International Journal of Pharmacology and Clinical Research (IJPCR)

ISSN:2521-2206

IJPCR |Volume 1 | Issue 2 | July – Dec- 2017
www.ijpcr.net

Research Article *Clinical Research*

Phytochemical investigation, screening of anti-inflammatory activity of *Asparagus gonocladosBaker* roots

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ABSTRACT

In India, *Asparagus gonocladosBaker* (Liliaceae) is commonly used as a phyto-therapeutic agent. The ethanolic extract of *Asparagus gonocladosBaker*, was evaluated for Anti-inflammatory effect, induced by intra-plantar administration of carrageenan to adult Albino rats. The percentage inhibition of paw edema of the alcoholic extract of the plant *Asparagus gonocladosBaker* were carried out at a dose of 100,200,300 mg/kg. There was significant inhibition ($p < 0.05$) in paw edema. Different physiochemical parameters such as Ash Value, Extractive Value, Moisture Content and Chemical evaluation were carried out as per W.H.O recommended physiochemical determination with authentic phyto chemical procedure.

Keywords: *Asparagus gonoclados Baker*, Physiochemical, Phytochemical, Extraction, Extractive values, Anti-Inflammatory.

INTRODUCTION

The World Health Organization (W.H.O) recognized this fact in the early 1970s and encouraged governments to effectively utilise local knowledge of herbal medicines for disease prevention and health promotion [1].

Inflammation is a protective tissue response to injury or destruction of tissue which serves to destroy, dilute, or wall off both the injurious agent and the injured tissues. It is a common clinical condition which affects about 2.1 million Indians and Americans. A large number of Indian medicinal plants have been used in the treatment

of Anti- inflammatory and they were reported to be effective with no side effects. In this back ground we decided to evaluate the Anti-inflammatory activity of *Asparagus gonocladosBaker* used as a medicine for inflammation.

Asparagus gonocladosBaker is distributed mainly in tropical and sub-tropical region. In India, it is distributed mainly in Western Ghat region, Assam, Meghalaya etc. The plant leaves are modified to spine 6-13 mm long. Flowers are white, sexual, regular, racemes, 2.5-7.5 cm long. Roots are bulbous and adventitious [2]**.** Further this plant is reported to have anti-oxidant, cooling, emolient, diuretic, nervine tonic, anti-diarrhoea, anti-cancer, anti- tuberculosis, anti-epileptic and anti-arthritic activities [3,4,5]. The drug is used in nearly 67 Ayurvedic preparations like Anuthaila, Brahma rasayana, Dhanwan- thararishta, Mahathikthaka kashaya, Narayana thaila,

Rasnadi kashaya, Sahacharadi thaila, Saraswath arishta, Shatabara panaka, Shatabara ghritha, Shatamulyadi lehya, Vasishtha rasayana and Vidaryadi ghritha to mention a few [6,7].

MATERIALS AND METHODS

Collection of Plant Material

Roots of *Asparagus gonoclados* were collected from in and around Agumbe, Karnataka, and authenticated by Dr. D. Rudrappa, H.O.D., S.R.N.M. National College of Applied Science, Shimoga. A voucher specimen of *Asparagus gonocladosBaker* has been deposited at the Dept. of Pharmacognosy, (Voucher specimen no NCP/13/2011-2012) National College of Pharmacy, Shimoga. (Authentification letter was enclosed)

Physiochemical studies [8]

Determination of Ash value

The total ash obtained was boiled with 25 ml of alcohol for few minute. The insoluble matter was collected on ash less filter paper, washed with hot water and ignited for 15 min at temperature not exceeding 450 ^{o}c. The difference in weight represents the alcohol-soluble ash. The percentage of alcohol - soluble ash was calculated with reference to the air-dried drug.

Determination of extractive value

About 5gms of air-dried, coarsely powdered roots of *A. gonoclados* were weighed accurately and separately macerated with 100 ml of alcohol in stoppered flask for 24 hrs, shaking frequently during the first 6 hrs and was The method is based on the extraction of active constituents present in the crude powder plant material, using ethanol as a solvent. Ethanol is moderately polar in nature and hence dissolves wide range of compounds belonging to different chemical classes. The extraction was done by hot percolation using soxhlet apparatus. The extraction vessel was made up of borosil glass, which contain round bottomed flask. The plant material to be extracted was packed in the soxhlet assembly and a condenser through which refluxing was done. Ethanol was used as solvent. Heat was supplied through a heating mantle. The extract was collected after evaporating the solvent using Rota evaporator. The extract was kept in airtight container at room temperature until further use.

Phyto-chemical studies

Preliminary phytochemical studies of the root extract was conducted as per the standard procedure [9, 10, 11, 12, 13].

