

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF H. ROSASINENSIS & T.CORDIFOLIA PLANTS

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ABSTRACT

The present study investigates *the ANTI-INFLAMMATORY ACTIVITY OF ethanolic extract of H. ROSA SINENSIS & T. CORDIFOLIA PLANTS* using CARRAGEENAN INDUCED PAW EDEMA induced in wistar rats. In traditional medicinal system different parts of the plant have been mentioned to be useful in a variety of diseases. The hibiscus plant material is widely used as a cardiogenic, anti-constipates, anti-hepatotoxic agents and plant TINOSPORA is used in the treatment of fever, jaundice, anti-poisoning agents. The flowers of hibiscus and leaves of tinospora are employed for the treatment of inflammation. The objective of the present study was to evaluate the anti-inflammatory activity of both ethanolic extracts of flowers of hibiscus and leaves of Tinospora on carrageenan induced paw edema in rats. Indomethacin used as a standard drug. For this activity Animals were divided into four groups of six animals. Group I served as control (0.9% normal saline, 5 mlkg⁻¹ b.w), Group II and III received extracts at the doses of 100 and 200 mgkg⁻¹ b.w respectively and group IV as standard (Indomethacin 5 mgkg⁻¹ b.w). The result showed the ethanolic extract of Hibiscus rosa sinensis and Tinospora cordifolia exhibited significant anti-inflammatory activity on the tested animals during experiment. In carrageenan induced rat paw oedema there was significant reduction in paw volume in Indomethacin standard group ($p < 0.05$) as compared to control group at 4-hour readings. There was dose dependent decrease in paw volume in ethanolic extract treated groups. The Ethanolic extract (100mg/kg and 200mg/kg) significantly ($p < 0.05$) and dose- dependently inhibited carrageenan-induced rat paw oedema (60.80 and 75.19%, respectively) when compared with control group after 1 hour of carrageenan injection.

KEYWORDS: Hibiscus rosa sinensis and Tinospora cordifolia, Indomethacin, Anti- inflammatory activity, edema.

1.1 INTRODUCTION**1.2 Disease^[1-2]**

Inflammation is an ordinary, fundamental, and defensive reaction to any poisonous boost that might compromise the host and may fluctuate from a confined response to an intricate reaction including the entire life form. Numerous substances called middle people are shaped or acknowledged either simultaneously or in progressive time groupings at the site of injury. Different cell sources are dependable to an etiological factor. These cell sources might incorporate neutrophils, basophils, pole cells, platelets, macrophages and lymphocytes. The arbiters of inflammation involved in the incendiary interaction and explained by the prior cells incorporate histamine, serotonin, plasma kinesis, lymphokines and prostaglandins. The cycle of inflammation can be summed up as follows.

- Beginning injury to tissues causing arrival of arbiters, histamine, serotonin, prostaglandins.
- An intense transient stage portrayed by nearby

vasodilatation and expanded slenderporousness.

- A postponed sub-intense stage, most conspicuously portrayed by invasion of leukocytes and phagocytic cells.
- A constant proliferative stage, wherein tissue degeneration and fibrosis happen.

1.3 Plant profile**1.3.a Hibiscus rosa sinensis^[3]**

Hibiscus Rosa-sinensis is a bushy, evergreen shrub or small tree growing 2.5–5 m (8–16 ft) tall and 1.5–3 m (5–10 ft) wide, with glossy leaves and solitary, brilliant red flowers in summer and autumn. The 5-petaled flowers are 10 cm (4 in) in diameter, with prominent orange-tipped red anthers (Dorling Kindersley, 2008). The flowers are large, conspicuous, and trumpet-shaped, with five petals and their colours can be white to pink, red, orange, peach, and yellow or purple that is 4–18 cm broad. The flowers from various cultivars and hybrids can be either a single flower or a double flower.



Fig. 1: Hibiscus Rosa Sinensis. Fig. 2: Dried Hibiscus Rosa Sinensis Flower.

