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PHARMACOGNOSTICAL PHYTOCHEMICAL SCREENING OF *ARISTOLOCHIA BRACTEOLATE* (ARISTOLOCHIACEAE)

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ABSTRACT: *Aristolochia bracteolata* is a small, glabrous shrub occurring in India. It belongs to the family Aristolochiaceae. The whole plant is used in various ailments like abortifacient, alterative, anthelmintic, antiperiodic, emmenagogue and purgative. The stem and root are having aristolochia acid (Alkaloid). The leaves and roots are used to rid the body of Guinea worm (a parasitic infection caused by a nematode). In present study was macroscopical, microscopy, physiochemical parameters (extractive values, crude fiber content, ash values, foreign organic matter), fluorescent analysis, plant cell inclusions reported. The ethanolic plant extraction was carried out by using a soxhlet apparatus. The extract was screened for phytochemical properties by using a color reaction test.

INTRODUCTION: Scientific Classification: ^{6,7}

Kindgom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Piperales

Family: Aristolochiaceae

Genus: *Aristolochia*

Species: *Aristolochia bracteolata*

Aristolochia bracteolata is a perennial, or rarely annual, mostly prostrate but occasionally climbing or suberect, unpleasantly smelling herb growing from 10 - 40cm long. The plant is usually gathered from the wild and is used locally in traditional medicine. It is sometimes cultivated for medicinal use in India. Grows along the banks of the ganges and in southern India. It is wasteland weed.

Vernacular Names:

- English: Worm killer, Indian birthwort
- Malayalam: Adhthinnappala, karalakam,
- Hindi-Kidamari, Tamil-Adu tinna palai,
- Telugu: Gudide Gaddithaigadapara
- Kannada: Sanajali-hullu
- Marathi: Gandhani
- Sanscrit: Pattra- banga



FIG. 1: ARISTOLOCHIA BRACTEOLATE

Cultivation: *Aristolochia bracteolata* is propagation by seed. A plant of the drier tropics and subtropics, usually growing where there is a

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distinct dry season. The flowers of many species in this genus form an intricate trap for pollinating insects. The insects are tempted to an area of the flower where they are unable to grip, and they slide down into the utricle - downward pointing hairs prevent them from climbing out. The trap helps to ensure the fertilization of the flowers and the insects are eventually released.

Medicinal Uses:

- The whole plant is very bitter and has abortifacient, alterative, anthelmintic, anti-periodic, emmenagogue and purgative properties. It should be used with great caution since the plant can be toxic to mammals. The stem and the root contain the aristolochic alkaloid acid.
- The dried, powdered root has been shown to increase the contractions of the uterus during labor. It has been used as a substitute for ergot.
- The leaves and roots are used to rid the body of Guinea worm (a parasitic infection caused by a nematode).
- The powdered roots are combined with castor oil (from *Ricinus communis*) and used in the treatment of colic, amenorrhoea, dysmenorrhoea, intermittent fever and worms.
- Externally, its juice is applied to foul and neglected ulcers to destroy insect larvae. It is also used to treat scorpion bites.

MATERIALS AND METHODS:

Plant Material: The plant of *A. bracteolate* was collected from Thirumalaisamudram 7 km away from Thanjavur (Tamil Nadu) in December 2010. The plants were identified by local people of that village and authenticated by Dr. N. Ravichandran, Asst. Professor, Drug Testing Laboratory, CARISM, SASTRA University Thanjavur, and the Voucher specimen is preserved in the laboratory for future reference.

Chemicals: All the reagents used were of analytical grade obtained from S.D. fine chemicals, Ltd, and Hi-Media, Mumbai.

Pharmacognostical Screening of Plants: Macroscopic characters and physiochemical parameters of *Aristolochia bracteolate* leaf and

leaf powder: The macroscopic evaluation was carried out for shape, size, color, odor, taste, and fracture of the drug. Different physiochemical values such as Ash value, extractive values, loss on drying, foreign organic matter, Crude fiber content, were determined and reported in **Table 1**.

Preparation of Extract from *Aristolochia bracteolate* leaf Powder: The leaves were dried under shade, powdered and passed through 40meshes and stored in a closed vessel for further use. The dried powder material (150 gm) was subjected to Soxhlet extraction with ethanol for continuous hot extraction for 24 h. The extracts were concentrated under reduced pressure to obtain the solid extracts residues. The percentage value of extract was 29 (% w/w).

Phytochemical Evaluation of Ethanolic Leaf Extracts of *Aristolochia bracteolate*: The ethanolic extract of *Aristolochia bracteolate* (Leaf) was subjected to preliminary phytochemical tests followed by the methods of Harbone (1998), and Trease and Evans (1983) and the phytoconstituents reported in **Table 2**.^{1, 2, 3}

Fluorescence Analysis Study of *Aristolochia bracteolate* Leaves Powder: Fluorescence analysis study of powdered drug material with different reagents was carried out to observe the color reactions reported in **Table 3**.

Study of Plant Cell Inclusions: Plant cell inclusions study of powdered drug material with different reagents was carried out to observe the color reactions reported in **Table 4**.

