

PROXIMATE ANALYSIS, PHYTO-CHEMICAL SCREENING AND ANTI-INFLAMMATORY ACTIVITY OF *COCCINIA INDICA*

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ABSTRACT

Coccinia indica, an annual creeper, is available all over India. In the present investigation, petroleum ether extract, 60% methanolic extract and aqueous extracts of the whole plant were made using hot extraction procedure using soxhlet apparatus. The qualitative phyto-chemical screening procedure was performed on each extract. Phyto-chemical study reveals that flavonoid was only present in the methanolic extract. The anti-inflammatory activity of each extract and that of a standard drug, diclofenac sodium were studied using Carageenan induced rat paw oedema model. The extracts and the standard drug were administered orally.

It was observed that 60% methanolic extract of *Coccinia indica* produced maximum anti-inflammatory activity even more than the standard drug, diclofenac sodium after 3 hours. Petroleum ether extract and the aqueous extract produced less percentage of inhibition in comparison the standard drug.

Key words: *Coccinia indica*, anti-inflammatory, petroleum ether, aqueous, flavonoid.

INTRODUCTION

Coccinia indica or ivy gourd is a plant that grows wild in many places in India and is extensively used as ayurvedic medicine. It is more commonly seen in areas like Bengal, Bihar and Orissa. This plant has been widely used in traditional Indian medicinal system (Ayurvedic, Unani, Siddha). Leaves and stems of this plant have traditional uses in the treatment of skin diseases, gastrointestinal disturbances and diseases, diabetes, urinary tract infection and respiratory tract related trouble. Roots of this plant have uses in reducing pain in joints, skin diseases, and gastrointestinal disturbances.

Niazi et al. have shown that the 50 mg/ Kg dose of aqueous extract of fresh leaves produces anti-inflammatory activity equivalent to 20 mg/Kg of diclofenac sodium against carageenan induced paw oedema in Wister rats but it was significantly pronounced at higher doses. They also established that the aqueous extract leaves at a dose of 300 mg/kg produces reduction in hyperpyrexia comparable to paracetamol. This extract also produced analgesic activity comparable to morphine [1]. The effect of aqueous and methanolic extract of ivy gourd leaves on aspirin induced Wister rat model was studied by Majumder *et.al.*. They found that the methanolic extract had a significant

antiulcer effect in a dose dependant manner while the aqueous extract produced the antiulcer effect insignificantly [2]. Mallick *et al.* highlighted significant antiglycemic activity of the aqueous methanolic (2:3) extract of *C. indica* [3]. *Coccinia indica* significantly reduces the effect on both glucose – 6- phosphate and fructose – 1,6- biphosphate in both normal and streptozocin induced Type I diabetic rats [3-7]. The fruit powder of *C. indica* has anti-inflammatory activity and produces resistance against pain after 30 minutes [8]. Sutar *et. al.* studied the anti-inflammatory activity of alcoholic and aqueous extract of *C. indica* against carageenan and dextran induced rat paw oedema. Both the extracts showed significant activity (* $p < 0.05$ & ** $p < 0.01$) compared with the 10 mg/Kg diclofenac sodium in both of these models [9].

As there is no reference of the anti-inflammatory aspect of the whole plant extract and particularly on 60% methanolic extract and petroleum ether extract, in the present investigation, a detailed study has been carried out on the anti-inflammatory activity of the aqueous, 60% methanolic, and petroleum ether extract of whole plant of *C. indica*. Besides these, the phytochemical investigation was also done on each extract.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Fresh plant/plant parts were collected randomly from the local region of Sodepore, West Bengal, India. The taxonomic identities of these plants were confirmed by Botanical Survey of India, Government of India, Hawrah-711103 and the voucher specimen of the plants were preserved. Fresh plant material was washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Drugs and Chemicals

Carrageenan was purchased from Merc Pvt.Ltd and Diclofenac Sodium was obtained from Zydus Cadilla Ltd. The analytical grade solvent and other chemicals of E.Merck were used.

Proximate Analysis of the whole Plant of *Coccinia indica*

The total ash value, acid soluble ash value, acid insoluble ash value, extractive values of *C. indica* in petroleum ether, 60% methanol and water and moisture content were determined by the following standard procedures:

Determination of Ash Values

Accurately weighed 5gms of powdered was taken in a dried silica crucible. It was incinerated at temperature 450°C, until free from carbon and then cooled. The weight of total ash was taken and the percentage of it was calculated with reference to the air dried sample.

