



## ANTI-INFLAMMATORY ACTIVITY OF LEAF EXTRACT OF *ASPARAGUS RACEMOSUS* WILLD

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### ABSTRACT

The aim of the present study was to explore the effect of leaf extract of *Asparagus racemosus* for its anti-inflammatory effect. Study on ethanol extract from the leaves of *Asparagus racemosus* Willd. of Indian origin revealed that ethanol extract displayed marked anti-inflammatory effect at a dose of 600 mg/kg causing a maximum inhibition of about 46 % in paw oedema induced by carrageenan. Phytochemical screening of the ethanol extract of leaves of *Asparagus racemosus* revealed that sterols and flavonoids type of compounds might be possibly responsible for the anti-inflammatory activity of the extract, which supports the folk medicinal utilization of this plant.

**Key words:** *Asparagus racemosus*, Asparagaceae, Anti-inflammatory, Carrageenan-induced pedal inflammation.

### INTRODUCTION

Plants, the first medicines for human beings, have played a remarkable role in health care since the ancient times. Traditional plant-based medicines still exert a great deal of importance to the people living in developing countries and also lead to discovery of new drug candidates for a variety of diseases that threaten human health. *Asparagus* is the name of a genus of plants, a member of the family Asparagaceae (formerly placed in the Liliaceae). The *Asparagus* genus is considered to be of medicinal importance because of the presence of steroidal saponins and sapogenins in various parts of the plant<sup>1</sup>. *Asparagus* is the Greek word for “stalk” or “shoot”. About 300 species of *Asparagus* are known to occur in the world in many countries in both; hemispheres and throughout temperate and tropical regions. Some of the European species are *A. officinalis*, *A. sprengeri* and *A. acutifolius*. *A. officinalis* is reported to be a popular vegetable consumed in many parts of the world<sup>2</sup>.

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Out of several species of '*Asparagus*' grown in India, *A. racemosus*, *A. gonaclades* and *A. adsendens* are most commonly used in indigenous medicine<sup>3</sup>. *A. racemosus* is commonly mentioned as a rasayana in the Ayurveda. Rasayanas are those plant drugs, which promote general well being of an individual by increasing cellular vitality or resistance.

A study of ancient classical Ayurvedic literature claimed several therapeutic attributes for the root of *A. racemosus* (Sanskrit : Shatavari) and has been specially recommended in cases of threatened abortion and as a galactagogue<sup>4</sup>. Root of *A. racemosus* has been referred as bitter-sweet, emollient, cooling, nervine tonic, constipating, galactagogue, aphrodisiac, diuretic, rejuvenating, carminative, stomachic, antiseptic<sup>5</sup> and as a tonic. Beneficial effects of the root of *A. racemosus* are suggested in nervous disorders, dyspepsia, diarrhoea, dysentery, tumors, inflammations, hyperdipsia, neuropathy, hepatopathy<sup>6</sup>, cough, bronchitis, hyperacidity and certain infectious diseases. However, no scientific proof, justifying all the above uses of the leaves of *A. racemosus* is available so far.

Based on the above-mentioned traditional uses of *Asparagus* genus, the present study was undertaken to evaluate anti-inflammatory effect of the ethanol leaf extract obtained through successive solvent extractions from *Asparagus racemosus* in order to prove the ethnopharmacological application of the plant for anti-inflammatory purpose in Indian folk medicine.

## EXPERIMENTAL

### Plant material

The plant material (*Asparagus racemosus* Willd.) was collected from Chikkala Village, near Nidadavolu, West Godavari Dist., A.P., India in September 2002. The identification of the plant sample was carried out by Dr. M. Venkaiah, Associate Professor, Department of Botany, A. U., Visakhapatnam. The specimen (voucher No. BMK/BGR-10/2003) was kept in the Herbarium of the Phytochemistry and Pharmacognosy Specialization, Andhra University, Visakhapatnam, Andhra Pradesh, India.

### Chemicals

All solvents (Hexane, chloroform and ethanol) were procured from Ranbaxy (India).

