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RESEARCH ARTICLE

Anti-Inflammatory and Analgesic Effects of Leaf Extracts of *Hibiscus Populnea* Linn.

Hareeshbabu E*[1], Molly Mathew[2]

*[1]*Vinayaka University, Salem, Tamilnadu, India.*

[2]*Malik Deenar College of Pharmacy, Kasaragod, India.*

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ABSTRACT

Hibiscus populnea or Indian Tulip Tree or Pacific Rosewood, has been traditionally used for the treatment of many diseases including inflammatory conditions. A number of chemical constituents namely, thespesin, gossypol, DL-gossypol, populnin, populneol, glycosides of quercetin, epoxylic acid, rutin, kaemferol-3-flucoside, lupenone, mansonone, myricyl alcohol, lipids and β sitosterol have been isolated from the plant. Many of these compounds are proved to have the claimed activities. But no work has been specifically carried out to establish the anti inflammatory effects of the ripe and older leaves. Hence in the present study an attempt was made, with the leaves, for the phytochemical screening of the methanol, pet. ether and aqueous extracts along with their anti-inflammatory and analgesic potential also. In order to study the anti-inflammatory effects, dextran-induced paw edema method and carrageenan - induced paw edema methods were used. Similarly analgesic activity was tested using acetic acid writhing. Anti arthritic study was also tried. Regarding carrageenan- induced paw edema, the level of activity of the petroleum ether extract was less than that of the reference drug and the methanolic extract exhibited the most potent inhibitory activity. But the activity of the aqueous extract was more than that of Indomethacin. From the results of the experimentally induced arthritic study it was seen that the activity of the petroleum ether extract was quite stable throughout the period of assay where as the methanolic extract was proved to be more active against the chronic phase than against the acute phase. The aqueous extract was seen to be a very potent anti inflammatory agent against both phases of the inflammatory process with more or less the same activity as that of indomethacin, the reference standard. The analgesic activities of aqueous and methanolic extracts were found to be very significant ($P < 0.001$).

KEYWORDS

Hibiscus populnea, acute toxicity, antiarthritic, anti-inflammatory, nociceptive effect.

INTRODUCTION

Plants have been used for thousands of years, based on experience and folk remedies and continue to draw wide attention for their role in the treatment of mild and chronic diseases.

***Address for Correspondence:**
Hareeshbabu E.
Vinayaka Missions University,
Salem, Tamilnadu
India.
E-Mail Id: hareeshbabue@yahoo.com

In recent times, focus on plant research has increased all over the world and a large body of evidence has been accumulated to highlight the immense potential of medicinal plants used in various traditional systems of medicine.[6,34,37]

Hibiscus populnea or Thespesia populnea, commonly known as the Portia Tree is a species of flowering plant in the mallow family, (Malvaceae) is a typical example of folk

remedy. It is a small tree or arborescent shrub that has a pantropical distribution, found on coasts around the world. However, the Portia Tree is probably native only to the Old World[11], and may have originated in India.[22] It is possibly indigenous to the Hawaiian Islands and elsewhere in the Pacific, but may have been spread by early Polynesians for its useful wood. The tree reaches a height of 6–10 m (20–33 ft), tall with a trunk diameter of 20–30 cm (7.9–12 in).[12] It grows at elevations from sea level to 275 m (902 ft)[8] in areas that receive 500–1,600 mm (20–63 in) of annual rainfall.[11] The Portia Tree is able to grow in a wide range of soil types that may be present in coastal environments, including soils derived from quartz (sand), limestone, and basalt; but it favors neutral soils (pH of 6-7.4).[12] Leaves are alternate, simple with long petioles of 5-10 cm (2-4 in). Flowers are showy, hibiscus like, single at upper leaf axils, to 8 cm (3 in) across: corolla is yellow with a red centre, turning maroon by nightfall; stamens are united into a column shorter than petals.[21]

