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In-vitro Anti-inflammatory and Antioxidant activity of crude extract of leaves and stem of *Asparagus racemosus* plant (Family-Liliaceae)

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Abstract

Inflammation is the body's defence against infection. Before starting the treatment of any infection, inflammation needs to be reduced for better treatment. Our body has antioxidants to protect our body from various diseases. Antioxidants to boost the immune system of our body. This study proves the antioxidant and Anti-inflammatory activities of leaves & stem extracts of *Asparagus racemosus*. The present study includes a detailed exploration of the pharmacological activities of alcoholic extracts of *Asparagus racemosus*.

Keywords: Extract, Drug, Activity, Extraction, Antioxidant activity, *In-vitro*.

INTRODUCTION

Asparagus racemosus was botanically described in 1799. Due to its multiple uses, the demand for *A. racemosus* is constantly on the rise day by day. Due to destructive harvesting combined with habitat destruction and deforestation, the plant is now considered endangered in its natural habitat. The name Shatavari means "curer of a hundred diseases". Asparagus is reputed to be a tonic. The plant is considered slightly sweet and is useful in the blood, kidney, liver, scalding urine, gonorrhoea, etc. Its content of sapogenin, sarsa sapogenins, flavonoids (kaempferol, quercetin and rutin) and polyphenols are the precursor of many pharmacologically active steroids. It is used as medicine in Ayurveda, Unani and Siddha. In Ayurveda, it is considered a female tonic and used in diseases including inflammations, tumours, bronchitis, nervous disorder, hyperacidity, diabetic retinopathy, UTI (Urinary Tract Infections) and certain infectious diseases. In modern Ayurvedic practices, the roots of the plant are considered to be effective as a stomach tonic, aphrodisiac, galactagogue,

antioxidant, antispasmodic, appetiser, anti-inflammatory, blood purifier, anti-dysenteric, anticancer, antitubercular, kidney problems and in throat complaints (Garde & Sarth 1970).

A racemosus is a woody climber growing to 1-2 m in height. The leaves are like pine needles, small and uniform; the flowers are white and have small spikes. This plant belongs to the genus Asparagus, which has recently moved from the subfamily Asparagae in the Liliaceae family to a newly created family, Asparagaceae (Oguntibeju 2018).

MATERIAL & METHODS

1.1 Collection of plant material

Leaves and stems of *Asparagus racemosus* were collected from Shubham Nursery of Bhopal. After the plant was collected, it was processed for cleaning to prevent the deterioration of the phytochemicals present in the plant. Soon after cleaning, the plant material was kept to dry under the shade. The primary purpose of drying is to remove the water content from the plant so that the plant material can be stored. The dried plant part was finely powdered using an electric grinder, sieved and packaged in polyethene bags until needed.

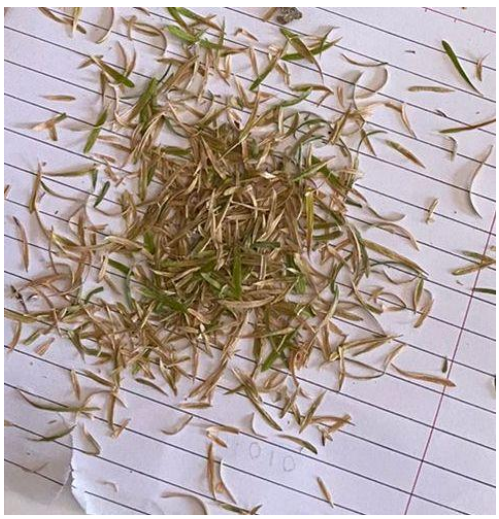

Figure 1: Dried leaves and stem of *Asparagus racemosus*

1.2 Extraction procedure

The following procedure was adopted for the preparation of extracts from the shade-dried and powdered herbs (Heinrich et al., 2012; Khandelwal, 2005; Kokate, 1994).

Extraction by maceration method

The air-dried and powdered leaves (20.5 grams) and stem (28.7 grams) of *Asparagus racemosus* were subjected to extraction with ethanol: water in a ratio of 70:30 v/v. Powdered plant materials were extracted using the maceration method. The resultant content was filtered with Whatman filter paper no.1 and kept for solvent evaporation to get the dry, concentrated extract. The dried crude concentrated extract was weighed to calculate the extractive yield, then transferred to glass vials (6×2 cm) and stored in a refrigerator (4°C) till used for analysis.

