic flower extract of *Plumeria pudica* and *Plumeria rubra*.

Content (mg/g)	*Plumeria pudica*	*Plumeria rubra*
Alkaloid content	38.12 ± 0.39	32.02 ± 0.23
Flavanoid content	44.26 ± 0.43	43.15 ± 0.33
Phenolic content	11.06 ± 0.26	12.03 ± 0.22

All the values were expressed in mean ± SEM, n = 3.
± Represent the inclusive range of values that reading might have in medicine, it means "with or without".

Table 2. Comparative study of the flower extracts denaturation process for *in vitro* proteins.

Concentration (µg/ml)	Anti-arthritic activity (% inhibition)		
	Bovine serum albumin		Standard drug: Diclofenac sodium
	Plumeria pudica	*Plumeria rubra*	
10	-	-	74.7 ± 0.21
25	-	-	80.4 ± 0.12
50	58.23 ± 0.031	53.12 ± 0.22	-
100	68.52 ± 0.123	61.66 ± 0.29	-
300	84.09 ± 0.234	76.52 ± 0.36	-
500	92.68 ± 0.311*	85.25 ± 0.46*	-

All the values were expressed in mean ± SEM, n = 3.

Table 3. Comparative study of flower extract of egg albumin *in vitro* method.

Concentration (µg/ml)	Anti-arthritic activity (% inhibition)		
	Egg Albumin		Standard drug: Diclofenac sodium
	Plumeria pudica	*Plumeria rubra*	
10	-	-	74.7 ± 0.15
25	-	-	80.4 ± 0.16
50	50.32 ± 0.01	41.32 ± 0.22	-
100	64.32 ± 0.21	53.39 ± 0.01	-
300	82.12 ± 0.21	73.36 ± 0.12	-
500	89.74 ± 0.31*	81.35 ± 0.17*	-

All the values were expressed in mean ± SEM, n = 3. * $p < 0.001$ when compared with standard values.

Egg albumin method

Table 3, Figures 1 and 2.

In vivo anti-inflammatory study: Mediated carrageenan paw edema

Table 4, Figure 3.

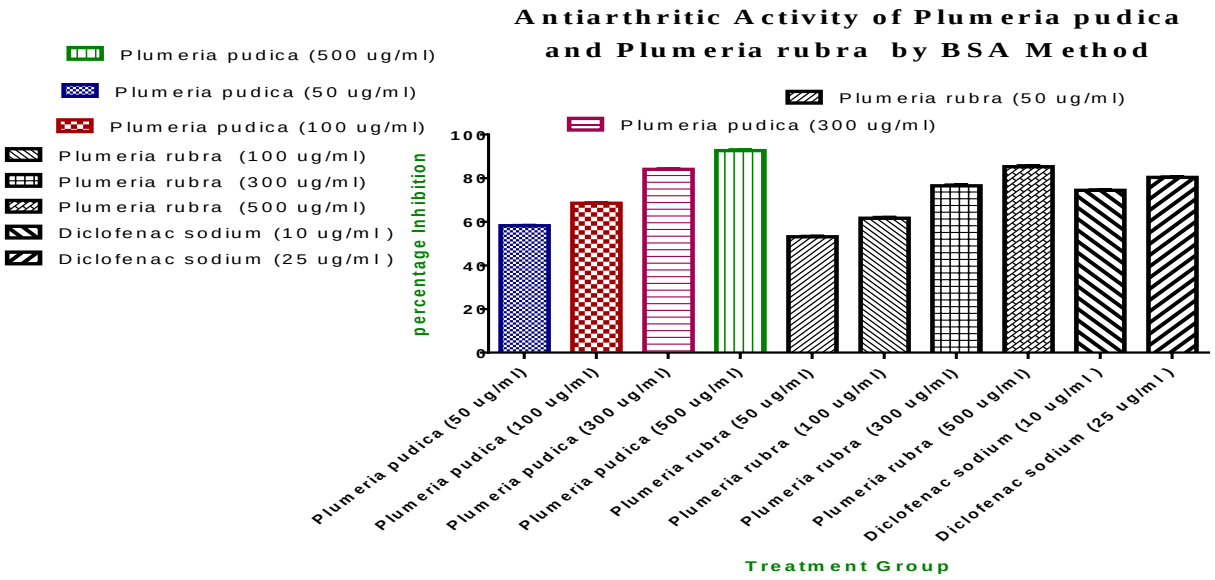

Figure 1. Antiarthritic activity of *Plumeria pudica* and *Plumeria rubra* by BSA method.

