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Evaluation of in vivo Anti-inflammatory Action of Leaf Extracts of *Plumeria pudica*

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ABSTRACT

The leaves of *Plumeria pudica* grow whirling around the stem and have a sessile base. The leaves are light green in color and the shape of the blade is spoon like, the apex is sharp. The extraction abilities of different solvents for recovering extractable components from leaves followed the order: methanol>water>chloroform>benzene. The results suggest the presence of alkaloids, saponin glycosides, phenolics, terpenoids, sterols, proteins and flavonoids in the leaf of the plant. All the extracts were subjected to *in vitro* determination of their anti-inflammatory potential using inhibition of albumin denaturation as well as anti-proteinase methods. All the concentration levels of the extracts showed the inhibition of albumin denaturation. The 500 µg/mL methanolic extract had shown the greatest inhibition capacity (64.36%) whereas the lowest inhibition capacity was exhibited by 100 µg/mL of the benzene extract (1.29%). The highest anti-proteinase activity was exhibited by methanolic extract solution at 500 µg/mL concentration, inhibiting 51.37% while the 100 µg/mL methanolic solution was able to inhibit only 13.01% protease activity. The methanolic extract was found to be the most potential in exhibiting anti-inflammatory action in the in vitro assays and hence it was used for *in vivo* anti-inflammatory screening at two dose levels. The methanolic leaf extract of *Plumeria pudica* (MLEPP) was able to reduce the inflammation in a dose dependent manner. The maximum inhibition of edema by MLEPP at 100 mg/kg dose was 28.78% at the end of the 4th hour while that with 200 mg/kg dose was 50.4%.

Key words: In-Vivo, Anti-Inflammtory, *Plumeria Pudica,*

1. INTRODUCTION

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants[1]. It is characterized by redness, swollen joints, joint pain, its stiffness and loss of joint function. Inflammation is currently treated by NSAIDs[2]. Unfortunately these drugs cause increased risk of blood clot resulting in heart attacks and strokes[3]. Inflammation is a normal, protective response to tissue injury caused by physical trauma, noxious chemicals or microbiological agents[4].

Plumeria pudica is used as medicinal plant native to Mexico, Central America, the Caribbean and South America spreaded throughout the tropics[5]. They are commonly known as "Temple tree" or "Champa" in India. Depending on location many other common names also exists like "Kembang kamboja" in Indonesia."Kalachuchi" in the Phillipines[6]. From the literature it was evident that the *Plumeria* species especially *Plumeria acuminata* has being studied widely for its pharmacological potential[7]. It was also found that the antioxidant potential, the related anti inflammatory and antidiabetic property of the plant has also being scientifically explored by researchers. But all the research was directed either towards leaves or the flowers of the plant[8]. Some studies of other species of Plumeria were also found but no study on leaf extracts of *Plumeria pudica* was revealed in the literature[9].

2. MATERIAL AND METHODS

2.1 Selection of the Plant

Plumeria pudica is planted at large in households for its decorative purpose. The perennial flowering capability of the plant and the ease of propagation have been the prime factors for the wide use of the plant. This ornamental plant was therefore considered as a potential candidate for the study.

2.2 Collection and identification of the plant material

The leaves of *Plumeria pudica* were collected from the local surrounding of Bhopal, Madhya Pradesh in the month of January and authenticated at Saifia Science College, Bhopal.

2.3 Pharmacognostic Study

The pharmacognostic investigation of the plant was performed for generating database of ornamental plants. The leaves of the plant were subject to microscopic and macroscopic analyses. The leaf type, venation and other macroscopic features were observed for and recorded. The transverse section of the leaf was studied for the presence of different types of cellular structures.

2.4 Preparation of the plant material

The collected plant leaves after authentication was washed with distilled water and was dried under shade. The completely dried leaves were converted to fine powder form using a blender at low speed. The powdered leaves were stored in air tight container till taken for further processes and investigation.