### Animals

Sprague Dawley rats weighing between 175-225 g were used. The animals were left for 7 days for acclimatization to animal room conditions and were maintained on standard pellet diet and water *ad libitum*. The food was withdrawn on the day before the experiment,

but allowed free access of water. Throughout the experiments, animals were processed according to the suggested national ethical guidelines for the care of laboratory animals.

### **Preparation of the extracts**

The plant material of *Asparagus racemosus* was dried under shade, ground mechanically to fine powders in a grinder and weighed accurately as 450.86 g. The powdered material was subjected to successive solvent extraction with ethanol using Soxhlet apparatus. The extract was concentrated under vacuum (50°C), dried completely and weighed (76 g). A portion of ethanol extract (10 g) was kept aside for anti-inflammatory activity and the remaining portion was fractionated with hexane and then with chloroform until the solvent used for extraction becomes colourless. The hexane, chloroform and residual ethanolic fractions were concentrated under vacuum (50°C), dried completely and weighed. Their weights were given as follows: Hexane fraction (25 g), chloroform fraction (20 g) and residual ethanolic fraction (16 g).

### **Phytochemical screening**

The ethanol extract and its fractions (hexane, chloroform and residual ethanolic fractions) were tested by the Libermann Burchard, Shinoda, foam, ferric chloride, Mayer's, Dragendorff's, and Molisch's tests to determine the presence of sterols, flavonoids, saponins, phenolic compounds, alkaloids and carbohydrates respectively.

Hexane fraction (5 g) was subjected to silica gel column chromatography eluting with hexane (100%), followed by the gradient mixtures of hexane/chloroform and finally by chloroform (100%), by gradient mixtures of chloroform/methanol and then finally by methanol (100%). All fractions were subjected to TLC for identification of phytoconstituents.

### **Toxicity evaluation in mice**

The ethanolic extract was tested for its acute and short term toxicity (if any) in mice. To determine acute toxicity, a single oral administration of ethanolic extract at doses of 0.25, 0.5, 0.75 and 1.0 g/kg were administered to different group of mice (2 mice were used for each group, control mice received 1% sodium CMC). Mortality and behavior of the animals were observed periodically for 48 hr. The animals were observed from initial and at 2 hr, 4<sup>th</sup>, 6<sup>th</sup>, 24<sup>th</sup>, and 48<sup>th</sup> hours following drug administration. The parameters observed were grooming hyperactivity, sedation, respiration rate and convulsion.

To study short term toxicity, 3 groups each containing 6 male mice (20-25 g body weight) were used. Group 1 was kept as control, Group 2 and Group 3 received 200 and 400

mg/kg ethanolic extract, respectively in 1 % sodium CMC. The drug was administered daily for 14 days (*p.o.*), control group received 1 % sodium CMC in the same manner. The behavior of the animals was observed daily for 1 hr for 14 days. Initial and final body weights, food and water intake, body temperature and state of tool were observed. No toxic effects or mortality were observed in animals with doses upto 1 g/kg.

### **Carrageenan induced rat paw oedema**

Five groups (A, B, C, D, and E) of Sprague Dawley rats (175-225 g) were taken and each group consists of four animals.

Group A was treated with drug vehicle, 0.5% sodium CMC (-ve control); Group B was treated with a standard non-steroidal anti-inflammatory drug, indomethacin 10 mg/kg (+ve control), Group C, D and E were treated with the suspension of crude ethanolic extract of *Asparagus racemosus* Willd. 200, 400 and 600 mg/kg, respectively (Experimental groups).

All doses were administered orally as per kg body weight 1 hr prior to induction of oedema. Oedema was induced by injecting 0.1 mL of 1% carrageenan in 0.9 % NaCl subcutaneously (*s.c.*) into the subplantar tissue of right hind paw of each rat. The left hind paw of the same rats received 0.1 mL of saline. Before the induction of oedema (i.e.) at 0 hr, initial thickness of the paws were measured using Zeitlin's apparatus<sup>7</sup> and recorded. The paw thickness measurements were taken at 1, 2, 3, 4, 5 and 6 hr after induction of oedema.