Determination of extractive value (% yield)

The % yield of each extract was calculated by using the formula:

$$\textbf{Percentage Yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}}$$

1.3 *In-vitro* antioxidant activity using the DPPH method

The spectrophotometer was used to measure the DPPH scavenging activity. The stock solution (6 mg in 100 ml methanol) was produced to give an initial absorbance of 1.5 ml in 1.5 ml methanol. After 15 minutes, there was a decrease in absorbance in the presence of sample extract at various concentrations (10-100 g/ml). 1.5 ml of DPPH solution was added to 3 ml of methanol, and the absorbance was measured at 517 nm for the control reading. 1.5 ml of DPPH and 1.5 ml of varying concentrations of the test sample were placed in a succession of volumetric flasks, and the final volume was adjusted to 3 ml using methanol. Three test samples were collected and processed in the same way. Finally, the average was calculated. After 15 minutes at 517 nm, the absorbance of DPPH with varied concentrations showed a final reduction (Parkhe and Jain, 2018).

$$\text{Radical scavenging activity (\%)} = (A_{control} - A_{test})/A_{control} \times 100$$

$A_{control}$ is the absorption (without extract) of the control, and A_{test} is the absorption in the presence of the extract/standard.

1.4 Evaluation of *in vitro* Anti-inflammatory activity

The anti-inflammatory activity of the *Asparagus racemosus* extract was evaluated by the protein denaturation method as described by (Padmanabhan and Jangle,2012). Diclofenac sodium, a powerful non-steroidal anti-inflammatory drug, was used as a standard drug. The reaction mixture consisting of 2 ml of different

concentrations of standard diclofenac sodium (100-500 µg ml-1) and 2.8 ml of phosphate-buffered saline (pH 6.4) was mixed with 0.2 ml of egg albumin (from fresh hen's egg) and incubated at (37±1)°C for 15 min. Denaturation was induced by keeping the reaction mixture at 70°C in a water bath for 10 min. After cooling, the absorbance was measured at 660 nm by using double distilled water as blank. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ Inhibition} = \frac{At\text{-}Ac}{Ac} \times 100$$

Where A_t = absorbance of a test sample

A_c= absorbance of control

The plant concentration for 50% inhibition (IC_{50}) was determined by plotting percentage inhibition with respect to control against treatment concentration.

RESULTS & DISCUSSION

1. Results of extractive values:

Table 1: Extractive values of *Asparagus racemosus*

S.No.	Extracts	Colour	Physical nature	% Yield (W/W)
1	Leaves	Green	Solid	2.75%
2	Stem	Brown	Solid	4.21%

2. Results of Antioxidant activity by using the DPPH method

Table 2: Absorbance of ascorbic acid and *Asparagus racemosus* using DPPH method

S. No.	Concentration (µg/ml)	Absorbance		
		Ascorbic acid	Leaves extract	Stem extract
1	10	0.623	0.841	0.789
2	20	0.532	0.763	0.674
3	40	0.441	0.544	0.524
4	60	0.352	0.513	0.436
5	80	0.223	0.374	0.399
6	100	0.109	0.302	0.217
	Control	**1.122**		

Table 3: % Inhibition of ascorbic acid and *Asparagus racemosus* using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition		
		Ascorbic acid	Leaves extract	Stem extract
1	10	44.47	25.04	29.68
2	20	52.58	32.00	39.93
3	40	60.70	51.52	53.30
4	60	68.63	54.28	61.14
5	80	80.12	66.67	64.44
6	100	90.29	73.08	80.66