General Chemical and Microchemical Tests: General chemical and Microchemical tests of powdered drug material with different reagents was carried out to observe the color reactions to identify the compound reported in **Table 5**.⁴

Leaf Constants: Vein islet number, vein termination number, stomatal number, and the stomatal index was carried out to observe microscopically reported in **Table 6**.⁵

RESULTS: Prostate herb and often the maximum height is 50 cm. Leaves are cordiform or reniform, 4-7× 5-9 cm, 5-nerved from the base. Bracts are cordate-orbicular, leaf margin entire or wavy.

Flowers dark purple, 5cm long the limb entire, 1-lipped; lip 3cm long, rolled back, emarginated. Fruits are *Capsule* 2.5×1.5cm in size, and the seeds are numerous and the seeds cordate in shape.

Transverse Section of Leaf *Aristolochia bracteolata*: The T.S. of leaf midrib consists of epidermis, cortex and vascular bundle. The epidermis made up of single rows, short ovoid cells and the outer cell wall contains cuticle. The cortex consists of 4 rows of short, ovoid parenchymatous cells.

The vascular bundles are single, globular shaped consists of xylem, cambium, and phloem. 2 rows of phloem cells surround the xylem cells. The lamina consists of single rows of elongated cells and the outer cell wall contains cuticle. The palisade cells are single rows, elongated with chloroplasts and

arranged without intercellular space. The spongy parenchyma cells are 2-3 rows polygonal with intercellular space. The bundle sheath xylem cells are spiral thickening. The epidermal cells of the leaf having anomocytic type of stomata.



FIG. 2: *ARISTOLOCHIA BRACTEOLATE*

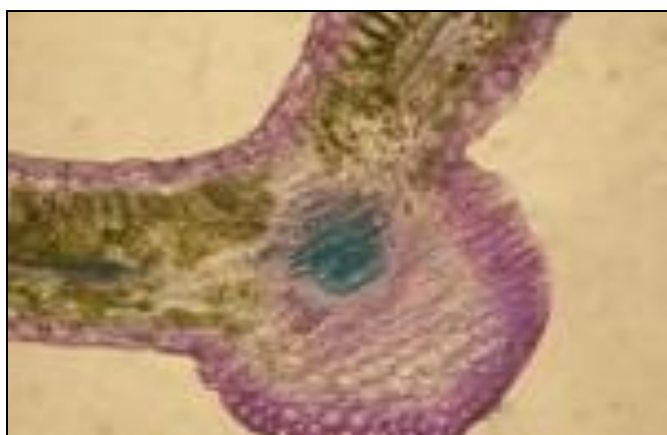


FIG. 3: T.S OF *ARISTOLOCHIA BRACTEOLATE* (MID RIB)

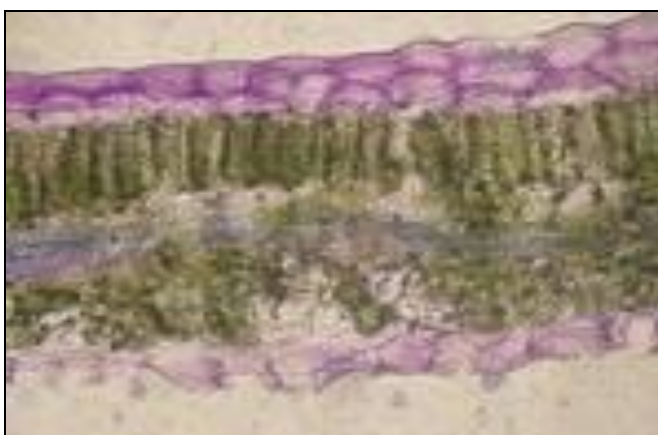


FIG. 4: T.S OF *ARISTOLOCHIA BRACTEOLATE* (LAMINA)

Physiochemical Parameters: The extractive value was highest in water and was recorded to be 24% w/w, and ethanol soluble extractive value was

about 14.4% w/w. The different ash values and the different physiochemical parameters were screened and are presented in the table.

TABLE 1: PHYSIOCHEMICAL PARAMETERS OF *ARISTOLOCHIA BRACTEOLATE* LEAF POWDER

S. no.	Parameters	<i>Aristolochia bracteolata</i>
1	Hexane Soluble extractive	8%
2	Pet ether Soluble extractive	2.4%
3	Chloroform Soluble extractive	4.5%
4	Acetone soluble extractive	4.8%
5	Ethanol soluble extractive	14.4%
6	Ethyl acetate soluble extractive	8%
7	Methanol soluble extractive	13.6%
8	Water-soluble extractive	24%
9	Foreign organic matter	2%
10	Loss on drying	3%
11	Crude fiber content	21%
12	Total Ash	5%
13	Acid-insoluble ash	2%
14	Sulphated ash	12%
15	Water Soluble ash	1%

TABLE 2: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF ETHANOLIC LEAF EXTRACTS OF *ARISTOLOCHIA BRACTEOLATE*

S. no.	Phytoconstituents	<i>Aristolochia bracteolate</i>
1	Alkaloids	+
2	Aminoacids	+
3	Anthraquinones	-
4	Carbohydrates	+
5	Flavonoids	+
6	Phenolic groups	+
7	Saponins	+
8	Steroids	-
9	Tannins	+