To determine the influence of the crude ethanolic extract of *Asparagus racemosus* Willd. on carrageenan-induced rat paw oedema, the paw thickness of each rat was found by subtracting initial thickness from that obtained every hour after carrageenan treatment. The percentage inhibition of paw oedema was calculated using the formula

$$\% \text{ Inhibition in paw thickness} = \frac{(Y_t - Y_0) \text{ control} - (Y_t - Y_0) \text{ treated}}{(Y_t - Y_0) \text{ control}} \times 100 \quad \dots(1)$$

Where,

$Y_t$  = Paw thickness at time  $t$  ( $t = 1, 2, \dots, 6$ ) after injection and

$Y_0$  = Paw thickness at 0 hr before injection.

### **Cotton pellet-induced granuloma**

Cotton pellet-induced granuloma in rats was induced according to D'Arcy method<sup>8</sup>.

The animals were divided into three groups of six animals in each group. Cotton pellets weighing  $20 \pm 1$  mg were autoclaved and implanted subcutaneously into both sides of the groin region of each rat. Group A was treated with drug vehicle, 0.5 % Sodium CMC (-ve control); Group B was treated with a standard non-steroidal anti-inflammatory drug, indomethacin 10 mg/kg (+ve control), Group C were treated with the suspension of crude ethanolic extract of *Asparagus racemosus* Willd. 400 mg/kg (Experimental group). All doses were administered orally as per kg body weight. On the 8th day, the animals were sacrificed and the pellets together with the granuloma tissues were carefully removed, dried in an oven at 60°C weighed and compared with control.

### Statistical analysis

Data obtained from animal experiments were expressed as mean  $\pm$  SEM. Statistical differences between the treatments and the control were evaluated by one way analysis of variance (ANOVA), followed by Dunnett's test.  $p < 0.05$  was considered to be significant.

## RESULTS AND DISCUSSION

### Phytochemical screening

The results of the preliminary phytochemical screening of ethanol extracts and its fractions of *Asparagus racemosus* have been presented in Table 1.

**Table 1: Preliminary phytochemical screening**

| Name of the test                                    | Ethanol Extract | Hexane fraction | Chloroform fraction | Residual ethanolic fraction |
|---|-----------------|-----------------|---------------------|-----------------------------|
| Leibermann-Burchard test (Sterols)                  | + ve            | + ve            | + ve                | - ve                        |
| Shinoda test (Flavonoids)                           | + ve            | + ve            | + ve                | - ve                        |
| Foam test (Saponins)                                | + ve            | + ve            | + ve                | + ve                        |
| Neutral FeCl <sub>3</sub> test (Phenolic compounds) | + ve            | - ve            | + ve                | - ve                        |
| Mayer's, Dragendorff's test (Alkaloids)             | - ve            | - ve            | - ve                | - ve                        |
| Molisch's test (Carbohydrates)                      | + ve            | - ve            | - ve                | + ve                        |

Column chromatography of hexane fraction of ethanol extract revealed the presence of sterols and flavonoids. The prominent components were considered to be sterols as they were turned to rose red-pink-blue-green after being exposed to Liebermann-Burchard test, and green-yellow-orange red after being exposed to Salkowski reaction a specific test for sterols.  $\beta$ -Sitosterol was crystallised from hexane fraction as colourless fine needles with m. p. 136 to 138 °C. Quercetin-3 glucuronide was soluble in acetone, ether, ethyl acetate and methanol. The compound was crystallised from methanol as yellow needles. The above compounds were confirmed with m. p. and Co-TLC with authentic sample. Several *Asparagus species*, contain sterols and flavonoids as one of its phytochemical constituents<sup>9-12</sup>. *Asparagus racemosus* plant also contains sterols and flavonoids as one of its phytochemical constituents<sup>13,14</sup>.