2.5 Extraction of leaves

The leaves powder prepared using the above procedure was used for extraction process. Hot continuous extraction was performed for extracting out the phytochemicals from the leaf powder. Briefly, 500 g of the leaf powder was evenly packed in the extractor of the soxhlet apparatus and extracted successively with various solvents of increasing polarity. The solvents used for extraction included benzene, chloroform, methanol and water. The extraction process was carried out for about 13 h for each solvent. The extracts were filtered while hot through Whatman filter paper to remove any un-dissolved material (debris or impurities). The extracts were concentrated by distillation to reduce the volume to one tenth. The concentrated extracts were then transferred to 100 ml beaker and the remaining solvents were evaporated on thermostatically heated water bath. The oleo-resinous extracts were collected and placed in desiccators to remove the excessive moisture**.** The dried extracts were stored in desiccators until used for further investigational procedures.

2.6 Preliminary phytochemical testing

All the extracts were subjected to qualitative phytochemical testing procedures for identifying the presence or absence of usual plant secondary metabolites. The test was performed for alkaloids, triterpenes/steroids, glycosides, tannins, flavonoids, saponins, and phenolic acids. The color, intensity of color or the precipitate formation was used as observational responses to the reactions occurring in these tests.

2.7 *In vitro* anti-inflammatory activity

2.7.1 Inhibition of albumin denaturation

Preparation of Phosphate Buffer Saline (PBS)

A solution of PBS was prepared by dissolving an accurately weighed quantity of 8 g NaCl, 0.2 g KCl, 1.44 g disodium hydrogen phosphate and 0.24 g potassium dihydrogen phosphate in deionized water to produce 1 L of solution.

The technique of inhibition of albumin denaturation reported by Kumari *et al* [12] was used with slight modifications. The volume of each component of the reaction mixture was reduced to half its volume.

The extracts were dissolved in DMSO and appropriately diluted to prepare solutions of 100, 200, 300, 400 and 500 µg/mL concentration. A solution of 1% BSA in deionized water was prepared for the test. Ibuprofen solution of concentration 1 µg/mL was used as the positive control.

The test containers were filled with 200 µL of BSA, 1400 µL of PBS and 1000 µL of the test solution (extract). Ibuprofen solution was used in the positive control and distilled water was used in the negative control vessels in place of extract.

The reaction mixtures were incubated at 37°C for 15 min and then heated at 70°C for 5 min. The mixtures were then allowed to cool to room temperature and the absorbance of constituent of each vessel were analyzed in UV-Visible spectrophotometer at 660 nm. The inhibition of percent denaturation of albumin was determined using the following formula:

$$\% \text{ Denaturation inhibition} = (1 - D/C) \times 100$$

Where D is the absorbance reading of the test sample, and C is the absorbance reading without test sample (negative control).

2.7.2 Anti-proteinase action

Preparation of Tris-HCl buffer

An accurately weighed quantity of 121.44 g of Tris was dissolved in 800 mL of distilled water. The pH of the solution was adjusted to 7.0 by addition of appropriate volume of concentrated HCl and the final volume of the solution was made up to 1 L with distilled water.

The technique of antiproteinase action reported by Oyedepo *et al*[13] and Sakat *et al*[14] was used with slight modifications. The reaction mixture was prepared with 0.06 mg trypsin, 1 mL 20 mM Tris-HCl buffer (pH 7.0) and 1 mL test sample of different concentrations (100 - 500 µg/mL). The mixture was incubated at 37°C for 5 min followed by the addition of 1 mL of 0.8% w/v solution of casein in water. The mixture was incubated additionally for 20 min. In order to stop the reaction, 2 mL of 70% perchloric acid was added to the mixture. The turbid suspension obtained after the reaction was centrifuged and the absorbance of the supernatant was recorded at 210 nm against buffer as blank. The percentage inhibition of proteinase inhibitory activity was calculated by the following formula:

% inhibition = (Abs control –Abs sample) x 100/ Abs control

2.8 Anti-inflammatory action using carageenan induced rat paw edema method

2.8.1 Animals

Healthy Wistar rats of either sex, weighing 180-250g were used for the study. The animals were housed in cages during the course of experimental period and maintained at 12 day and night schedule with a temperature [17-26°C] maintained at standard experimental condition. The animals were fed with standard rodent pellet feed and water *ad libitum*. The animals were fasted 12 hours before the experiment with free access to only water. The protocol was approved by the institutional ethical committee.

