

Physico-Phytochemical investigation and Anti-inflammatory screening of *Capsicum annum* L. and *Hemidesmus indicus* (Linn.) R.Br.

ABSTRACT

Capsicum annum L. (Family: Solanaceae) and *Hemidesmus indicus* (Linn.) R.Br. (Family: Asclepiadaceae) are commonly used in Tamilnadu for treating various ailments in the native system of medicine. The hydroalcoholic extracts of both plants at dose level of 100 mg/kg body weight showed demonstrable anti-inflammatory activity in the carrageenan-induced hind paw model in rats. Nevertheless, the overall anti-inflammatory activity exhibited by the extracts are found to be less as compared with that of standard drug indometacin. Preliminary physico-phytochemical analysis of the plants in question were attempted. The results are highlighted and discussed.

Key words: *Capsicum annum* L., *Hemidesmus indicus*(Linn.)R.Br., Physico-phytochemical characters, anti-inflammatory activity, carrageenan-induced hind paw edema rat model, ethnomedicine.

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INTRODUCTION

Capsicum annum L. (Fam. Solanaceae) is known as Raktamaricha in Sanskrit and Milagai in Tamil. It is an annual shrub with many angular branches, leaves are simple, flowers white or violet in clusters. It is cultivated throughout the year in both tropical and temperate regions. The plant shows intensified flowering and fruiting during March and May and this period is considered quite appropriate for the collection of plant specimens.

The fruits are useful in cephalgia, gout, arthritis, seatica, anorexia, dyspepsia, flatulence, cough, cardiac debility, malaria and intermittent fevers, cholera, indolent fevers and other vitiated conditions of Kapha¹.

Hemidesmus indicus (Linn.) R.Br. (Family: Asclepiadaceae) is a prostrate woody, perennial herb with simple leaves. Flowers are greenish on the outside and purple on the inside. Roots are dark brown and have pleasant taste and smell. This plant is represented by single genus and single species (Monogenetic). It is a wild plant showing intensified flowering during November to February and time of collection of root was done during months of September October.

It is known as Sariba in Sanskrit and Nannari in Tamil. The roots are useful for treating vitiated conditions of Pitta, burning sensation, Leukoderma, Leprosy, skin diseases, asthma, bronchitis, hemicrania, epilepsy, helminthiasis, diarrhea and haemorrhoids, syphilis, fever and inflammation.¹

The medicinal properties *C. annum* L. and *H. indicus*(Linn.)R.Br., are well explored.^{2,3} A comprehensive review on plants showing anti-inflammatory activity was attempted by earlier workers who stated that inflammation was termed as King of Misereries.^{4,5}

Capsicum annum L. leaves and fruits are used for abortion and to correct menstrual disorders by some tribal communities of India and *H. indicus*(Linn.)R.Br., root is useful for treating sexual disorders and impotence.⁶ H. root is useful for anti-ulcerogenic⁷, *C. annum* L., fruit powder is mixed with honey and applied to site of dog bite to check hydrophobia in cattle.⁸ *C. annum* L., fruit powder is mixed

with coconut oil and warmed up. This mixture is applied as a remedy for ear problems by Irulas of The Nilgiris and H. root is chewed as a remedy for urinary problems by them.⁹ H. root paste is applied to cure skin problems.¹⁰ *H. indicus*(Linn.)R.Br., roots confers protection from Rifampicin and isoniazid induced hepato toxicity.¹¹

Inflammation is a tissue-reaction to infection, irritation to foreign substance and forms an integral part of host defense mechanism. Inflammation which was recognized as a simple allergic reaction for many decades is currently being considered to underlie pathophysiology of a much broader spectrum of diseases.¹² There are four cardinal signs of inflammatory conditions and they are redness (rubor), heat (calor), swelling (tumor) and pain (dolor).¹³

There are several tissue factors or mechanisms that are known to be implicated in the inflammatory reactions such as histamine, bradykinin, and prostaglandins¹⁴ and inflammation is a homeostatic phenomenon. The following documented research data in regard to plants under study deserve a mention here¹⁵⁻¹⁹.

In the light of skimpy data on anti-inflammatory potential of above plants used in traditional system of medicine, the present work was undertaken to screen anti-inflammatory potential of the study plants to throw more light in this direction. Hence, this present work to supplement additional information on the topic.

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MATERIALS AND METHODS

(A) Collection of plant materials

The fruits of *Capsicum annum* L., and roots of *Hemidesmus indicus* (Linn.) R.Br., were collected during the months of March and April from the forest areas located in and around Thanjavur district. The collected specimens were authenticated by a Botanist (SR) by comparing them with Regional Herbarium of Botanical survey of India, Coimbatore. The Voucher specimens were deposited in the herbarium of Survey of Medicinal plants and Collection Unit (CCRH), Ooty as well as in the herbarium of Dept. of Botany, A.V.V.M. Sri Pushpam College, Poondi for future reference. The work was carried out in the Dept. of Pharmacology, Periyar College of Pharmaceutical sciences, Tiruchirapalli 620 021 (T.N). The clearance of animal ethical committee has been obtained from the college.