Hibiscus populnea has traditionally been used for the treatment of rheumatic and other inflammatory affections in the tropical regions. Also it is used in dysentery, and haemorrhoides, ringworm affections and other skin diseases.[4,8,27] Many chemical constituents have been isolated from the plant namely, thespesin, gossypol, DL-gossypol, populnin, populneol, glycosides of quercetin, epoxylic acid, rutin, kaemferol-3-flucoside, lupenone, mansonone, myricyl alcohol, lipids and β sitosterol.[24] Two new sesquiterpenoid quinones, namely thespesone and thespone were isolated from the heart wood of the plant.[28] Mansonones C, D, E and F also are recently isolated.[21]

The plant as a whole or in parts is used for the treatment of many diseases. A decoction of the plant is used in the treatment of cutaneous infections, skin and liver diseases.[32] Also it is used in cough, influenza and relapses of illness. Cold infusion of bark is used in dysentery, diabetes, gonorrhoea, pelvic infection, ulcers and so on. Stem extract is used in breast cancer. Inner bark is used in constipation and typhoid fever. Crushed fruit is used in the problems of abdominal swelling.[24] The aqueous extract generally exhibits a high degree of antibacterial activity which confirms its traditional therapeutic claim.[26] The bark, leaves and flowers are useful in cutaneous infections such as scabies, psoriasis, eczema and guinea worms.[10] The wood is used industrially for furniture making. Hibiscus populnea is not only an interesting source of antimicrobial activities but also it is a potential source of phenolic antioxidants.[3] As no work has been reported on the leaves, this particular study was taken up to establish a scientific base to its claimed therapeutic effect.

MATERIALS AND METHOD

Plant Material

Old and ripe leaves (yellow in colour) of *Hibiscus populnea* were collected from Thiruvananthapuram District. It was identified by Dr. Chandramohan Nair K., Botanist, University College, Thiruvananthapuram, Kerala. A voucher specimen was deposited in the herbarium of College of Pharmaceutical Sciences, Medical College, Thiruvananthapuram where this work was carried out. (MTCPS/Herb HP 04)

The plant material was air dried, powdered, defatted with petroleum ether (Pet.ether) and later extracted with methanol in a Soxhlet extractor. The solvents were removed under vacuum in a rotary evaporator until the residues were completely dry. An aqueous extract customarily employed in folk medicine and which was reconstituted into a 10% infusion was also prepared. The aqueous extract was lyophilized to facilitate handling.

Test Animals

Anti-inflammatory activity against experimentally induced arthritis was investigated in female Wister rats weighing 150 $\pm$ 25 g. Female OFI mice weighing 25 $\pm$ 5 g were used to study inhibition of carrageenan induced paw oedema and acute toxicity studies.

The animals were housed in standard cages under 12 hour light and dark cycle and fed with

a standard pellet diet. Groups of 6 test animals were used. (The study protocol was approved by the institutional ethical committee). The animals were kept for 2 weeks to get acclimatized with laboratory conditions. Then they were fasted overnight before the experiment, but with water *ad libitum*.

Phytochemical Study

Phytochemical screening of the various extracts were carried out to ascertain their qualitative composition using commonly used reactions and readily performed chromatographic techniques to identify the major natural chemical groupings.[14, 16, 29, 38]

Pharmacological Studies

Acute Toxicity Studies

For acute toxicity studies, groups of 10 mice were selected. All the extracts were subjected for acute toxicity studies by following the OECD guidelines No. 425 of CPCSEA and $1/10^{th}$ of the LD_{50} dose was selected for the pharmacological activity.[13]

Anti-inflammatory Activity

Experimentally Induced Arthritis

Freund's adjuvant induced arthritis was used to assess the anti-arthritic activity in albino rats.[23] Animals were divided into groups of six each. Group I served as control which received 1% carboxymethyl cellulose (CMC). Group II served as standard receiving Indomethacin. Groups III, IV and V served as test groups receiving the extracts. Arthritis was induced in the rats by injecting 0.1 ml of Complete Freund's Adjuvant (CFA) (Sigma, St.Louis, U.S.A) which is a suspension of killed *Mycobacterium tuberculosis* bacteria (0.5%w/w) homogenized in liquid paraffin into the left hind paw subcutaneously. Drug treatment was started from the initial day, ie, from the day of adjuvant injection (0 day), and continued till 20 days. Paw volume was measured on 5^{th}, 10^{th}, 15^{th} and 20^{th} day with the help of a plethysmometer.