2.8.2 Carageenan induced rat paw edema method

The carageenan induced rat paw edema method was used for evaluating the anti-inflammatory activity of the Methanolic leaf extract of *Plumeria pudica* (MLEPP).

Paw oedema was induced by subcutaneous injection of 0.1mL (1% solution) of Carrageenan into the plantar surface of the right hind paw of the rat. The test sample was administered in dose of 10 mg/kg in different groups of animals, 30 min prior to carrageenan injection. Ibuprofen (10 mg/kg i.p.) was used as a standard anti-inflammatory drug which was administered 30 min prior to carrageenan injection. Animals were divided into 4 groups (n = 6) as follows

Group -- I - Control - treated with vehicle (normal saline)
Group -- II - Standard drug – Ibuprofen
Group – III– MLEPP was administered in dose of 100 mg/kg.
Group – IV– MLEPP was administered in dose of 200 mg/kg.

Paw diameters were measured immediately before the administration of the Carrageenan and thereafter at 1, 2, 4 and 6 h using vernier caliper. The results obtained were compared with control group. The percentage inhibition of paw inflammation exhibited by each group was calculated by using following formula:

% inhibition = C-T/ C x 100

C= Paw volume (mm) in vehicle treated group (control),
T= Paw volume (mm) in drug treated group

3 RESULTS AND DISCUSSIONS

3.1 Pharmacognostic Study

The leaves of *Plumeria pudica* grow whirling around the stem and have a sessile base. The leaves are light green in color and the shape of the blade is spoon like, the apex is sharp. The midrib runs centrally through the leaf- blade from its base to the apex producing thinner lateral veins, which in their turn give rise to even thinner veins or veinlet.

The transverse section of the leaf exhibits the presence of epidermis on both the surfaces. The outer walls of the epidermis have thin cuticle. The mesophyll (tissue enclosed between both the epidermis) consists of elongated or columnar cells called as the palisade parenchyma. Starch grains are visible in the parenchymal layer. Irregularly arranged cells with large intercellular space called as the spongy parenchyma are also present. The vascular bundles consisting of xylem and phloem are present in the midrib section of the leaf in Figure below:

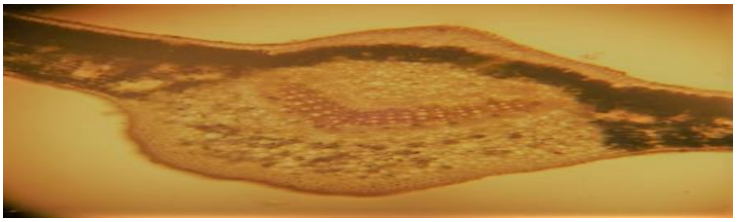

Figure 5.2Transverse section of the leaf of *Plumeria pudica*

3.2 Extraction Yields

The extraction yield of the leaf using different solvents is presented in Figure 5.3. The extraction abilities of different solvents for recovering extractable components from leaves followed the order: methanol>water>chloroform>benzene. No similar study was found in any of the scientific reports made on the plant species.