The samples were washed with distilled water and dried under shade, mechanically pounded to get coarse powder and passed through 40 number sieve meshes. The sample powders were processed in such a way that they are suitable for both powder studies and phytochemical analysis.

(B) Preparation of extracts²⁰⁻²¹

The coarse powder 150 grams of the given samples were extracted with 600 ml of ethanol (95%) by continuous hot percolation using Soxhlet apparatus until the completion of extraction procedure. The successive extractions were done separately for each solvent namely Chloroform, Ethanol, Ether, Benzene, Hexane and water. The powder solvent ratio employed for the present study was 1:4. On completion, the extracts were filtered and the solvents were removed by distillation and dried under reduced pressure and controlled temperature 50°-60°. They were refrigerated until use. Both fruit and root powder samples were subjected to various analyses such as organoleptic characters²², fluorescence studies²³, physico-chemical properties²⁴, preliminary phytochemical screening^{25,26}. The anti-inflammatory activity of the hydroalcoholic extracts was evaluated as described below.

Animal studies

Albino rats of either sex weighing 150 to 200 g belonging to Wistar strain were used in this study. The animals were procured from a registered animal dealer of the College (Sri Venkateswara Enterprises, Bangalore). The rat pellet feed was also supplied by the same firm (Hindustan Lever Company). The animals were acclimatized to the laboratory condition by subjecting them to dark and light cycles for 12 hours period before commencement of work. All the animals were given food and water ad libitum.

Evaluation of anti-inflammatory activity²⁷

- Group I served as control (5 ml/kg Normal Saline)
- Group II received hydro alcoholic extract of *C. annum* fruit (100 mg/kg body weight)
- Group III received hydro alcoholic extract of *H. indicus* root (100 mg/kg body weight)
- Group IV served as reference standard drug (10 mg/kg b.w).

0.1 ml of 1 per cent w/v carrageenan solution in normal saline was injected to all the groups. Carrageenan is a sulphated polysaccharide obtained from sea weed (Rhodophyta). It causes inflammation by releasing histamine, 5HT, bradykinins and prostaglandins that produce inflammation and Oedema. The extracts were administered orally by using a catheter. Indomethacin reference drug was given orally.

After one hour of the administration of drugs, 0.1 per cent w/v carrageenan solution in normal saline was injected to the sub plantar tissue of the left hind paw of the rat and the right hind paw served as normal. The paw volumes of the rats were measured using the Digital plethysmograph (Ugo basile, Italy) at intervals of 0 min, 30, 60, 120 min and 180 min (Fig.I and II).

The mean paw oedema volume of the extract treated groups was compared both with control group and the standard drug Indomethacin treated groups. Thus, oedema volume in control (Vc) and extract treated groups (Vt) was computed. The percentage inhibition was calculated using the formula as shown below.

$$\text{Percentage inhibition} = \frac{V_c - V_t}{V_c} \times 100$$

where, Vc-Edema volume of control group
Vt Edema volume of test group

The results are tabulated and analysed using student's 't' test to know the level of statistical significance.

Statistical analysis

All the values represent the mean and standard error of six animals. The student unpaired 't' test was employed for examining the statistical significance at 1% and 5% probability levels.

RESULT AND DISCUSSION

Tables 1-5 narrate the results of physico-phytochemical data on the fruit (*Capsicum annum* L.) and root (*Hemidesmus indicus* (Linn.) R.Br.). Both are characterized by rough texture. The fruit appears yellow in colour and root light green. *C. annum* L., fruits possess hot taste with pungent irritating odour. *H. indicus* (Linn.) R.Br., root has aroma and palatability. These powders when viewed under U.V. light at 365 nm appear yellowish red (*Capsicum*) and brown (*Hemidesmus*) (No photographic evidence are provided here). Under normal light, they are yellow and light brown respectively. After treating with various chemical reagents, they displayed wide-ranging colour variations.

The ethanol extraction values are higher for the fruit and root samples (31.09%). The root records higher water extractive value than fruit indicating the presence of more water soluble constituents in it. Except the moisture content value fruits exhibit consistently higher values for total ash, insoluble ash and mineral contents. Preliminary phytochemical screening showed the presence of glycosides, phytosterols, saponins, tannins and phenolic compounds, proteins and free amino acids fats and volatile oils in the root. Fruits contain alkaloids, carbohydrates phytosterols and volatile oils. The distinctive absence of flavonoids in the fruit and root samples of the study plants is noteworthy feature here.

