

Phytochemical and pharmacological evaluations of *Aristolochia bracteolata* Lam.

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ABSTRACT

Innumerable plants were used in Indian Systems of Medicine like Siddha, Ayurveda and Unani. There are lacuna were found in many of these plants in their scientific evaluation like pharmacological potential. *Aristolochia bracteolata* is a common Indian medicinal plant belonging to the family Aristolochiaceae easily available in all seasons. The plant is mainly used in skin diseases, snake bite, arthritis and diabetes in Siddha system of medicine. Alcoholic and aqueous extracts were prepared by using Soxhlet apparatus. Preliminary phytochemical screening and pharmacological evaluations like anti inflammatory and antipyretic activities were analyzed by standard methods. The phytochemical screening indicated that the presence of flavonoid and tannin in rich status and the pharmacological evaluation of the drug plant *Aristolochia bracteolata* has a significant anti-inflammatory and antipyretic properties.

Key words: *Aristolochia bracteolata*, Phyto Chemical, Anti Inflammatory, Anti Pyretic.

INTRODUCTION

Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on human body. The use of medicinal plants as traditional medicines is well known in rural areas of many developing countries [1]. Traditional healers claim that their medicine is cheaper, more effective and impart least side effects as compared to synthetic medicine. Screening of medicinal plants for pharmacological and elementological activities are important for finding potential new compounds for therapeutic use.

Inflammation is the reaction the living tissues to injury; it comprises systemic response and local response. Loss of function occurs depends on the site and extent of injury. Pyrexia is caused as a secondary impact of infection or other diseased states. It is the body's natural defense to create an environment where infectious agent or damaged tissue cannot survive [2].

Aristolochia bracteolata is a common medicinal plant that belongs to the family Aristolochiaceae. The plant commonly called as Worm killer in English and Aadutheendapalai in Tamil. Among several other colloquial names, Dhumra-patra is common in North India. It has been reported to be distributed throughout the South India, Bengal, Upper Gangetic Plain, Ceylon and Tropical Africa. Traditionally, *Aristolochia bracteolata* has been reported to be used for inflammatory diseases, fever and insect bites. The whole plant was used as purgative, anthelmintic,

antipyretic, anti inflammatory agents. The plant contain Aristolochic acid has many medicinal properties in various disease conditions [3].

The present study was designed to evaluate the preliminary phytochemical screening and the pharmacological activities of anti inflammatory and anti pyretic of the medicinal plant *Aristolochia bracteolata*.

MATERIALS AND METHODS

2.1. Collection of plants

The study plant *A. bracteolata* were collected in Kangaikondan near Tirunelveli and identification was done by using Flora of the Presidency of Madras and the Flora of Tamil Nadu and Carnatic [4]-[5]. The voucher specimen was kept in our department herbarium, Department of Siddha Medicine, The Tamil University, Thanjavur for further reference.

2.2. Preparation of extracts

The plant powder (350g) was soxhlet- extracted with 350 ml of absolute alcohol. The alcohol extract was evaporated to dryness at reduced temperature and pressure in a rotary evaporator to obtain the crude extract. For the water extract, 25g of the powder was stirred continuously in 350 ml distilled water for 6 hours at room temperature, filtered and the filtrate was used for the experiments.

2.3. Qualitative Phytochemical analysis

Qualitative phytochemical analysis of alcoholic and aqueous extracts were carried out using standard procedure to identify the phytochemical constituents, alkaloid, flavonoid, tannin& phenol, starch, sterol and saponins [6]-[7].

2.4. Phytochemical screening for different compounds

2.4.1. Test for alkaloid

0.5g of aqueous and ethanol extracts was mixed in 8ml of 1% HCl, warmed and filtered. 2ml of the filtrate were treated separately with Maeyar's reagent and Dragendorff's reagent., after which it was observed whether the alkaloids were present in the appearance of cream and orange colour precipitates in response to the above reagents respectively.