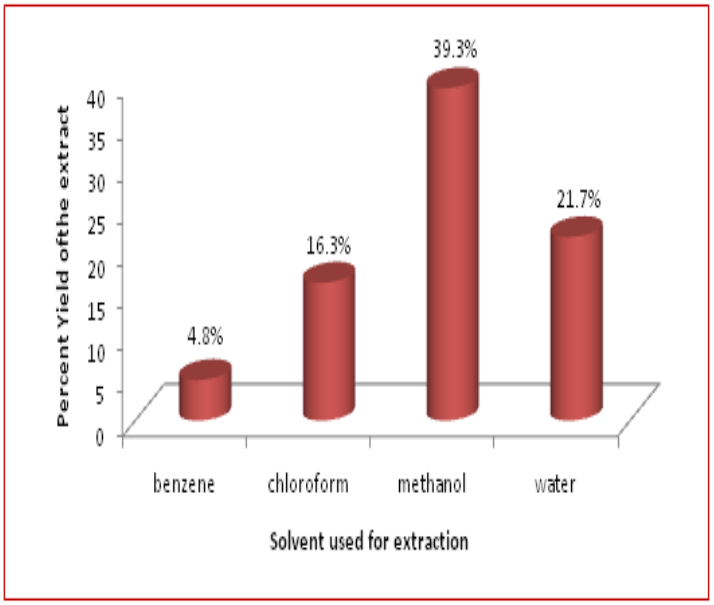

Figure 5.3: Extraction yield of leaf in different solvents

3.3 Phytochemical Evaluation

For detecting the presence alkaloids, glycosides, tannins, saponins, flavonoids and terpenoids a small fraction of all the dried extracts were subjected to the phytochemical evaluation tests as depicted in the section 4.6. Small amount of each extract was resuspended into distilled water/ethanol for the carrying out the testing procedures. All the extracts were tested for the presence of various categories of phytochemicals and the results are presented in Table below.

The findings suggest the presence of alkaloids, saponin glycosides, phenolics, terpenoids, sterols, proteins and flavonoids in the leaf of the plant. The presence of ursolic acid, stigmast-7-enol and lupeol in the leaves of the plant has also been reported in their review on *Plumeria*.

Table 5.1: Phytochemical screening of *Plumeria pudica* leaf extracts

Chemical Tests	Observation	Benzene extract	Chloroform extract	Methanolic extract	Aqueous extract
Alkaloids					
Mayer's reagent	cream colour precipitate	-	-	-	+
Hager's reagent	yellow colour precipitate	-	-	-	+
Wagner's reagent	reddish brown precipitate	-	-	-	+
Dragendorff's reagent	reddish brown precipitate	-	-	-	+
Glycosides					
Froth test	Frothing is seen	-	+	+	-
Kedde's Test	No color	-	-	-	-
Bontrager's Test	Rose pink or red color in the ammonical layer not found	-	-	-	-
Keller-Kiliani	No color in acetic acid layer	-	-	-	-
Phenols/Tannins					
Ferric chloride	Blue green color	+	+	+	+

Gelatin Solution	White precipitate	+	+	+	+
Alkaline reagent test	Yellow to red precipitate	-	+	+	+
Vanillin HCl test	Purplish red color	-	-	-	+
Flavonoids					
Shinoda test	red color	-	+	+	+
Alkaline reagent test	Yellow color that turns red on acidification	-	+	+	+
Zinc HCl reductino test	red color	-	+	+	+
Proteins					
Millon's Test	No precipitation	-	-	-	+
Ninhydrin Test	Violet color not obtained	-	-	-	+
Sterols/triterpenoids					
Liberman - Burchard Test	Brown ring at junction Upper layer turns green	+	-	-	-
Salkowski Test	Yellow color in lower layer	+	-	-	-

3.4 *In vitro* anti-inflammatory action

3.4.1 Inhibition of Albumin Denaturation

Protein denaturation has been significantly correlated with the occurrence of the inflammatory response and may lead to various inflammatory diseases including arthritis. Tissue injury during life might be due to denaturation of the protein constituents of cells or of intercellular substance. Hence, the ability of a substance to inhibit the denaturation of protein signifies obvious potential for anti-inflammatory activity.

All the concentration levels of the extracts showed the inhibition of albumin denaturation. The 500 µg/mL methanolic extract had shown the greatest inhibition capacity (64.36%) whereas the lowest inhibition capacity was exhibited by 100 µg/mL of the benzene extract (1.29%). The inhibition protein denaturation by 100 µg/mL solution of standard drug Ibuprofen was found to be 67.73%. Of all the extracts, the best inhibition of denaturation of albumin was exhibited by the methanolic extract of the *Plumeria pudica* leaves.