Table 1: Taxonomical hierarchy.^[4]

<i>Kingdom</i>	<i>Plantae</i>
<i>Division</i>	<i>Tracheophyta</i>
<i>Subdivision</i>	<i>Spermatophytina</i>
<i>Class</i>	<i>Magnoliopsida</i>
<i>Superorder</i>	<i>Rosanae</i>
<i>Order</i>	<i>Malvales</i>
<i>Family</i>	<i>Malvaceae</i>
<i>Subfamily</i>	<i>Malvoideae</i>
<i>Tribe</i>	<i>Hibisceae</i>
<i>Genus</i>	<i>Hibiscus</i>
<i>Species</i>	<i>H. Rosa-sinensis</i>

Medicinal Uses^[5]

Hibiscus rosa sinensis is known as China rose belonging to the Malvaceae family. This plant has various important medicinal uses for **treating wounds, inflammation, fever and coughs, diabetes, infections caused by bacteria and fungi, hair loss, and gastric ulcers** in several tropical countries.

1.3.b Tinospora Cordifolia^[6]

It is a large, deciduous, extensively-spreading, climbing shrub with several elongated twining branches. Leaves are simple, alternate, and exstipulate with long petioles up to 15 cm (6 in) long which are roundish and peltate, both at the base and apex with the basal one longer and twisted partially and half way around. It gets its name

heart-leaved moonseed by its heart-shaped leaves and its reddish fruit. Lamina are broadly ovate or ovate cordate, 10–20 cm (4–8in) long or 8–15 cm (3–6 in) broad, seven nerved and deeply cordate at base, membranous, pubescent above, whitish tomatos with a prominent reticulum beneath. Flowers are unisexual, small on separate plants and appearing when the plant is leafless, greenish-yellow on auxiliary and terminal racemes. Male flowers are clustered, but female flowers are usually solitary. It has six sepals in two series of three each. The outer ones are smaller than the inner. It has six petals which are smaller than sepals, obovate, and membranous. Fruits aggregate in clusters of one to three. They are ovoid smooth drupelets on thick stalks with sub terminal style scars, scarlet or orange coloured.



Fig. 3: *Tinospora Cordifolia* Plant.Table 2: Taxonomical hierarchy.^[7]

Kingdom	<i>Plantae</i>
Subkingdom	<i>Tracheophyta</i>
Super division	<i>Supermatophyta</i>
Division	<i>Magnoliophyta</i>
Class	<i>Magnoliopsida</i>
Subclass	<i>Polypetalae</i>
Order	<i>Ranales</i>
Family	<i>Menispermaceae</i>
Tribe	<i>Tinosporeae</i>
Genus	<i>Tinospora</i>
Species	<i>Cordifolia</i>

Medicinal uses^[8]

Tinospora cordifolia has an importance in traditional ayurvedic medicine used for ages in the treatment of fever, jaundice, chronic diarrhea, cancer, dysentery, bone fracture, pain, asthma, skin disease, poisonous insect, snake bite, eye disorders.

2. MATERIAL AND METHODS**2.1 Collection of *H. Rosa Sinensis* & *T. Cordifolia* Plants-**

The flowers of *H. rosa sinensis* plant were collected from medicinal garden of the Shri Ram group of college, Banmore, Madhya Pradesh India. And leaves of *T. cordifolia* plant were collected from local area of Gwalior district, Madhya Pradesh, India.

2.2 Authentication-Flowers & leaves the selected *H. Rosa sinensis* & *T. Cordifolia* plants collected from medicinal garden of the Shri Ram group of college, Banmore, Gwalior, M.P And leaves of *T. cordifolia* plant were collected from local area of Gwalior Identified and authentication by **Dr. S.N Dwivedi, Department of Botany Javata P.G College, A.P.S. University, Rewa (486001) M.P INDIA** And date of authentication 01/02/2021

Voucher Specimen Number: J/BOT./2021-TC/19, J/Bot./2021-Hc/20

2.3 Chemical and Reagents: - Carrageenan, ethyl alcohol, acetone, chloroform, Mayer's reagent, Wagner's reagent, Dragendorff's reagent, Molisch's Reagent, Hager's reagent, Borntrager's Reagent, Benedict's Reagent.