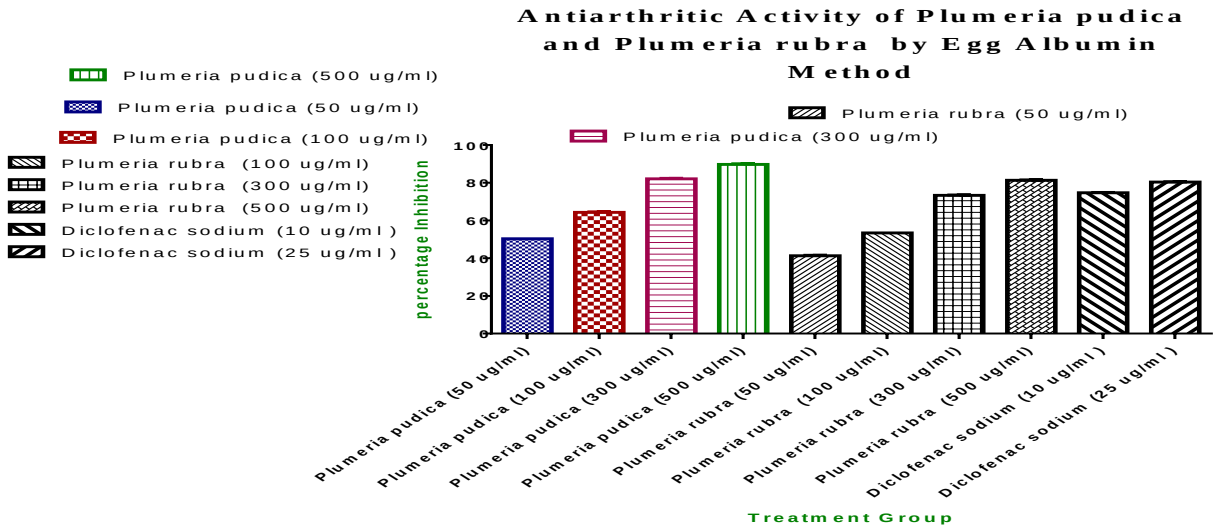

Figure 2. Antiarthritic activity of *Plumeria pudica* and *Plumeria rubra* by egg albumin method.

Table 4. Comparative analysis of the mean increase in paw diameter using the Carrageenan paw edema induced method between *P. pudica* ethanol flower extract and *P. rubra* ethanol flower extract.

Treatment	Mean increase in paw diameter (mm)				
	0 h	1st h	2nd h	3rd h	4th h
Control	0.34 ± 0.21	0.52 ± 0.44	0.91 ± 0.12	1.2 ± 0.07	0.86 ± 0.77
Indomethacin	0.26 ± 0.13	0.3 ± 0.01	0.46 ± 0.12	0.58 ± 0.01	0.9 ± 0.02
Extract of *Plumeria pudica* (150 mg/kg)	3 ± 0.12	0.3 ± 0.01	3.2 ± 0.13	2.9 ± 0.09	2.9 ± 0.08
Extract of *Plumeria pudica* (300 mg/kg)	2.9 ± 0.11	3.3 ± 0.08	3.1 ± 0.14	2.8 ± 0.08	2.8 ± 0.08*
Extract of *Plumeria rubra* (150 mg/kg)	2.5 ± 0.17	3.3 ± 0.08	2.9 ± 0.16	2.7 ± 0.19	2.6 ± 0.09
Extract of *Plumeria rubra* (300mg/kg)	2.8 ± 0.11	3.2 ± 0.14	2.8 ± 0.2	2.7 ± 0.2	2.8 ± 0.21*

N= 3 Units are expressed as mg/kg, analyzed with ANOVA are expressed as mean ±SEM following the Dunett test. All the values were expressed in mean ± SEM, n = 3. * $p < 0.001$ when compared with standard values.