Table 5.2: Albumin denaturation inhibition activity

Treatment	**100 µg/mL**	**200 µg/mL**	**300 µg/mL**	**400 µg/mL**	**500 µg/mL**
Ibuprofen	67.73	-	-	-	-
Methanolic Extract	11.36	22.35	35.64	53.83	64.36
Aqueous Extract	9.27	14.63	23.89	31.76	40.37
Chloroform Extract	5.61	9.39	16.57	23.19	27.58
Benzene Extract	1.29	4.58	9.62	14.84	18.55

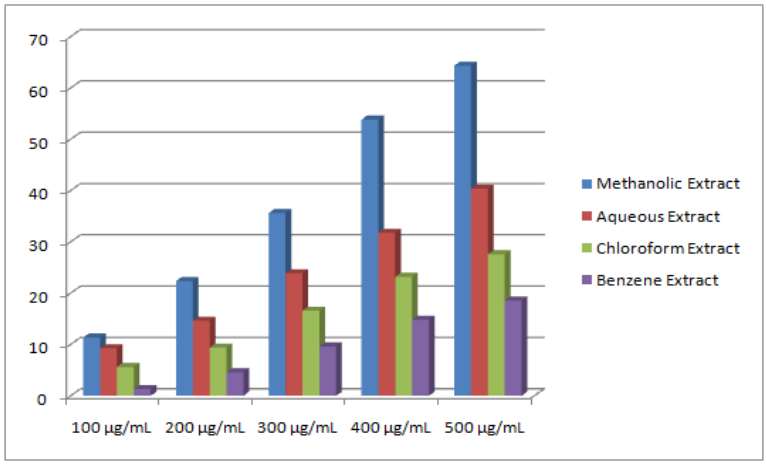

Figure 5.4: Comparative percent inhibition of albumin denaturation by various leaf extracts of *Plumeria pudica*

3.4.2 Anti-proteinase Acitivity

Dys regulated release of mast cell proteases is known to have a pivotal role in the pathogenesis of a number of inflammatory conditions including asthma, abdominal aortic aneurysm formation, vessel damage in atherosclerosis and hypertension, arthritis, and ischemia/reperfusion injury.

The antiprotease activity exhibited by the extracts of *Plumeria pudica* leaf at various test concentrations. The highest inhibition capacity was exhibited by methanolic extract solution at 500 µg/ml concentration, inhibiting 51.37% while the 100 µg/ml methanolic solution was able to inhibit only 13.01% protease activity. Ibuprofen solution at 100 µg/mL concentration was able to inhibit 59.84% of protease activity. Benzene extract exhibited the poorest antiproteinase action.

Table 5.3: Antiproteinase activity

Treatment	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml
Ibuprofen	59.84	-	-	-	-
Methanolic Extract	13.01	21.85	33.69	44.92	51.37
Aqueous Extract	10.56	14.34	19.62	25.77	34.43
Chloroform Extract	7.28	11.63	15.81	20.22	26.75
Benzene Extract	3.77	4.18	6.29	8.95	11.02