2.4 Apparatus: - Plethysmometer, Soxhlet Apparatus, Electronic Balance, Heating Mantle, Petri Dish/ Glass rod, Funnel/ Measuring Cylinder.

2.5 Extraction Process^[9]

Extraction is also known as the hot continuous extraction process. The Soxhlet extractor setup consists of a round bottom flask, siphon tube, distillation path, condenser, cooling water inlet, cooling water outlet, heat source and thimble. In this method, sample dried, powdered, grinded

into small particles and placed in a porous bag or "thimble" made from a strong filter paper, which is placed, in thimble chamber of the Soxhlet apparatus. This method is not suitable for thermolabile compounds as extended heating may lead to degradation of compounds. This method maintains a relatively high extraction temperature with heat from the distillation flask. A fine coarse powder was obtained which was sieved through #40 to obtain uniformity. The powdered obtained was extracted in ethanol. Primarily the powdered root was subjected to petroleum ether or hexane in order to defeat the crude drug. As defatting, helps to remove the oily, fatty materials, and other low polar common compounds from the plants that helps to facilitate further processing easily with the yield of 8.87%. Two hundred grams of the powdered root was subjected to Soxhlet assembly in 60 % ethanol (200 g/1.5 L). The extract was then concentrated to dryness under pressure giving a dark greenish solid with a yield of 16.4 g (8.2%). The extracts were then made to powder under reduced pressure.

3.1 Qualitative Analysis: Different biochemical parameters like reducing sugar, Flavonoid, Terpenoid, Tannin, Saponin, Anthraquinone, glycosides, alkaloids etc. were tested.

3.1.a Test for Alkaloids^[10,11]

Mayer's Test: Extract treated with Meyer's reagent gives cream coloured precipitate which indicates the presence of Alkaloids.

Dragendorff's Test: Addition of Dragendorff's reagent (solution of Potassium Bismuth Iodide) to the extract gives reddish brown coloured precipitate.

3.1.b Test for Carbohydrates

Fehling's Test: Extract on boiling with equal proportions of Fehling's A and Fehling's B solution gives yellow to brick red coloured precipitate.

Molisch's Test: To the extract added few drops of Molisch's reagent and 1-2 ml conc. Sulphuric acid slowly through the sides of the test tube. Development of

a violet ring at the junction, indicate the presence of carbohydrates.

3.1.c Test for Proteins

Biuret Test: Extract on treatment with 4% Sodium hydroxide and 1% Copper sulphate solution gives violet or pink colour.

3.1.d Test for Glycosides

Borntrager's Test: Extract when shaken with Benzene gently for few minutes. The organic layer on treatment with Ammonia, give rose pink color in Ammoniacal layer.

3.1.e Test for Saponins

Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foams indicates the presence of saponins.

3.2 Experimental Animals

Studies were carried out using Wistar rats of either sex

weighing 180–200 g. They were obtained from central animal facility of **Shri Ram College of Pharmacy, Banmore, M.P., India (SRCP/M.Pharm/IAEC/74/20-21)** and was maintained in polypropylene cages on rodent pellet condition of controlled temperature ($22\pm 2^\circ\text{C}$) and acclimatized to 12/12 h light/dark cycle. Free access to food and water was allowed until 2h before the experiment. The care and maintenance of the animals was as per the approved guidelines of the "Committee for the purpose of control and supervision of experiments on animals (CPCSEA)". All experiments on animals were conducted according to the guidelines of establishment's ethical committee on animal experimentation.

The rats were randomly allocated into five groups of five rats each for the four Different experimental animal models. We have used Rat models for testing Anti-inflammatory.

3.3 Chemicals: Carrageenan, Standard drug (Indomethacin).

3.4 Experimental Design.

Group	Name of Group	Treatment	Dose
I	Control	Normal Saline	0.9ml/kg
II	Standard Drug	Indomethacin	5mg/kg
III	Test Group(A)	<i>T. Cordifolia</i> leaves extract and <i>H. Rosa-sinensis</i> flower extract	100mg/kg. (Both extract)
IV	Test Group(B)	<i>T. Cordifolia</i> leaves extract and <i>H. Rosa-sinensis</i> flower extract	200mg/kg (Both extract)

4. RESULTS AND OBSERVATION

The phytochemicals present in the plant material was extracted. The solvent, ethyl alcohol was used for the separation of chemical component.

constituents employing standard screening test. Conventional protocol for detecting the presence of steroids, alkaloids, tannins, flavonoids, glycosides, etc., was used.