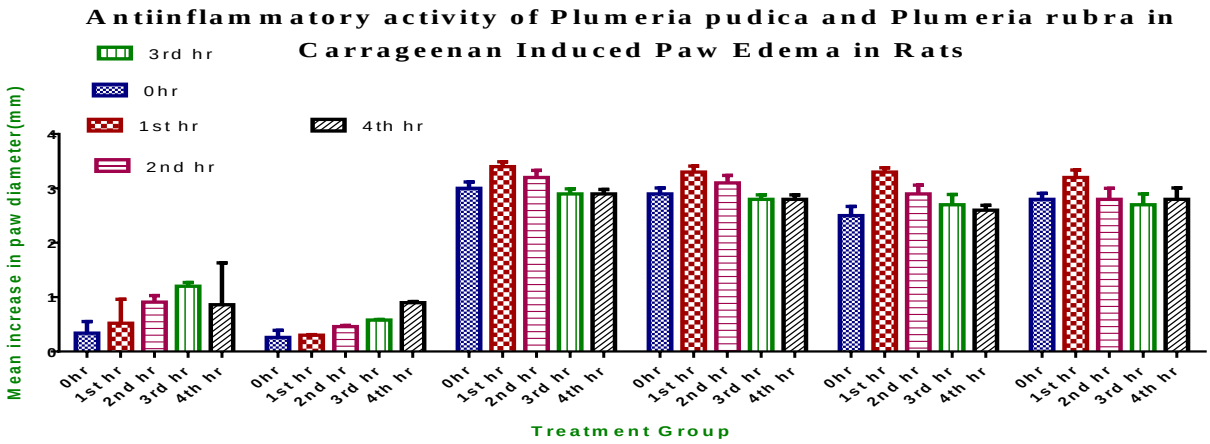

Figure 3. Antiarthritic activity of *Plumeria pudica* and *Plumeria rubra* in carrageenan induced paw edema in rats.

In-vivo anti-inflammatory activity: Induced arthritis of formaldehyde in rats

Table 5, Figure 4.

Table 5. Between ethanolic flower extracts of *Plumeria pudica* and *Plumeria rubra* comparative analysis of the mean increase in paw volume using Formaldehyde induced paw edema.

	1 h after HCHO administration	2nd day	4th day	6th day	8th day	10th day	14th day
Indomethacin	0.26±0.13	0.3±0.01	0.46±0.02	0.86±0.23	0.89±0.25	0.9±0.19	0.85±0.11
Before drug administration	2.4±0.06	2.2±0.07	20.21.4±	2.6±0.19	2.6±0.19	2.5±0.16	2.4±0.11
Extract of *Plumeria pudica* (150 mg/kg)	3.3±0.12	3.5±0.1	2.6±0.2	3±0.12	20.11.9±0.11	20.1.4±0.1	2.2±0.09
Extract of *Plumeria pudica* (300 mg/kg)	3.6±0.19	3.4±0.09	2.4±0.15	2.9±0.16	2.6±0.12	2.3±0.11	2.3±0.09
Extract of *Plumeria rubra* (150 mg/kg)	3.3±0.12	3.1±0.17	3.3±0.17	3.1±0.15	2.9±0.13	2.8±0.11	2.7±0.86*
Extract of *Plumeria rubra* (300 mg/kg)	3.2±0.12	3.1±0.12	3±0.1	2.9±0.09	2.7±0.12	2.6±0.16	2.5±0.14*

All the values were expressed in mean ± SEM, n = 3. * p < 0.001when compared with standard values.

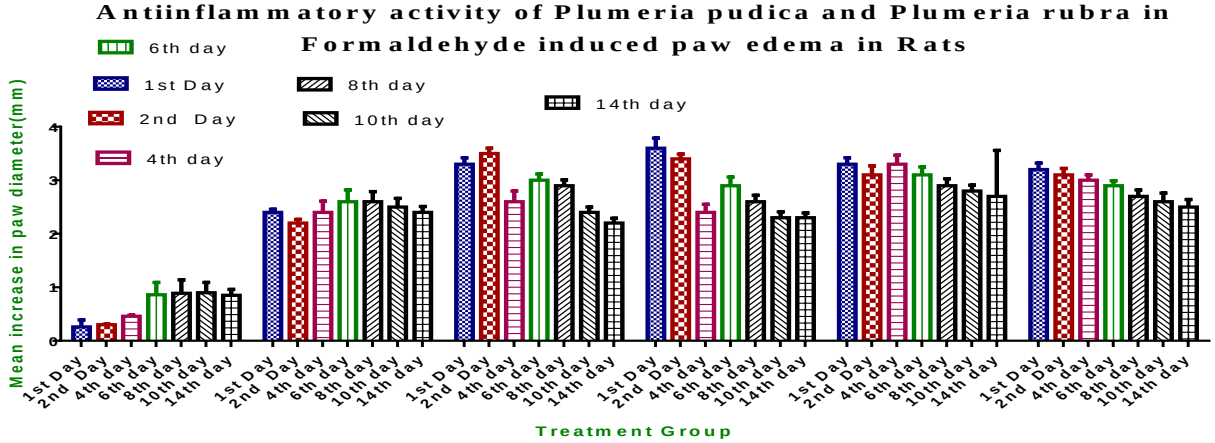

Figure 4. Antiarthritic activity of *Plumeria pudica* and *Plumeria rubra* in formaldehyde induced paw edema in rats.

DISCUSSION

P. pudica and *P. rubra* flowers have a wide range of medical uses to provide a scientific rationale for the plants we looked into the anti-arthritic and anti-inflammatory therapeutic properties of both organisms, performed chemical analyses, and compared them to the control group and standard drugs. Both species had flavonoids, saponins, triterpenoids, tannins, carbohydrates, glycosides, and proteins, according to the phytochemical study. All of the chemical constituents may play a role in the study reduction of arthritis and inflammation.