2.4.2 Test for Flavonoids

A few drops of 1 % NH₃ solution is added to the aqueous and ethanol extract of the plant sample in a separate test tube. A yellow colouration is observed if flavonoid compounds are present.

2.4.3. Test for Phenol

Each 1ml of extracts was mixed in 2ml of chloroform followed by a few drops of 10% aqueous ferric chloride solution was added. Formation of blue or green colour indicated the presence of phenols.

2.4.4. Test for Tannin

0.25 g of aqueous and ethanol extracts were dissolved in 10ml distilled water and filtered. 1% aqueous ferric chloride solution was added to the filtrate separately. The appearance of intense green, purple, blue or black colour indicated the presence of tannins in the test samples.

2.4.5. Test for Saponins:

2ml of each extracts were added 6ml of water in a separate test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

2.4.6. Test for Sterols:

1ml of extract was treated with drops of chloroform, acetic anhydride and con. H₂SO₄ and observed for the formation of dark pink or red colour. The colour conform the presence of sterols.

2.5. Pharmacological Studies

2.5.1. Animals

Albino rats (180-250 mg) of either sex maintained at the animal house, Department of Siddha Medicine, The Tamil University, Thanjavur were used. The animals were housed under standard conditions of temperature and light and fed on standard diet and given water *ad libitum*.

2.5.2. Anti inflammatory activity

This study was carried out using the Cotton pellet method [8]. Cotton pellet, each weighing 10 mg were prepared and sterilized in an autoclave for about an hour under 15 pounds atmospheric pressure. Eighteen rats weighing between 180-250 gm were selected and divided into six groups each group containing three rats. The rats were anaesthetized with ether and the cotton pellets were implanted subcutaneously in the groin, two on either side. From the day of cotton pellet implantation, the drug was administered as follows. Group I received normal saline (2ml/kg) and served as control, group II and III received 100 and 200mg/kg of alcoholic extract, group IV and V received 100 and 200mg/kg of aqueous extract orally respectively. While group VI, the rats received salicylic acid (150mg/kg) as control. On the eighth day, the rats were sacrificed and the pellets were removed, dried to concordant weight in an incubator at 60-80C. The weight of the granulation tissue was then determined separately.

2.5.3. Anti pyretic activity

The antipyretic activity was performed by Yeast induced pyrexia method [9]. The body temperature of each albino rat was recorded by measuring rectal temperature (RT). Fever was induced in the rats by injecting 15% Brewer's yeast at a dosage of 1ml/kg body weight subcutaneously. The rectal temperature of each rat was again recorded after 24 hours of yeast administration. Thirty rats were selected and grouped into six each group consist of five rats and treated as follows. Group I received distilled water, group II paracetamol, group III and IV received 100 and 200mg/kg of alcoholic extract and group V and VI received 100 and 200mg/kg aqueous extract respectively. Rectal temperatures of all rats were then recorded by inserting digital thermometer into the rectum at 1hr intervals for 4hrs.

RESULTS AND DISCUSSION

The detailed investigations of preliminary phytochemicals in aqueous and ethanol extracts are shown in table I. The aqueous and ethanolic extracts of the medicinal plant *Aristolochia bracteolata* contain phenols, flavonoids, tannin and saponin. Sterol and alkaloid are absent in both extracts. These metabolites have varying pharmacological effects on animals [10]. Previous studies [11] demonstrate that *Aristolochia bracteolata* showed the presence of phenols, flavonoids, glycosides, lignin and saponins and also the methanolic extract showed the presence of phenols, glycosides, terpenoids, sterols, lignin and saponins. The previous phytochemical analysis and the present research analysis show nearly the similar results.