4.1 Phytochemical Screening^[12-15]

The extract was screened for the presence of various

Table 4: Phytochemical Screening.

S. NO.	Test	Extract (ethanol)	
		<i>H. Rosa sinensis</i> (Flower)	<i>T. Cordifolia</i> (Leaf)
1.	Alkaloids	+	+
2.	Carbohydrates	+	+
3.	Proteins	+	-
4.	Amino acids	-	+
5.	Glycosides	+	+
6.	Flavonoids	+	-
7.	Phytosterols	+	-
8.	Fats and oils	+	-
9.	Phenolics and tannins	-	-
10.	Volatile oils	-	-

(+) Indicates positive result, (-) Indicates negative result

4.2 Evaluation of Anti-Inflammatory Activity^[16-17]

4.2.a Carrageenan Induced Paw Edema in Rats

In the present study, anti-inflammatory activity was

determined in wistar rats of either sex according to the method of Winter et al. All groups were injected with 1% carrageenan (in 1 % CMC) solution into the sub-

plantar region of rat right hind paw. The first group is referred as Control Group as they received normal saline, whereas third and fourth groups received low dose (100mg/kg) and higher dose (200mg/kg) respectively of *Tinospora Cordifolia* and *Hibiscus* through oral gavage. Second group served as standard, received Indomethacin (5mg/kg) through oral gavage. Before 1 hour of injecting of carrageenan the rats were treated with different doses of *Tinospora Cordifolia* and *Hibiscus Rosa-sinensis* Indomethacin and normal saline.

The volume of paw oedema was measured by displacement of water column in Plethysmometer (Orchid Scientific) immediately after carrageenan injection at 1, 2, 3, and 4 hours.

% Inhibition of paw oedema = $\frac{O_c - O_t}{O_c} \times 10$

Where, O_c = represent average increase in paw volume (average inflammation) of the control group of rats at a given time.

O_t = was the average inflammation of the drug treated (i.e., plant extracts or test drug aspirin) rats at the same

time. The difference in the initial 0h and volume at +1h indicate paw edema at 1h following carrageenan administration. Accordingly paw edema at +1, +2 +3, and +4 hours was calculated.

4.2.b Plethysmometer^[18-19]

A Plethysmometer designed for precise and rapid screening of small rodents for inflammation or oedema of the paw. The volume is determined by weight and specific gravity, based on the principles of Archimedes. When the animal paw is dipped into the water in the cell, down to the point pre-marked on the paw, the exact volume of the paw will be displaced. A finger rest enables stable measurement to be made.

It is designed to provide a highly useful tool in the measurement of small volume changes. This test is typically used to follow the evaluation of inflammatory response experimentally induced in rodents and to screen potential anti-inflammatory or anti-oedema properties of pharmacological substances.



Fig 4: - Plethysmometer.

4.3 Evaluation^[20]

The mean reaction time for each treated group was determined and compared with that obtained for each group before treatment. Percentage increase in reaction time (I %), was derived, using the formula $I\% = \left\{ \frac{I_t - I_o}{I_o} \right\} \times 100$, Where I_t = reaction time at time, t, and I_o = reaction time at time zero (0 h). The animals were subjected to the same test procedure at +1, +2, +3, +4 and +5 hr. after the administration of test/standard/control drug.

4.4 RESULTS

In carrageenan induced rat paw oedema there was significant reduction in paw volume in Indomethacin

standard group ($p < 0.05$) as compared to control group at 4-hour readings. There was dose dependent decrease in paw volume in ethanolic extract treated groups. The Ethanolic extract (100mg/kg and 200mg/kg) significantly ($p < 0.05$) and dose-dependently inhibited carrageenan-induced rat paw oedema (60.80 and 75.19%, respectively) when compared with control group after 1 hour of carrageenan injection.