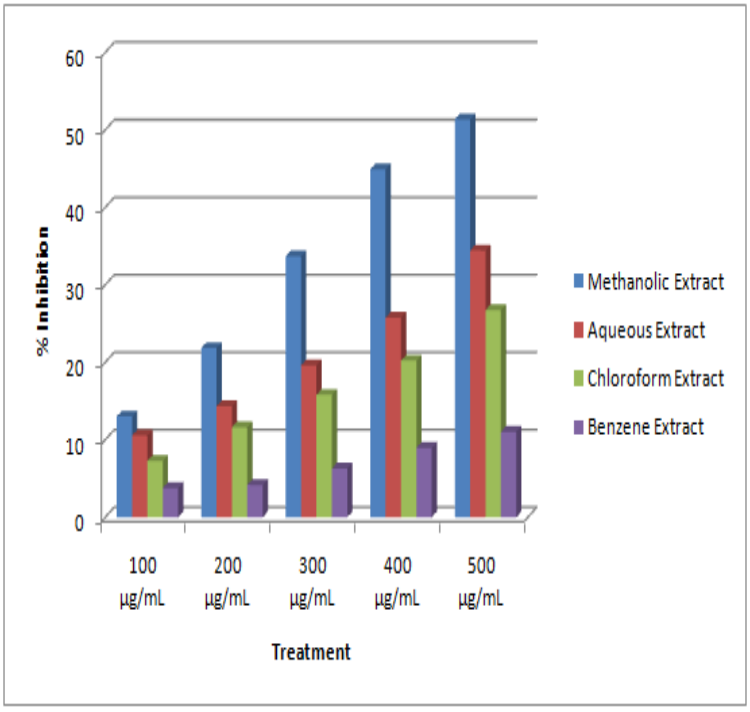

Figure 5.5: Comparative percent inhibition of proteinase by various leaf extracts of *Plumeria pudica*

3.5 *In vivo* anti-inflammatory action

Carrageenan-induced acute inflammation is one of the most widely applied test procedures for screening of anti-inflammatory agents in animals. As shown in the table 5.4, MLEPP was able to reduce the inflammation in a dose dependent manner. The maximum inhibition of edema by MLEPP at 100 mg/kg dose was 28.78% at the end of the 4th hour while that with 200 mg/kg dose was 50.4%.

This is to certify that the project entitled Evaluation of in vivo anti-inflammatory action of leaf extracts of *plumeria pudica* by Mr. Ankush Sharma has been approved by IAEC Reg. No. is 585/PQ/a11/2022/CPCSEA, dated:09/05/2022.

Table 5.4: Rat paw edema in rats

Group	Change in Paw thickness (mm)			
	1h	2h	3h	4h
Normal Saline	1.674 ± 0.025	3.02 ± 0.072	3.28 ± 0.086	4.10 ± 0.047
Ibuprofen	0.84 ± 0.007	1.26 ± 0.01	1.19 ± 0.014	1.03 ± 0.025
ELEPA (100 mg/kg)	1.33 ± 0.059	2.59 ± 0.110	2.73 ± 0.076	2.92 ± 0.063
ELEPA (200 mg/kg)	1.19 ± 0.025	2.14 ± 0.014	2.19 ± 0.003	2.01 ± 0.025

Results are reported as meane ± SD (n=6)

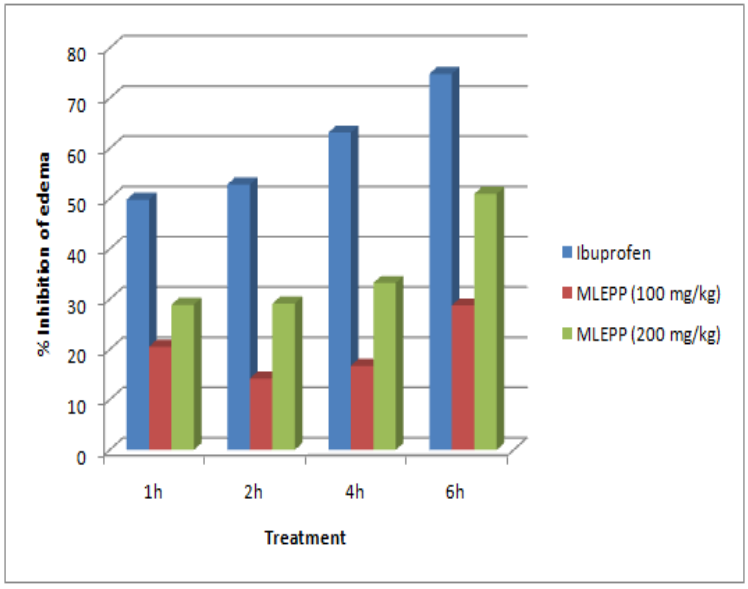

Figure 5.6: Comparison of anti-inflammatory effect of Ibuprofen and MLEPP

Carrageenan-induced acute inflammation is one of the most suitable test procedures to screen anti-inflammatory agents. As shown in the table, MLEPP was not able to inhibit edema significantly in the early hours and in low dose but was able to

inhibit edema considerably at 4h when the dose of 200 mg/kg was administered. The anti-inflammatory effect of MLEPP was not very significant as compared to Ibuprofen.