Proteins act as a fuel supply for the human body because they are an essential food. When proteins are exposed to heat, an organic solvent, a strong acid or base, or a concentrated inorganic salt, their tertiary and secondary structure, as well as their biological functions, are lost. In conditions such as rheumatoid arthritis, cancer, and diabetes, which are all inflammatory conditions, denaturation of protein induces the development of auto-antigens. As a consequence, inflammatory activity can be decreased by inhibiting protein denaturation (Wills, 1969). Protein denaturation is the source of inflammation. Plant extracts with anti-inflammatory activity were tested for their ability to prevent protein denaturation, which was used to investigate the mechanism of action for anti-inflammatory activity. They showed that bovine serum and egg albumin were effectively inhibited. When both species were given a 500 g/ml concentration, the maximum amount of inhibition was obtained (91 and 90% respectively), but when the extracts were compared, *P. pudica* had a higher percentage of inhibition than *P. rubra*.

A preclinical study in inflammatory arthritis employs a variety of animal models (Veras et al., 2015; Mehta et al., 2012). The rat models of formaldehyde-mediated paw edema and carrageenan-induced arthritis are well-established and have been widely used in preclinical research to evaluate the anti-arthritic and anti-inflammatory potential of various agents (Costa et al., 2004). The current research looked at the anti-arthritic properties of ethanolic flower extract of *P. pudica* and *P. rubra*. Previous research has shown that the standard medication diclofenac sodium prevented adjuvant-induced arthritis. The non-steroidal anti-inflammatory drug diclofenac was used as a reference since it is widely prescribed for arthritis treatment and works primarily by inhibiting cyclooxygenase and prostaglandin development (Furst and Manning, 2001).

The carrageenan-induced rat paw edema model is a good way to analyse anti-inflammatory drugs, and it has been used a lot to see how effective they are at reducing swelling (Takayama et al., 2011). Prostaglandins, leukotrienes, histamine, bradykinin, TNF-, and other inflammatory and proinflammatory mediators (prostaglandins, leukotrienes, histamine, bradykinin, TNF-, and others) are released when carrageenan is ingested (Kumar et al., 2016). Carrageenan is a sulphur-containing polysaccharide found in red edible seaweeds belonging to the Rhodophyceae family. It is mostly used in the food industry and is biphasic, causing inflammation. It is clarified that inhibiting the release of early mediators in the first phase and cyclooxygenase in the second phase results in pain reduction by inducing nociception, depending on the mechanism. Efficient anti-inflammatory drugs that have been clinically used to evaluate the anti-edematous activity of several natural products have shown that the second phase is vulnerable to these. For the study, different doses of 100 mg/kg (low dose) and 200 mg/kg (high dose) were used. According to the findings, the higher dose (200 mg/kg) of both extracts resulted in the greatest reduction of paw edema in the rats. When compared to *P. rubra* extract, *P. pudica* extract yielded better results.

Formaldehyde-induced arthritis is used to assess the effectiveness and efficacy of drugs/substances with anti-arthritic properties that may mimic human arthritis symptoms in certain ways. After injecting HCHO into the foot, a proliferative process is elicited by COX mediators, resulting in chronic inflammation. Indomethacin, a non-selective NSAID, effectively inhibits COX-1 and COX-2 isoform enzymes after being injected into the rat hind paw and ankle. All of the groups were given orally to all of the rats every day for 14 days (Costa et al., 2004). The paw volume of rats was measured using a plethysmometer at 0, 1, 2, 4, 6, 8, 10 and 14 days after drug administration. In comparison to *P. rubra*, a higher dose of *P. pudica* (300 mg/kg) resulted in a greater reduction in paw volume (Nair et al., 2011).

CONCLUSION

The current study compares the anti-arthritic and anti-inflammatory activity of ethanolic flower extracts of *P. pudica* and *P. rubra in-vitro* and *in vivo*. Secondary metabolites in the plant species such as terpenoids, phenols, and proteins, may contribute to the biological effect of reducing inflammation and arthritis. When compared to control, placebo, and standard drug, the ethanolic flower extract of both species showed the most therapeutic activity against arthritis and inflammation. We concluded that the ethanolic flower extract of *P. pudica* and *P. rubra* have therapeutic efficacy for anti-arthritic and anti-inflammatory activities in albino rats both in-vitro and in vivo after analyzing the findings. In comparison to *P. rubra* extract, *P. pudica* extract produced better results. As a result, herbal medicine has opened up new possibilities for developing broad-spectrum uses of *P. pudica* and *P. rubra*, as well as lying the groundwork for developing new novel potent drugs to treat arthritis and inflammation.

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