Determination of acid insoluble Ash Values

The total ash obtained was boiled for 5 mins with 25 ml of 2N HCl, filtered and the insoluble matter was collected on ash less filter paper. Then it was washed with hot water, ignited in tarred crucible cooled and the residue obtained was weighed. Finally the percentage of acid insoluble ash was calculated with reference to the air dried drug.

Determination of acid soluble Ash Values

The total ash obtained was boiled with 25 ml of water for few minutes. The insoluble matter was collected on ash less filter paper, washed with hot water and ignited for 15 minutes at temperature not exceeding 450°C. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug.

Determination of Extractive Values

Determination of Petroleum Ether Soluble Extractive Value

Accurately weighed powder (5 g) was taken and a thimble pack was prepared. The crude drug in the pack was extracted with solvent petroleum ether (40 – 60° C in a continuous extraction (Soxhlet) apparatus for 6 h. The extract was filtered; and the filtrate was evaporated and dried at 105°C to a constant weight.

Determination of Methanol Soluble Extractive Value

Accurately weighed powder (5 g) was taken and macerated with 100 ml of 60% methanol for 24 h. The contents were frequently shaken during the first 6 h and allowed to remain for 18 h. After 24 h, the extract was filtered and 25 ml of the filtrate was evaporated. The extract was dried at 105°C to a constant weight.

Determination of Water Soluble Extractive Value

Water soluble extractive value was determined using the procedure described for methanol soluble extractive, except that chloroform water was used for maceration.

Determination of moisture Content

The Loss on Drying Test is designed to measure the amount of water and volatile matters in a sample when the sample is dried under specified conditions. Accurately weighed 5gms of powdered was taken in a china dish. It was kept for 30 minutes in a hot air oven at 105 - 110°C. The percentage of moisture content was then calculated with reference to the air dried drug at different times.

Extraction of Plant Material

The dried coarse powder (600g) of the whole plant of *Coccinia indica* was extracted in a soxhlet apparatus serially with petroleum ether, chloroform, 60% methanol and water for 72 hours. The resulting extracts were filtered and concentrated at 50 - 60°C temperature under reduced pressure. Each extract was then kept in a sealed tube at 4-8°C temperature in a refrigerator.

Phyto-chemical Screening

Each dried extract was subjected to test for the presence of carbohydrate, acidic compound, alkaloid, flavonoid, glycoside, and tannin by adopting the standard procedures of analysis [10 -11].

Animals

Adult Wister rats weighing between 160 - 180g of either sex were used for pharmacological study. The animals were

housed in poly vinyl cages in departmental animal house in a well ventilated room at 22±02°C having light and dark cycles of 10 and 14 hours respectively for one week before and during the experiment. They were fed with standard rodent pellets and provided with drinking water *ad libitum*. To keep the hydration rate constant, food and water were stopped 12 hours before the experiments. The ethics for use of experimental animals were followed carefully.

Anti-inflammatory Study

In this experiment, carrageenan-induced rat hind paw edema was used as the animal model of acute inflammation according to Winter *et al.* [12] and described previously (Saha *et al.* 2007) [13]. The experimental procedure was approved by the authorized ethical committee.

The rats were divided into five groups containing five rats in each group (one control, one standard, one petroleum ether extract, one 60% methanolic extract and one aqueous extract). The extracts were suspended in 2% Tween80 and each extract was administered orally at 200mg/kg body weight one hour before the carrageenan injection. Diclofenac sodium at the dose of 10 mg/kg body weight was used as standard anti-inflammatory agent.

The Rats were injected with 0.1 ml of 1% suspension of carrageenan with 2% gum acacia in normal saline, in the left hind paw of the rats 1h after the oral administration of test materials. The paw was marked with marker at the level of lateral malleolus. The paw volume was measured by plethysmometer at 1, 2, and 3 hours after the carrageenan injection.

The percentage of inhibition of edema was calculated using formula:

$$\% \text{ Inhibition of edema} = (V_c - V_t / V_c) \times 100$$

Where V_t = Paw volume in test group animals. V_c = Paw volume in control group animals.

RESULTS AND DISCUSSION

The data obtained after the proximate analysis and extractive values in different solvents mentioned above are shown in Table 1. It shows that all the experimental

values are well within the limits prescribed by Indian Ayurvedic Pharmacopoeia.