Carrageenan-induced paw edema model in rats is known to be sensitive to cyclo-oxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents, which primarily inhibit the cyclo-oxygenase involved in prostaglandin synthesis. Therefore, it can be inferred that the inhibitory effect of MLEPP on carrageenan-induced inflammation may be due to inhibition of enzyme cyclo-oxygenase leading to inhibition of prostaglandin synthesis.

4. CONCLUSION

The objective of the present study was to assess the anti-inflammatory potential of different leaf extract of *Plumeria pudica* using the *in vitro* and *in vivo* models. The results obtained led to the conclusion that *Plumeria pudica* leaves contain high amounts of potential phytoconstitutents that lead to anti-inflammatory activity. The ease of availability of the plant and easy adaptability to all climatic conditions make the evergreen flowering plant a good source of natural anti-inflammatory compounds. Further studies related to deducing the mechanism of anti-inflammatory action would be carried out in future to establish a standardized herbal formulation for management of various inflammatory conditions.

CONFLICTS OF INTERESTS

There are no conflicts of interests.

REFERENCES

1. Kumar S, Bajwa BS, Singh K, Kalia AN. Anti-Inflammatory Activity of Herbal Plants: A Review. International Journal of advances in Pharmacy, Biology and Chemistry. 2013; 2(2): 2277-4688.
2. Saso L, Valentini G, Casini ML, Grippa E, Gatto MT, Leone MG . Inhibition of heat-induced denaturation of albumin by nonsteroidal antiinflammatory drugs (NSAIDs): Pharmacological implications. Archives of Pharmacal Research. 2001; 24(2): 150-158.
3. Opie EL. On the relation of necrosis and inflammation to denaturation of proteins. Journal of Experimental Medicine. 1962; 115: 597-608
4. Achoui M, Appleton D, Abdulla MA, Awang K, Mohd MA, et al. (2010) In Vitro and In Vivo Anti-Inflammatory Activity of 17-O-Acetylacuminolide through the Inhibition of Cytokines, NF-kB Translocation and IKKb Activity. PLoS ONE, 2010; 5(12):e15105. doi:10.1371/journal.pone.0015105.
5. Nandkarni KM, Indian Materia Medica, Popular Prakashan, Bombay 1976;1:993.
6. Kirtikar and Basu, The Wealth of India ,NISCAIR & CSIR, New Delhi 2006;8:164-166.
7. Santana LdAB, Aragão DP, Araújo TdSL, de Sousa NA, de Souza KLM, Oliveira LES *et al*. Antidiarrheal effects of water-soluble proteins from Plumeria pudica latex in mice. Biomed Pharmacother. 2018; 97: 1147-1154.
8. Chaveerach A, Tanee T, Patarapadungkit N, Khamwachirapithak P, Sudmoon R. Cytotoxicity and genotoxicity of Allamanda and Plumeria species. Science Asia. 2016; 42: 375-381.
9. Shinde PR, Patil PS, Bairagi VA, Phytopharmacological review of *Plumeria* species. Scholars Acad J Pharm. 2014; 3(2): 217-227.
10. Tohar N, Mohd MA, Jantan I, Awang K. A comparatives tudy of the essentiaol ils of the genus PlumeriaLinn.f rom Malaysia. Flavour Fragr. J. 2006; 21: 859-86.
11. Sunitha K, Ch. Naga Rani, Antibacterial activity of methanolic extracts of flowers of plumeria acuminata. J Global Trends Pharm Sci. 2018; 9(1): 4978-4980.
12. Kumari S, Yasmin N, Hussain MR, Babuselvam M , In vitro antiinflammatory and anti-arthritic property of Rhizopora mucronata leaves. Internat J Pharm Sci Res.(2015); 6: 482-485.
13. Oyedepo OO, and Femurewa AJ, (1995). Anti-protease and membrane stabilizing activities of extracts of *Fagra zanthoxiloides, Olax subscorpioides and Tetrapleura tetraptera*. Internat J Pharmacog, 33: 65-69.
14. Sakat S, Juvekar AR, Gambhire MN (2010). *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. Internat J Pharma Pharmacological Sc, 2: 146-155.