Table 7: Effect of Ethanolic extract of *Tinospora Cordifolia* and *Hibiscus Rosa-sinensis* carrageenan induced rat paw oedema (Mean paw volume).

Group	Paw volume (mean±S.E)	Paw volume(ml)				
		1hr	2hr	3hr	4hr	5hr
Control	1.212 ± 0.015	1.761 ± 0.185	2.370 ± 0.127	2.157 ± 0.183	2.151 ± 0.012	2.100 ± 0.185

Indomethacin 5mg/kg	1.195± 0.016	1.602±0.221	1.237±0.127	1.140±0.268	0.578±0.183	0.318±0.176
Combina tion-I	1.196±0.065	1.687±0.094	1.577±0.164	1.467 ±0.09	1.300±0.127	0.921±0.182
Combination-II	1.208±0.018	1.601±0.117	1.470±0.216	1.340±0.176	1.180±0.120	0.799±0.127

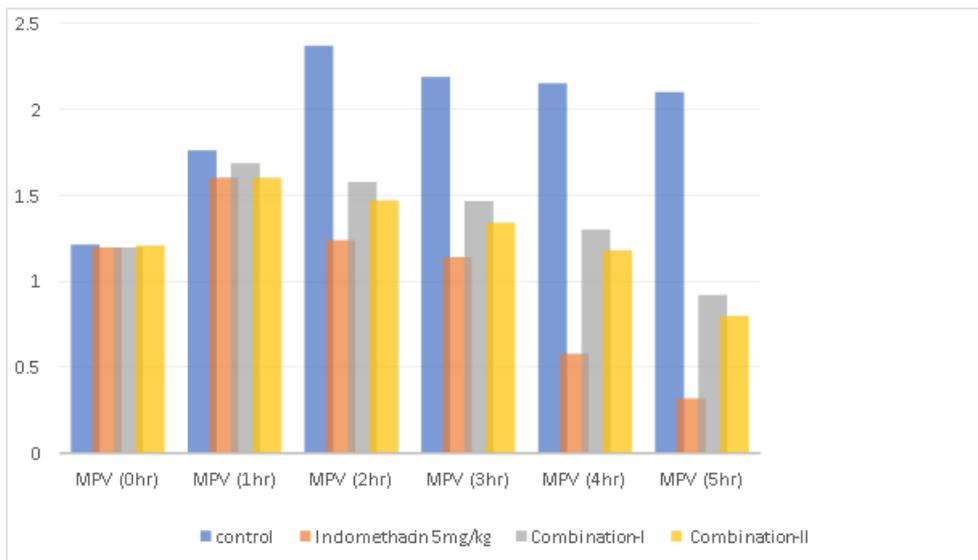


Fig. 5: Mean Paw volume (MPV).

Table 8: Effect of Ethanolic extract of *Tinospora Cordifolia* and *Hibiscus Rosa-sinensis* carrageenan induced rat paw edema (Percentage of inhibition).

Treatment Group	(%) inhibition (0hr)	(%) inhibition (1hr)	(%) inhibition (2hr)	(%) inhibition (3hr)	(%) inhibition (4hr)	(%) inhibition (5hr)
Control	-	-	-	-	-	-
Indomethacin 5mg/kg	1.40 %	9.02 %	47.14 %	47.14 %	73.12%	84.85%
Combination-I	1.32 %	4.20 %	33.45 %	31.98 %	39.56%	60.80%
Combination-II	0.33 %	9.08 %	37.97 %	37.87%	45.14%	75.19%