IC $_{50}$ value	**18.77**	**50.92**	**42.21**

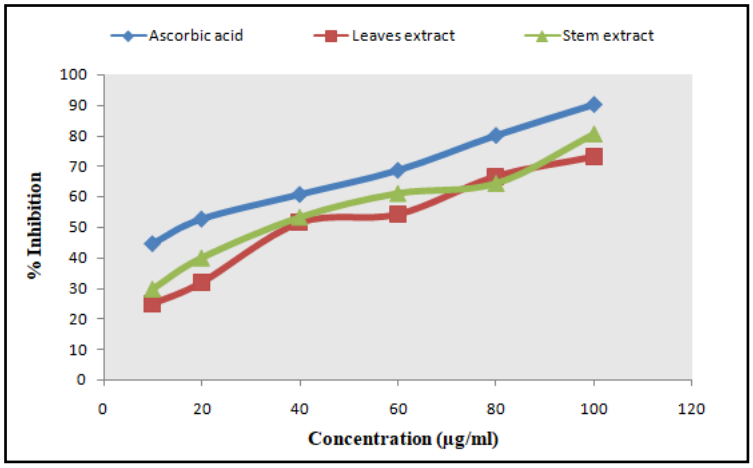

Figure 2: % Inhibition of ascorbic acid and *Asparagus racemosus* using DPPH method

2.3 Results of *in vitro* Anti-inflammatory activity

Table 4: Absorbance of Diclofenac sodium and *Asparagus racemosus*

S. No.	**Concentration (µg/ml)**	**Absorbance**		
		Diclofenac sodium	**Leaves extract**	**Stem extract**
1	100	0.461	0.742	0.702
2	200	0.378	0.712	0.668
3	300	0.157	0.656	0.592
4	400	0.103	0.564	0.479
5	500	0.089	0.475	0.383
Control	**0.851**			

Table 5: % Inhibition of Diclofenac sodium and *Asparagus racemosus*

S. No.	**Concentration (µg/ml)**	**% Inhibition**		
		Diclofenac sodium	**Leaves extract**	**Stem extract**
1	100	45.82	12.80	17.50
2	200	55.58	16.33	21.50
3	300	81.55	22.91	30.43
4	400	87.89	33.72	43.71
5	500	89.54	44.18	54.99
IC$_{50}$ value		**116.38**	**600.71**	**469.39**

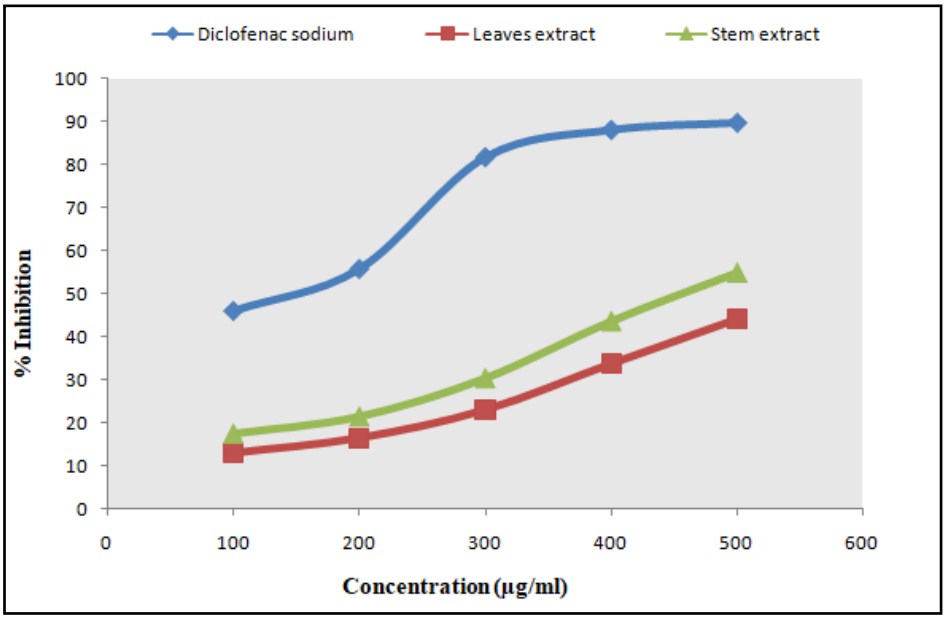

Figure 3: Graph of *in vitro* anti-inflammatory activity

CONCLUSION

Different parts *of* the *Asparagus racemosus* have been extensively studied for its medicinal properties. Its leaves and stems have also proved to possess various pharmacological properties and potent therapeutic agents. Further research is required to formulate the extract as a drug in the near future. In the current scenario, the major problem is the use of synthetic medicines. So, the traditional knowledge about the medicinal herbal plants is very essential.

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