Table 1
Organoleptic characters

Characters	<i>Capsicum annum</i> L.,(Fruit powder)	<i>Hemidesmus indicus</i> (Linn.)R.Br., (Root powder)
Colour	Yellow	Light green
Taste	Hot	Palatable
Odour	Pungent and irritating	Aroma
Texture	Rough	Rough

Table 2
Fluorescence properties of study samples

Treatment	Day light		UV light (365 nm)	
	<i>C. annum</i> L. Fruit	<i>H. indicus</i> (Linn.) R.Br., Root	<i>C. annum</i> L. Fruit	<i>H. indicus</i> (Linn.) R.Br. Root
Powder as such	Yellow	Light Brown	Yellowish Red	Brown
Sample +1 N NaOH	Yellowish white	Dark brown	Yellow	Blackish brown
Sample+1 N HCl	Light yellow	Brown	Yellow	Dark brown
Sample + 50% H ₂ SO ₄	Reddish black	Dark brown	Dark reddish black	Dark brownish black

Table 3
Extractive values for samples in different solvent systems

Solvent	<i>C. annum</i> L. Fruits (%)	<i>H. Indicus</i> (Linn.)R.Br. Root (%)
Chloroform	21.03	17.09
Ether	24.51	19.93
Benzene	20.52	26.05
Hexane	28.56	29.14
Water	19.84	24.01
Ethanol	30.51	31.09

Table 4
Physico-chemical properties of samples

Character	<i>C. annum L.</i> Fruits (%)	<i>H. Indicus(Linn.)R.Br.</i> Root (%)
Moisture content	7.40	9.20
Total ash	4.96	3.93
Insoluble ash	3.26	3.24
Total mineral content	1.70	0.69

Table 5
Phytochemical constituents of samples

S.No.	Type of constituents	<i>C. annum L.</i>	<i>H. indicus(Linn.) R.Br.</i>
1.	Alkaloids	+	-
2.	Carbohydrates	+	-
3.	Glycosides	-	+
4.	Phytosterols	+	+
5.	Saponins	-	+
6.	Fixed oils and fats	-	-
7.	Tannins and phenolic compounds	-	+
8.	Proteins and free amino acids	-	+
9.	Gums and Mucilage	-	-
10.	Flavonoids	-	-
11.	Lignin	-	-
12.	Volatile oils	+	+

(-) Absent (+) Present

Table 6 shows the results of anti-inflammatory screening of extracts which revealed good anti-inflammatory potentials of hydroalcoholic extracts of *C. annum L.*, (fruits) and *H. indicus(Linn.)R.Br.*, (roots) in the carrageenan induced hind paw oedema model used here. Inflammation caused by carrageenan, an Inflammogen was significantly reduced by the two extracts ($P < 0.001$). At the same oral dose levels of 100mg/kg b.w of fruit and root given, root showed enhanced anti-inflammatory activity than fruit which was evident from their respective percent inhibition values in comparison with control ($P < 0.001$). However, the overall anti-inflammatory activities of the extracts was found to be less than that of standard drug Indometacin. This preliminary finding awaits further studies on a larger set of data at different dose levels and in various models of inflammation to comment further on this trend.

It has been reported^{27,28} that edema which results after carrageenan inflammation is a biphasic event. The initial phase is attributed to the release of histamine and serotonin. The edema maintained in the interval of first and second phase is due to Kinin like substances. The second phase is believed to be accelerated by substances like prostaglandins. Many workers opine that the second phase of edema is very sensitive to drugs like hydrocortisone, phenylbutazone and Indomethacin.

To sum up, it can be concluded the two extracts under study possess significant anti-inflammatory activity at a dose of 100 mg/kg b.w in the model chosen for this work.

Table 6
Effect of hydroalcoholic extracts on carrageenan-induced hind paw edema in rats

Treatment	Dose mg/kg. bw (p.o)	Paw volume increase after 3 hr (ml) Mean ± SE	Per cent inhibition (%)	t value
Control	5 ml/kg	102.82 ± 8.32	-	-
Capsicum annum L.	100 mg	62.96 ± 4.80	38.76	4.15*
Hemidesmus indicus(Linn.)R.Br.	100 mg	54.36 ± 5.20	47.13	4.93**
Indomethacin (Standard drug)	10 mg	38.20 ± 2.62	62.84	8.79**

All values represent Mean ± SE; n = 6, df – 10;
 * 0.01>P<0.001 – significant; ** P<0.001 – significant
 p.o = oral route; b.w – body weight



Fig.I.
 Carrageenan solution injected in the albino rat



Fig.II.
 After Carrageenan induced in left Paw of albino rat

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