Each extract was subjected to phytochemical screening and the following chemical constituents were found to present in the extracts as shown in Table 2. Petroleum ether extract was found to contain sterol. Tannin, saponin glycosides and alkaloids were present in the chloroform extract. 60% Methanolic extract was found to contain sterol, tannin, flavonoids, saponin glycosides, carbohydrates and alkaloids. Aqueous extract of *C. indica* showed the presence of sterol, tannin, saponin glycosides, carbohydrates and alkaloids

Aqueous extract, petroleum ether (40 – 60 °C) extract and 60% methanolic extract were studied for anti-inflammatory activity. The results of the anti-inflammatory study after 3 hours are shown in Table 3. It reveals that all the extracts show a significant reduction in carageenan induced rat paw oedema at the dose of 200mg/kg body weight. However, both aqueous and petroleum ether extract showed 40.79% and 60% methanolic extract showed 57.24% whereas the standard diclofenac sodium at the dose of 10mg/Kg body weight showed 51.97% inhibition of oedema after 3 hours of administration.

Carageenan induced rat paw oedema is a suitable experimental animal model for evaluating anti-inflammatory activity of natural products [17]. It is believed to be a triphasic phenomenon. The first phase (one hour after carageenan administration) involves the release of serotonin and histamine from mast cells. The second phase (after 2 hours) is associated with the production of bradykinin and the third phase is mediated by prostaglandins, cyclooxygenase products and lipoxigenase

products [15]. The metabolites of arachidonic acid formed via the cyclooxygenase and lipoxigenase pathways represent two important classes of inflammatory mediators, prostaglandins (products of the cyclooxygenase pathway) especially prostaglandin E2 is known to cause or enhance the cardinal signs of inflammation, similarly, leukotriene B4 (product of lipoxigenase pathway) is a mediator of leukocyte activation in the inflammatory cascade [19].

CONCLUSION

From the present study, it can be concluded that 60% methanolic extract of *Coccinia indica* possesses more anti-inflammatory activity than the aqueous and petroleum ether (40 – 60° C) extract at the dose of 200mg/Kg body weight. 60% methanolic extract produces more reduction in rat paw oedema than the standard drug, diclofenac sodium at the dose of 10mg/Kg body weight. This better inhibition can be due to the presence of flavonoids as no other extract other than 60% methanolic extract contained flavonoid which can be responsible for the inhibition of prostaglandin synthesis. This study demonstrates the significance of this plant as anti-inflammatory agent. However, further study on the flavonoids isolated from 60% methanolic extract is necessary.

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Table 1: Physical Evaluation of *Coccinia Indica*

S. No	Parameter	Values (% w/w)
1	Loss on Drying	14
2	Total Ash Values	20.77
3	Acid insoluble ash Value	1.42
4	Water soluble ash Value	7.65
5	Water soluble extractive Value	15.5
6	Methanol soluble extractive Value	13.6
7	Petroleum ether soluble extractive Value	2.5

Table 2: Phyto-chemical constituents of different extracts of *Coccinia indica*

S. No.	Chemical Constituent	Petroleum Ether Extract	Chloroform Extract	60% Methanolic Extract	Aqueous Extract
1	Sterols	+	+	+	-
2	Tannins	+	+	+	+
3	Flavonoids	-	-	+	-
4	Glycosides	-	+	+	+
5	Phenols	-	-	+	+
6	Acidic compounds	-	-	-	-
7	Carbohydrates	-	-	-	+
8	Saponins	-	+	+	+
9	Alkaloids	-	+	+	+

+ Sign indicates presence

- Sign indicates absence

Table 3: Anti-Inflammatory Activity of Various Extracts of *Coccinia Indica* On Carageenan Induced Rat Paw Oedema

Group / Treatment	Dose	Mean Paw Oedema \pm S.E.M	% of Inhibition after 3 hours
Group I / Control	0.2 ml	0.76 \pm 0.0025	-
Group II / Standard	10mg/Kg	0.37 \pm 0.0054	51.97**
Group III / Petroleum Ether	200mg/Kg	0.45 \pm 0.0029	40.79*
Group IV / Aqueous	200mg/Kg	0.45 \pm 0.00015	40.79**
Group V / 60% Methanolic	200mg/Kg	0.33 \pm 0.00005	57.24**

Results are mean \pm S.E.M. (n=5) *P<0.05 & **p<0.01 compared to control**REFERENCES**

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