The pharmacological study was conducted using pet.ether, methanol and aqueous extracts. The organic extracts were suspended in 1% carboxymethyl cellulose (1% CMC). The lyophilized aqueous extract was reconstituted in distilled water. All the 3 extracts at a dose of 200 mg/kg body wt and reference drug-Indomethacin in 1% CMC - 10 mg/kg - were given orally before CFA administration daily throughout the experiment 1 hour before measurement of paw volume. The percentage inhibition was calculated using the formula: Percentage inhibition = $100 \times (V_c - V_t) / V_C$ where Vc = mean increase of paw volume of control animals and V_t = mean increase of paw volume of treated animals.

Plantar Oedema

Oedema was induced in the mice according to the method of Winter et al, 1962 as modified for mice by Sugishita et al, 1981. Paw oedema was produced by subcutaneous injection of 0.05ml of (3% w/v) carrageenan in saline solution into the sub plantar region of the left hand paw. The same doses as for the experimentally induced arthritis were given orally 1 hour before carrageenan administration. Paw volume was measured by water plethysmography before injection of the phlogistic agent and at 0, 3, 5 and 7 hour afterwards. The percentage inhibition of inflammation was calculated as in the case of experimentally induced arthritis.

Dextran Induced Paw Oedema

The animals were treated as in the case of carrageenan induced paw oedema model, except that in place of carrageenan, Dextran (0.1ml, 1% w/v in normal saline) was used.[39]

Analgesic Activity

Acetic Acid Induced Writhing Response in Mice

The antinociceptive activity of the extracts was assessed using writhing test - abdominal constriction test.[36] Exact 0.5 ml of 1% acetic acid dissolved in 0.9% saline was administered intraperitoneally 30 minutes after the administration of the drug extracts. The number of contractions of abdominal muscles together with stretching of hind limbs was cumulatively counted over a period of 30 minutes beginning 5

minutes after the injection of acetic acid. The extracts were administered at dose of 200 mg/kg intraperitoneally 30 minutes prior to acetic acid injection. A significant reduction in the number of writhes by the drug treatment compared to vehicle control animals was considered as the analgesic response and the percentage inhibition of writhing was calculated using the ratio: (Control mean – treated mean) X 100/control mean. Aspirin (100 mg/kg i.p.) was the reference standard.

Statistical Analysis

The experimental results were expressed as the mean ± SEM. Data were assessed by the method of analysis of ANOVA followed by student's t-test. $P < 0.05$ was considered as statistically significant.

RESULTS

Yield of the Extracts

The percentage yields based on the dried starting materials were 2.34% for petroleum ether, 12.22% for methanol and 11.82% for the lyophilized aqueous extract. (All percentages are w/v).

Phytochemical Screening

Phytochemical screening revealed the presence of sterols, tannins, sugars, anthocyanins and flavones. It gave negative results for coumarins, anthraquinones, cardiac and cyanogenetic glycosides and alkaloids. Anthocyanidine and flavaonoids are probably responsible for the pharmacological activity detected because these compounds are reported to be bioactive in various inflammatory conditions .[17, 30]

Pharmacological Studies

Acute Toxicity Studies

After acute toxicity study of pet.ether, methanol and aqueous extracts no death was reported up to a dose of 2000mg/kg body weight. Hence the LD_{50} could not be determined. For further studies $1/10^{th}$ of the maximum dose was utilized. The results of the biological assays have been expressed with respect to the control group and compared with the reference drug as standard.