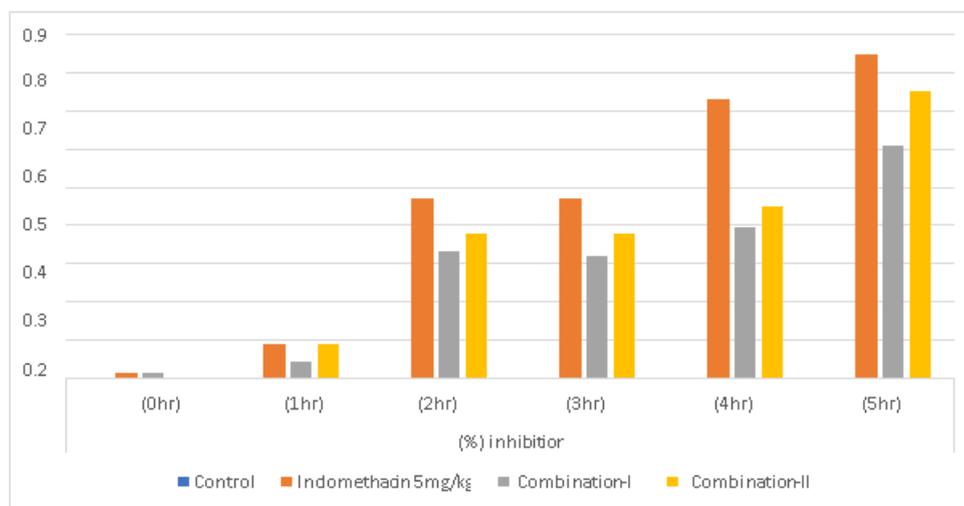


Fig. 6: Effect of Ethanolic extract of *Tinospora Cordifolia* and *Hibiscus Rosa-sinensis* carrageenan induced rat paw edema (Percentage of inhibition).

5. DISCUSSION

The carrageenan induced rat paw edema is considered as the most suitable in vivo model to study anti-inflammatory drugs both steroidal and nonsteroidal since it involves several mediators. In this study, anti-

inflammatory effects of ethanolic extract of *Tinospora Cordifolia* & *Hibiscus Rosa-sinensis* are investigated after sub-plantar injection of carrageenan in mouse paw. This study showed that the crude extracts (ethanol) of *Tinospora Cordifolia* & *Hibiscus Rosa-sinensis* possess

anti-inflammatory activities in rat model. The observation that the ethanolic extract of *Tinospora Cordifolia* & *Hibiscus Rosa-sinensis* exhibited greater anti-inflammatory activity, more of the active principle (s) responsible for the anti-inflammatory activity might be present in higher concentration in the ethanolic extract. The presence of edema is one of the prime signs of inflammation. In addition, it is a method that been frequently used to assess the anti-edematous effect of natural products. Furthermore, carrageenan is devoid of apparent systemic effects and exhibits a high degree of reproducibility. The local injection of carrageenan induces inflammatory reactions in two different phases. Though it is difficult to speculate the time of onset for anti-inflammatory activity, ethanol extract of the dried leaves of *Tinospora Cordifolia* and flowers of *Hibiscus Rosa-sinensis* for anti-inflammatory activity showed rapid onset, i.e., starting from 1hr. The duration, however, seems to be fairly long. To get full pictures of their pharmacokinetic profiles, further investigation should be undertaken. Both extracts and fractions of *Tinospora Cordifolia* & *Hibiscus Rosa sinensis* showed persistent anti-inflammatory activity during the five hours observation time. This study may give hint about the onset and duration of action of the extracts.

6. CONCLUSION

The results of this study show that dried leaves and flowers extracts of *T. Cordifolia leaves extract* & *H. Rosa-sinensis flower* exhibited anti-inflammatory activity with ethanol extract having higher activity. Phytochemical test of *Tinospora Cordifolia leaves extract* & *Hibiscus Rosa-sinensis flower* extracts the alcoholic extracts revealed the presence of Carbohydrate, Alkaloid and Glycosides, saponins. Further investigation, however, should be pursued after isolation and characterization of the active principle in order to come up with the active compound responsible for the anti-inflammatory properties of the *T. Cordifolia* & *H. Rosa-sinensis*. However, other studies need to be performed to confirm the exact mechanism and anti-inflammatory activity of the plant in Carrageenan Induced Paw Edema inflammatory models. Proper regulatory mechanism is recommended to ensure safety & efficacy of herbal products. The wide range of chemical structures provided by natural sources is under investigation for their chemical as well as pharmacological screening. Evaluation of Indian traditional medicine is possible through the proper exploitation of wide biodiversity and great ancient treatise with light of modern tools & techniques.

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