### Carrageenan-induced pedal inflammation

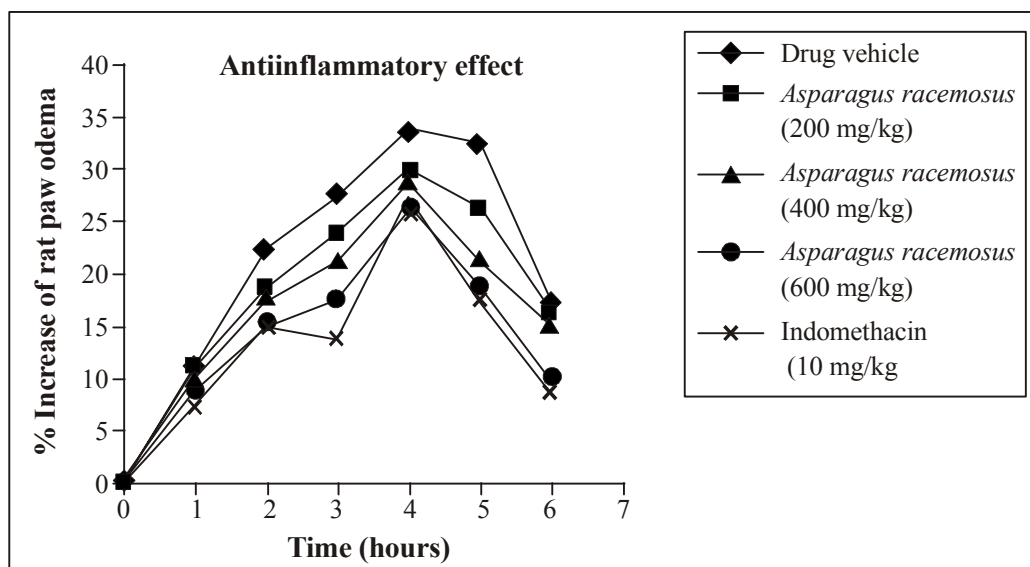
Effect of ethanol extract from *Asparagus racemosus* after Soxhlet extraction of the crude plant material by Carrageenan-induced pedal inflammation in rats are shown in Tables 2 and 3. The results are presented as mean  $\pm$  SEM., n = 4. The values are significant at p < 0.05. The percentage of maximal paw oedema produced during six hours was calculated and plotted a linear graph (Fig. 1).

**Table 2: Effect of the ethanolic extract of *Asparagus racemosus* Willd. on carrageenan-induced rat paw oedema**

| Treatment                  | Dose (mg/kg) | Mean oedema thickness |                 |                 |                 |                 |                 |
|----------------------------|--------------|-----------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                            |              | 1 hr                  | 2 hr            | 3 hr            | 4 hr            | 5 hr            | 6 hr            |
| Control                    | 0            | 0.23 $\pm$ 0.03       | 0.45 $\pm$ 0.05 | 0.55 $\pm$ 0.05 | 0.68 $\pm$ 0.05 | 0.65 $\pm$ 0.06 | 0.35 $\pm$ 0.10 |
| <i>Asparagus racemosus</i> | 200          | 0.23 $\pm$ 0.03       | 0.38 $\pm$ 0.02 | 0.48 $\pm$ 0.02 | 0.60 $\pm$ 0.04 | 0.53 $\pm$ 0.06 | 0.33 $\pm$ 0.06 |
| <i>Asparagus racemosus</i> | 400          | 0.20 $\pm$ 0.00       | 0.35 $\pm$ 0.03 | 0.43 $\pm$ 0.02 | 0.55 $\pm$ 0.03 | 0.43 $\pm$ 0.08 | 0.30 $\pm$ 0.09 |
| <i>Asparagus racemosus</i> | 600          | 0.18 $\pm$ 0.03       | 0.30 $\pm$ 0.00 | 0.35 $\pm$ 0.03 | 0.45 $\pm$ 0.03 | 0.35 $\pm$ 0.03 | 0.20 $\pm$ 0.04 |
| Indomethacin               | 10           | 0.15 $\pm$ 0.03       | 0.30 $\pm$ 0.04 | 0.28 $\pm$ 0.04 | 0.50 $\pm$ 0.04 | 0.38 $\pm$ 0.02 | 0.23 $\pm$ 0.05 |