Experimentally Induced Arthritis

The results of the anti-inflammatory activity of the organic extracts and aqueous extracts of *Hibiscus populnea* against Complete Freund's adjuvant– induced arthritis (expressed as increase in paw volume and percentage inhibition of inflammatory process) is expressed in Table 1.

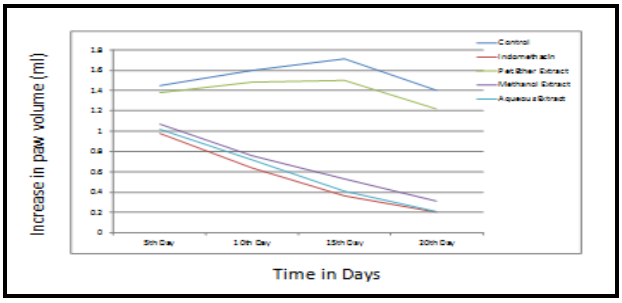

Figure 1: Percentage inhibition of CFA induced paw volume by *Hibiscus populnea* leaf extracts

The results have confirmed the inhibitory effect of both acute and chronic phases of arthritis by the Pet. ether and Methanolic as well as aqueous extracts. The activity of the petroleum ether extract, though not significant, was quite stable throughout the period of assay. The methanolic extract was proved to be more active against the chronic phase than against the acute phase. ie, on the 5^{th} day the inhibition of oedema was 20.21%; but as days advanced the percentage of inhibition was also increasing reaching a level of 77.85 on the 20^{th} day. The aqueous extract was seen to be a very potent anti inflammatory agent against both phases of the inflammatory process with more or less the same activity as that of indomethacin, the reference standard.

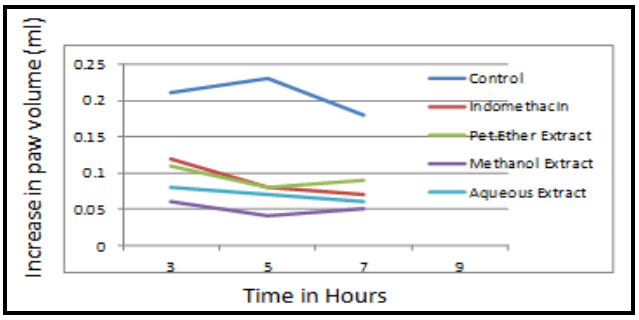

Figure 2: Effect of Pet.Ether, Methanol and Aqueous extracts of Hibiscus populnea on plantar Oedema in mice

Table 1: Percentage inhibition of CFA induced paw volume by *Hibiscus populnea* leaf extracts

Treatment/dose (mg / kg)	Increase in paw volume in ml (mean ± SEM)				Mean of % inhibition
	5th day	10th day	15th day	20th day	
Control	1.45±0.12	1.60±0.12	1.71±0.11	1.40±0.05	-
Indomethacin (10 mg / kg)	0.98±0.13 (32.41)	0.64±0.12* (60.00)	0.36±0.12* (78.94)	0.20±0.12* (85.71)	64.27*
Pet.ether ext. (200 mg / kg)	1.38±0.12 (05.17)	1.48±0.11 (07.50)	1.50±0.11 (12.28)	1.22±0.11 (12.85)	09.45
Methanol ext. (200 mg / kg)	1.07±0.14 (20.21)	0.76±0.12* (52.50)	0.53±0.10* (69.00)	0.31±0.10* (77.85)	54.89*
Aqueous ext. (200 mg / kg)	1.02±0.10 (29.66)	0.72±0.14* (55.00)	0.41±0.12* (76.02)	0.21±0.11* (85.00)	61.32*

Value in parenthesis represents % reduction of paw volume v/s control group *p<0.01

Table 2: Effect of Pet.ether, Methanol and Aqueous extracts of *Hibiscus populnea* on plantar Oedema in mice