Animal used

Healthy young Albino Wistar rats weighing between 150- 200 gm (8 to 10 weeks old) were used for acute toxicity study to determine LD_{50} of ethanolic extract. The temperature in the experimental room was around 25^{o}C. Lightning was artificial, the sequence being 12 hour dark, 12 hour light. The conventional laboratory diet was fed, with an unlimited supply of drinking water. Approval from the Institutional Animal Ethical Committee (IAEC) of National College of Pharmacy (Shimoga, Karnataka) was taken prior to the experiments. (IAEC approval Certificate was enclosed). Allowed to stand for 18 hrs. Then it was filtered and 25ml of the filtrate was evaporated to dryness in a tarred flat bottom shallow dish, dried at 105 ^{o}c and weighed. The percentage of alcohol-soluble extracts was calculated with reference to the air-dried drug.

Determination of moisture content

About 5 gm of powdered roots of *A. gonoclados* were weighed accurately and was taken separately in a china dish. It was kept at 105 – 110^{o}C for 30min in a hot air oven; the residue was cooled and weighed. The percentage of moisture content was then calculated with reference to the air-dried drug.

Extraction of Plant Material

Ld50 determination

Lewis RM et al. has been evaluated, acute oral toxicity for ethanolic extract of A. gonoclados. They examined the ethanolic root extract preparation which showed a low toxicity. Basing on the observation of animals, 3.0gm/kg body weight of the extract was found to be safe and is taken as the maximum tolerated dose (MTD) [14].

Experimental design

The animals were weighed, numbered and marked and both hind paws. The initial paw volume of each rat was noted by plethysmometer. The animals were divided in five groups each consisting five animals. The study was conducted for a period of 24 hours [15, 16]. Group I-Positive Control (received 0.1ml of 1%**w/v** suspension of carrageenan in normal saline along with ordinary drinking water and commercial pellet diet) G**1**
Group II- Standard (received 0.1ml of 1%**w/v** suspension of carrageenan in normal saline along with ordinary drinking water , commercial pellet diet and 10mg/kg Diclofenac Na.) G**2**
Group III- (received 0.1ml of 1%**w/v** suspension of carrageenan in normal saline along with ordinary drinking water, commercial pellet diet and 100mg/kg alcoholic extract of A. Gonoclados) G**3**
Group IV- (received 0.1ml of 1%**w/v suspension** of carrageenan in normal saline along with ordinary drinking water, commercial pellet diet and 200mg/kg alcoholic extract of A. Gonoclados) G**4**
Group V- (received 0.1ml of 1%**w/v** suspension of carrageenan in normal saline along with ordinary drinking water, commercial pellet diet and 300mg/kg alcoholic extract of A. Gonoclados) G**5**

Statistical analysis

The results are expressed as Mean ± SEM for five animals in each group. Difference between groups were assessed by one way analysis of variance (ANOVA) with post test followed by Dunnett compare all vs control using Graphpad-5 Instat Software for windows. Post hoc testing was performed for inter-group comparison using the least significance difference (LSD) test. Significance at p- values <0.05 has been given respective symbol in the table.

RESULTS

Physiochemical studies

The powder of roots of *Asparagus gonocladosBaker* were subjected to evaluate its alcohol soluble ash, alcohol soluble extractive value and moisture content. Each determination was carried out and the values were taken. The results are reported in table 1.

Table No.1 Percentage yield of values

Name of the Plant	VALUES (%w/w)		
	Alcohol Soluble ash	Alcohol soluble extractive	Moisture Content
Asparagus gonoclados	5.07	31.96	0.65

Qualitative chemical examination of extract

Ethanolic extract was subjected to qualitative chemical evaluation to detect the chemical constituents present in them. The results are tabulated in Table No.2

Table No.2 Qualitative Chemical examination of extract of *A. gonoclados Baker* roots

Chemical Constituent	Tests	Ethanolic Extract
Alkaloids	1.Mayer's test	+ve
	2. Wagner's test	+ve
	3. Dragendorff test	+ve
Glycoside	1. Modified B. T	+ve
	2. Legal's test	+ve
	3. Liebermann-Burchard's Test	+ve
Saponin	1.Froth's Test	-ve
Phytosterol	1. Liberman Buchard	+ve
	2. Salkowski test	+ve

Phenolics and Taniins	1. FeCl3 test	+ve
	2. Lead acetate sol.	+ve
	3. Shinoda test	+ve
	4. Vanillin HCl Test	+ve
Proteins and Amino acids	1. Millons Test	-ve
	2. Biuret Test	-ve
Fixed Oil And Fats	1. Stain Test	+ve
	2. Soap Test	+ve
Carbohydrates	1. Molisch's Test	-ve
	2. Fehling's Test	-ve
	3. Barfoed's Test	-ve