**Table 3: Effect of the ethanolic extract of *Asparagus racemosus* Willd. on % inhibition**

| Treatment                  | Dose (mg/kg) | % Inhibition |       |       |       |       |       |
|----------------------------|--------------|--------------|-------|-------|-------|-------|-------|
|                            |              | 1 hr         | 2 hr  | 3 hr  | 4 hr  | 5 hr  | 6 hr  |
| Control                    | 0            | -            | -     | -     | -     | -     | -     |
| <i>Asparagus racemosus</i> | 200          | 0.00         | 16.67 | 13.64 | 11.11 | 19.23 | 7.14  |
| <i>Asparagus racemosus</i> | 400          | 11.11        | 22.22 | 22.73 | 18.52 | 34.62 | 14.29 |
| <i>Asparagus racemosus</i> | 600          | 22.22        | 33.33 | 36.36 | 33.33 | 46.15 | 42.86 |
| Indomethacin               | 10           | 33.33        | 33.33 | 50.00 | 25.93 | 42.31 | 35.71 |

**Fig. 1: Anti-inflammatory activity of *Asparagus racemosus* leaf extract on carrageenan-induced rat paw oedema**

The results of this study indicate that the leaf extract of *Asparagus racemosus* possess acute anti-inflammatory activity against phlogistic agent carrageenan. Inflammation induced by carrageenan involves three distinct phases of the release of the mediator; including serotonin and histamine in the first phase i.e. 0 - 2 h, kinins in the second phase i.e.

3 h, and prostaglandin in the third phase i.e. > 4 h<sup>15</sup>. The ethanol extract of *Asparagus racemosus* significantly inhibited paw oedema induced by carrageenan in the third phase, suggesting an inhibitory effect on the release of prostaglandin. This antiedematous response was also significantly reduced in rats pre-treated with indomethacin, a known cyclooxygenase inhibitor. They act by inhibition of cyclooxygenase and, therefore, inhibit the production of gastric prostaglandins. This leads to a reduction in production of gastric mucus and an increase in mucosal permeability<sup>16</sup>.

Phytochemical screening of ethanolic extract and its hexane fraction revealed that the leaves of *Asparagus racemosus* contains sterols and flavonoids. Sterols and flavonoids compounds have been found to exhibit antiinflammatory effects<sup>17-19</sup>. The presence of sterols and flavonoids in the ethanolic extract may account for its observed pharmacological activities.

The results suggested that the ethanol extract of *Asparagus racemosus* Willd. at the oral dose 600 mg/kg, and a standard drug, indomethacin, 10 mg/kg dose were found to inhibit the carrageenan-induced rat paw oedema more significantly, when compared to drug vehicle treated group.

#### Cotton pellet-induced granuloma

Effect of ethanol extract of *Asparagus racemosus* by cotton pellet granuloma in rats are shown in Table 4. The results are presented as mean  $\pm$  SEM., n = 6. The values are significant at  $p < 0.05$ . Ethanol extract of *Asparagus racemosus* Willd. showed significant anti-inflammatory activity at dose of 400 mg/kg, when compared to drug vehicle treated group.

**Table 4: Effect of the ethanolic extract of *Asparagus racemosus* Willd. on cotton pellet-induced granuloma**

| Treatment                             | Dose (mg/kg) | Wt of cotton pellet (mg) | % Inhibition |
|---------------------------------------|--------------|--------------------------|--------------|
| Control                               | 0            | 125.8 $\pm$ 0.36         | ---          |
| <i>A. racemosus</i> ethanolic extract | 400          | 93.5 $\pm$ 0.86*         | 25.65        |
| Indomethacin                          | 10           | 75.1 $\pm$ 0.78*         | 40.29        |

Values are Mean  $\pm$  SEM, n = 6; \*  $p < 0.05$ , when compared to control treated group



To conclude, the results showed anti-inflammatory activity of the leaf extract of *Asparagus racemosus* Willd., which supports the folk medicinal utilization of the plant.

Further work is in progress to identify the possible mechanism of action and to identify the lead molecules responsible for this anti-inflammatory activity.

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