Treatment/Dose (mg/kg)	Increase in paw volume (ml) (mean± SEM)		
	3 h	5 h	7 h
Control	0.21 ± 0.009	0.23 ± 0.010	0.18 ± 0.005
Pet.ether extract (200)	0.11± 0.008)* (47.62)	0.08± 0.014* (65.22)	0.09±0.011* (50)
Methanol Extract (200)	0.06±0.005* (71.43)	0.04± 0.010* (82.61)	0.05 ± 0.009* (72.22)
Aqueous extract (200)	0.08±0.004* (61.90)	0.07± 0.009* (69.57)	0.06 ± 0.008* (66.67)
Indomethacin (10)	0.12± 0.006)* (42.86)	0.08± 0.006* (65.21)	0.07± 0.007* (61.11)

Value in parenthesis represents % reduction of paw volume v/s control group *p<0.01

Plantar Oedema

Carrageenan induced paw oedema was satisfactorily inhibited by the pet.ether, methanol and aqueous extracts of *Hibiscus populnea*. (Table 2)

The level of activity of petroleum ether extract was similar to or even more than that of the reference drug up to a period of 5 hours. Later it started slowly declining. The aqueous extract was a bit more effective than Indomethacin.

The methanolic extract exhibited the most potent inhibitory activity.

Dextran Induced Paw Oedema

In this study also aqueous extract showed maximum effect. But other extracts also show some activity. (Table 3)

Acetic Acid Induced Writhing in Mice

Table 4 shows the analgesic property of the extracts ($p < 0.001$). The petroleum ether, methanol and aqueous extracts exhibited

significant inhibition at the rate of 36.00, 56.66 and 71.92%.In the study of analgesic activity also maximum effect is produced by aqueous extract. This may be due to the presence of polyphenolic compounds present in the extract.

Table 3: Effect of Pet.ether, Methanol and Aqueous extracts of *Hibiscus populnea* on Dextran induced paw Oedema

Treatment/Dose (mg/kg)	Paw volume (ml)	% inhibition
Control	0.630 ± 0.050	------
Indomethacin (10)	0.246 ± 0.020	60.95*
Pet.ether extract (200)	0.415 ± 0.040	34.13
Methanol extract (200)	0.340 ± 0.040	46.03*
Aqueous extract (200)	0.286 ± 0.020	54.60*

Values are mean ± SEM

Experimental groups were compared with control; *p<0.01

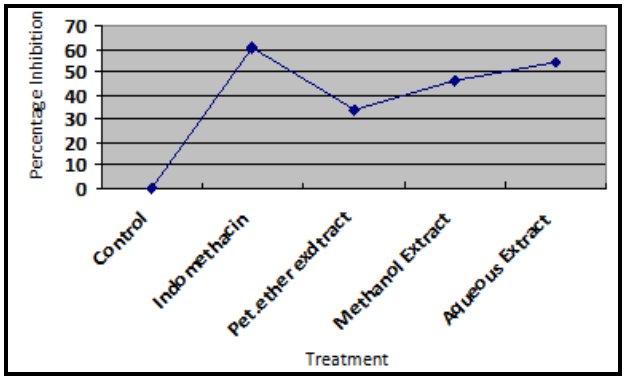

Figure 3: Effect of Pet.ether, Methanol and Aqueous extracts of Hibiscus populnea on Dextran induced paw Oedema

Table 4: Effect of Pet.ether, Methanol and Aqueous extracts of *Hibiscus populnea* on Acetic acid induced writhing test

Treatment / Dose (mg/kg)	No.of writhing /30 seconds	% inhibition
Control	32.05 ± 0.50	------
Aspirin (100)	3.21 ± 0.20	89.98*
Pet.ether extract (200)	20.51 ± 0.20	36.00
Methanol Extract (200)	13.89 ± 0.40	56.66*
Aqueous extract (200)	09.65 ± 0.30	71.92*