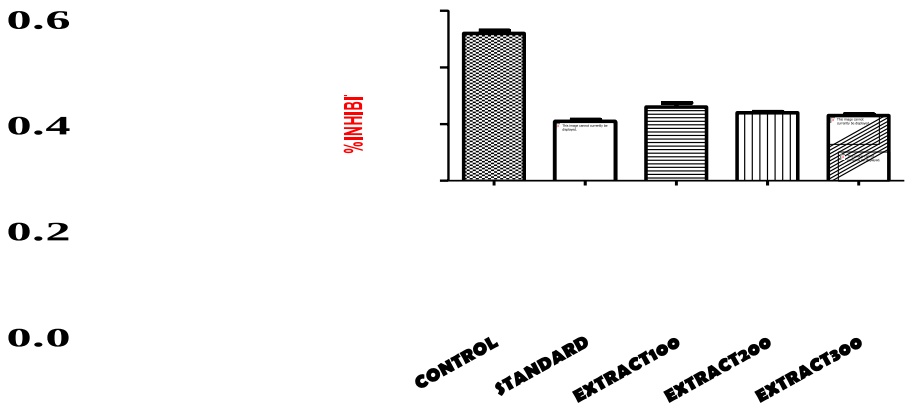
Anti inflammatory activity of ethanolic extract

The rats were treated with intra peritoneal administration of ethanolic extract of the plant *A. gonoclados Baker*reduced acute paw oedema volume as compared to the control. The percentage inhibition of paw edema of the ethanolic extract of the plant *A. gonoclados Baker*at a dose of 100, 200 and 300 mg/kg was found to be 50.00, 53.73 and 55.96 percentages when compared to control. However, it exhibited a dose dependent activity. There was significant inhibition ($P < 0.05$) in paw edema volume at a dose of 100, 200 and 300 mg/kg body weight (Table 3).

Table No 3: Effect of Ethanolic Extract of the plant A. gonoclados Bakeron carragenan induced paw edema in rats

Treatment	Dose (mg/kg)	Change in paw volume (Mean ± SEM)	% Inhibition
Control	------	0.52 ± 0.01	------
Diclofenac Sodium	10	0.21 ± 0.006**	59.61
Ethanolic Extract	100	0.26 ± 0.015**	50.00
Ethanolic Extract	200	0.24 ± 0.004**	53.73
Ethanolic Extract	300	0.23 ± 0.005**	55.96

All the values are expressed as mean ± SEM (n = 5) **P < 0.05 significant compared to control



DISCUSSION

The present study aimed at evaluation of Asparagus gonocladosBaker roots on pharmacognostic, pharmacological and phytochemical parameters. Asparagus gonocladosBaker roots were subjected for various standardization parameters. The present study was attempted to evaluate the anti-inflammatory activity of the roots of A. gonoclados Baker. The powder of Asparagus gonocladosBaker roots were

subjected to evaluate their Treatment alcohol soluble ash, alcohol soluble extractive value, moisture content and phytochemical evaluation. Ash values are helpful in determining the quality and purity of crude drugs. It gives an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. Extractive values are useful for evaluation of crude drugs and gives idea about the nature of chemical constituents present in them. In some cases the amount of drug soluble in a given solvent is an index of purity. Extractive values are primarily useful for the determination of exhausted or adulterated drug. The moisture content of the drug should be controlled and minimized in order to prevent decomposition of crude drugs either due to chemical change or microbial contamination. Phytochemical screening of alcoholic extract of *Asparagus gonocladosBaker* roots reveals the presence of alkaloids, flavonoids, tannins, phenolic compounds, phytosterols, fixed oils and saponins.

Anti inflammatory activity

Carrageenan induced inflammation in the Wister Albino rats were treated with intra peritoneal administration of various doses of extract of the plant *A. gonoclados Baker*reduced acute paw oedema volume as compared to the control. The ethanolic extract, at a dose of 300 mg/kg b.w. showed most significant inhibition, as compared to control. The phytochemical investigation reveals that, the ethanolic extract contain polar and non-polar group which may be responsible for the anti-inflammatory activity.

CONCLUSION

In the present study, roots of*Asparagus gonoclados Baker* were subjected for various physiochemical parameters which would help in standardization. The preliminary phytochemical investigation of roots of *Asparagus gonocladosBaker* ethanolic extract showed the presence of alkaloids, flavonoids, tannins, phenolic compounds, phytosterols, fixed oils and saponin. Significantly ethanolic extract has shown better anti-inflammatory activity, which might be due to the combination of polar and non polar constituent. From this study, we can conclude that *Asparagus gonocladosBaker*is having good anti-inflammatory activity.

Acknowledgment

The authors wish to thank to Dr. G. Chakravarthi (Principal, St'Mary's Pharmacy College) for providing facilities and precious guidance to carry out work and author is also thankful to Dr. V. Ram Mohan Gupta (Principal, Pulla Reddy Institute of Pharmacy) for their valuable support during this work.

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