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 dependent 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]. Sutur 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% methanol 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 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 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 malleolous. 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: % Inhibition of edema = (Vc-Vt/Vc) x100 Where Vt = Paw volume in test group animals. Vc = 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 [15]. The metabolites of arachidonic acid formed via the cyclooxygenase and lipoxygenase 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 lipoxygenase 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 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.

**ACKNOWLEDGEMENT**

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<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameter</th>
<th>Values (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Loss on Drying</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>Total Ash Values</td>
<td>20.77</td>
</tr>
<tr>
<td>3</td>
<td>Acid insoluble ash Value</td>
<td>1.42</td>
</tr>
<tr>
<td>4</td>
<td>Water soluble ash Value</td>
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<td>5</td>
<td>Water soluble extractive Value</td>
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</tr>
<tr>
<td>6</td>
<td>Methanol soluble extractive Value</td>
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</tr>
<tr>
<td>7</td>
<td>Petroleum ether soluble extractive Value</td>
<td>2.5</td>
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</table>
Table 2: Phyto-chemical constituents of different extracts of Coccinia indica

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Chemical Constituent</th>
<th>Petroleum Ether Extract</th>
<th>Chloroform Extract</th>
<th>60% Methanolic Extract</th>
<th>Aqueous Extract</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Sterols</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Glycosides</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Phenols</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Acidic compounds</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Carbohydrates</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Saponins</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

* Sign indicates presence
- Sign indicates absence

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

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

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

REFERENCES


7. Shibib BA, Khan LA and Rahman R. Hypoglycaemic activity of Coccinia indica and Momordica charantia in diabetic rats: depression of the hepatic gluconeogenic enzymes glucose-6-phosphates and fructose-1,6-bisphospatase and elevation of both liver and red-cell shunt enzyme glucose-6-phosphate dehydrogenase, J of Biochemistry.1993;292:267-270.