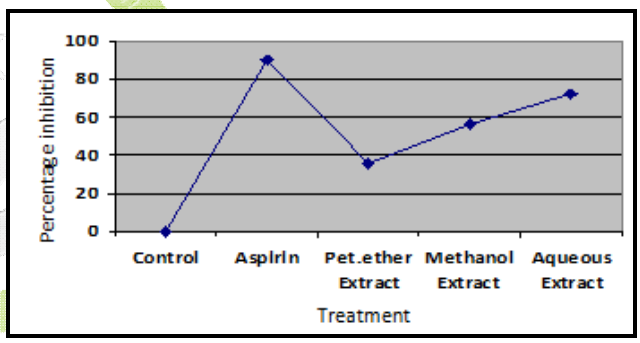

Figure 4: Effect of Pet.ether, Methanol and Aqueous extracts of *Hibiscus populnea* on Acetic acid induced writhing test

DISCUSSION AND CONCLUSION

The findings of this study help to establish a scientific basis for the use of *Hibiscus populnea* leaf extracts as an antiarthritic and anti-inflammatory agent in folk medicine. The results obtained have demonstrated the anti-inflammatory activity of the drug against both acute and delayed phases of inflammation. We have used two different rodent species to bioassay anti-inflammatory actions because Freund's adjuvant arthritis in rats is the best known animal model for the study of secondary inflammation[33]; mice appear to be resistant to development of arthritis.[15] Exclusively acute

inflammation was studied in mice by carrageenan injection.

The inflammatory response is a complex process with several characteristic features that include the activation of monocytes, granulocytes and the release and activation of inflammatory mediators, complement system and humoral mediators.[31] The inflammatory process begins with a stimulus that causes the release of prostaglandins from cells. Stimuli such as lipopolysaccharides can induce an enzyme responsible for the production of nitric oxide (inducible nitric oxide synthase or (iNOS) and another enzyme known as Cyclooxygenase-2 (Cox-2). Cox-2 acts by producing prostaglandins, particularly prostaglandin E_2 (PGE_2). In turn PGE_2 acts inside the cell to produce various types of qualitative cytokines, which are pro inflammatory agents that complete the process by bringing active leukocytes to the injury site.[25]

CFA induced arthritis in rats is a well established experimental model that has features similar to the human rheumatoid arthritis In addition it is achronic inflammatory model for development of potential analgesic and / or anti-inflammatory drugs useful for arthritis treatment. Freund's adjuvant is a mixture of dead Mycobacteria with liquid paraffin The method involves both infectious and immunological factors responsible for development of arthritis.(Kulonen,1970) Hence it gives an important means which can detect the efficacy of the test drug in controlling both the infectious as well as immunological factors. In the control group paw swelling increased up to 14th day. On the 14th day the paw volume was maximum. This was due to immunological response to the dead Mycobacterium present in CFA. The test drug inhibited the paw swelling and the inhibition was observed even after 14th day showing the efficacy of the drug in control of the immunological factors. This finding indicates the potential of the drug.[1]

The acetic writhing test is normally used to study the peripheral analgesic effects of drugs. Although this test is nonspecific (e.g., anticholinergic, antihistaminic, and other agents also show activity in the test), it is widely used for analgesic screening. In acetic acid induced abdominal writhing, the nociceptive mechanism is believed to be due the release of arachidonic acid via Cyclooxygenase and prostaglandin via synthesis. In the present study all the extracts produced significant antinociceptive effect and it might be due to inhibition of the synthesis of arachidonic acid metabolites.[9]

Flavonoids are known to inhibit the enzyme prostaglandin synthesis, more specifically the endoperoxide.[2, 7] *Hibiscus populnea* leaf extracts, especially methanol and aqueous extracts, are rich in flavonoid contents. Hence the significant anti-inflammatory activity of the extracts might be due to the presence of flavonoids.

So it is concluded that the pet.ether, methanol and aqueous extracts of *Hibiscus populnea* exhibit significant anti-inflammatory activity against both acute and delayed phases of inflammation. Also they produce significant analgesic effect. Further detailed investigation is underway to determine the exact phytoconstituents which are responsible for the above said effects.

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