Table.1 Qualitative Phytochemical Screening of leaf extracts of *A. bracteolata*

S.No.	Phytochemical constituents	Reagents used	Aqueous extract	Ethanolic extract
01.	Alkaloid	a.Mayer's b.Dragendorff's	-	-
02.	Flavonoid	NaoH	+	+
03.	Phenols	Chloroform/FeCl ₃	+	+
04.	Tannins	FeCl ₃	+	+
05.	Saponins	Foam test	+	+
06.	Sterol	Chloroform +Acetic anhydride +Con . H ₂ So ₄	-	-

Table . 2 Anti inflammatory activity of *Aristolochia bracteolata* by Cotton Pellet method

S. No.	Group	Dosage	Computation		
			Mean Wight of the Granulation Tissue	%of inflammation	Mean % of inhibition
1.	Control	2 nd normal Saline/kg	240 mg	100%	-
2.	Standard	100 mg/kg	84mg	35%	65%
3.	E.E. A.b	100 gm/kg	177mg	74%	36%
4.	E.E. A.b	200 mg/kg	144 mg	60%	40%
5.	A.E. A.b	100 mg/kg	172 mg	72%	28%
6.	A.E.A.b	200 mg/kg	158 mg	68%	32%

E.E.A.b-Ethanolic extract of *Aristolochia bracteolata*.

A.E.A.b-Aqueous extract of *Aristolochia bracteolata*.

Aqueous and ethanolic extracts of *A.bracteolata* was investigated for their anti inflammatory activity (Table II). Extract at 200mg/kg exhibited significant anti inflammatory activity. Ethanolic extract was found to be more effective than aqueous extract . Anti oxidant investigations of the ethanol extract showed good free radical scavenging activity, thereby supporting its anti inflammatory properties [12]. Previous studies demonstrate that *A.bracteolata* appears to be efficient in acute and sub acute inflammatory [13]-[14]-[15]. In our study report on rat granulation oedema supports the above study. Flavonoides and phenolic compounds in plant have been reported to exert multiple biological effects including microbial, antioxidant and anti inflammatory[16-18]. Saponin is a mild detergent, used in hyperglycemia, antioxidant, anticancer and anti inflammatory [19]. Anti inflammatory activities may be due to the strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids and phenols [20].

Both extracts of the plant *A.bracteolata* at 200mg /kg exhibited significant antipyretic activity. The activity was compared to the standard drug paracetamol. Such reduction of rectal temperature of the animals appears to be due to the presence of bioactive compound in them. The anti pyretic activity observed can be attributed to the presence of flavonoids have been reported to exhibit anti pyretic effects [21]-[22]. The secondary metabolites shows medicinal activities as well as exhibit physiological activity [23]. The phytochemical analysis of the extracts showed the presence of tannins and flavonoids.

The present research work, the results indicated that the aqueous and ethanolic extracts of *Aristolochia bracteolata* has significant anti inflammatory and anti pyretic activity due to the presence of their secondary metabolites like alkaloids, flavonoids, saponins and phenols.

Table 3: Anti pyretic effect of *Aristolochia bracteolata*

S. No	Group	Dosage mg/Kg	Temperature 18 hours after Yeast Induced Pyrexia	Temperature in C°			
				1 st hr	2 nd hr	3 rd hr	4 th hr
1	Control	2 ml Distilled water	38.65±0.15	37.08±0.17	37.33±0.15	37.2±0.15	37.11±0.07
2	Standard	Paracetamol 100 mg/kg	38.01±0.19	37.02±0.19	37.26±0.07	36.65±0.15	35.23±0.08
3	E.E. A.b	100 gm/kg	38.26±0.11	37.03±0.12	37.78±0.18	36.43±0.11	36.18±0.25
4	E.E. A.b	200 mg/kg	38.06±0.21	37.08±0.05	37.11±0.10	36.9±0.13	35.82±0.23
5	A.E. A.b	100 mg/kg	38.15±0.11	37.26±0.18	37.92±0.95	37.16±0.15	36.70±0.19
6	A.E.A.b	200 mg/kg	38.25±0.19	38.20±0.10	37.3±0.06	36.9±0.02	35.90±0.21

E.E.A.b-Ethanolic extract of *Aristolochia bracteolata*.

A.E.A.b-Aqueous extract of *Aristolochia bracteolata*.

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