+ = Present, - = Absent

TABLE 3: FLUORESCENCE ANALYSIS STUDY OF *ARISTOLOCHIA BRACTEOLATE* LAM., LEAVES POWDER

S. no.	Sample	Colour in Daylight	Colour in UV
1	Powder	Green	Green
2	Powder + 0.1N Sodium hydroxide	Dark green	Pale green
3	Powder + Acetic anhydride	Dark green	Pale green
4	Powder + 0.1N Hydrochloric acid	Pale green	Dark green
5	Powder + water	Pale green	Dark green

TABLE 4: STUDY OF PLANT CELL INCLUSIONS OF *ARISTOLOCHIA BRACTEOLATE*

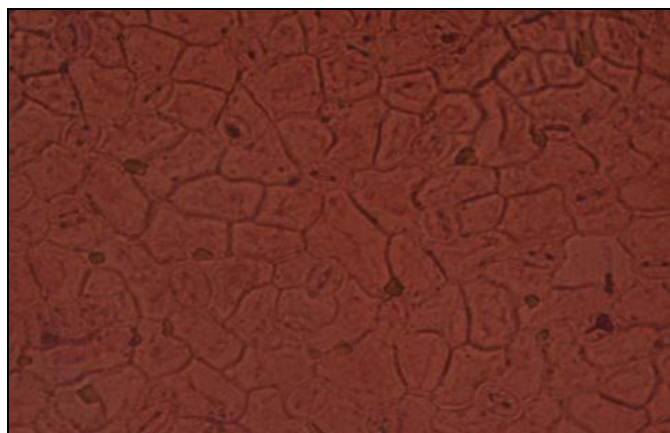
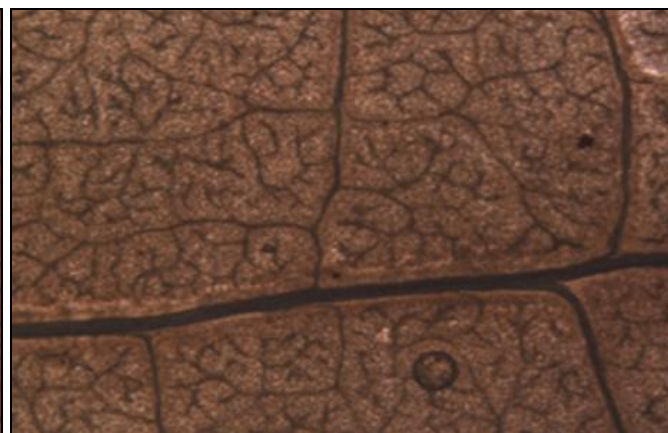
S. no.	Test	Result	Colour
1	Cellulose	+	Pale yellow
2	Lignin	+	Deep blue
3	Suberin	+	Deep yellow
4	Chitin	+	Violet
5	Starch	+	Blue
6	Mucilage	+	Pink
7	Proteins	+	Brick red
8	Alkaloids	+	Reddish brown
9	Tannins	+	Bluish-black
10	Calcium oxalate	+	Needle-shaped crystals
11	Calcium carbonate	+	Needle-shaped crystals

TABLE 5: GENERAL CHEMICAL AND MICRO CHEMICAL TESTS FOR LEAF POWDER OF *ARISTOLOCHIA BRACTEOLATE*

S. no.	Test	Results
1	Test with water /aqueous extract	+
2	Test For Tannins	+
3	Test for Anthraquinine	-
4	Test for Mucilage	+
5	Test for Carbohydrate	+
6	Test for alkaloids	+

TABLE 6: LEAF CONSTANTS OF *ARISTOLOCHIA BRACTEOLATE*

S. no.	Parameters	Results
1	Vein islets number	90±7.61
2	Vein termination number	99 ± 4.81
3	Stomatal number	21 ±0.55
4	Stomatal index	24 ±0.97

**FIG. 5: STOMATA****FIG. 6: VEIN ISLETS AND TERMINATES**

DISCUSSION: The plant was screened for its macroscopic, microscopic, physiochemical parameter, fluorescence analysis, general and microchemical analysis for crude powder and plant cell inclusions showed that they all within the limit. Extraction was carried out by using a Soxhlet apparatus. The extractive values are determined by using the chemicals in order of polarity wise. The extractive value was highest in water and was recorded to be 24.0% w/w, and ethanol soluble extractive value was about 14.4% w/w. The lowest value non-polar solvent pet ether 2.4% w/w, and hexane like 8% w/w. The different ash values like total ash 5% w/w, Acid insoluble ash 2%w/w, and sulphated ash 12% w/w. The *Aristolochia bracteolate* leaf powder reported the potential fluorescent property with different chemical reagents. Mucilage, alkaloids, and tannins identified the general chemical and microchemical analysis. Ethanolic extract was made by using soxhlet apparatus; finally, get the ethanolic extract was tested with chemical reagents color reaction based. The presence showed the alkaloids, flavonoids, carbohydrates, phenolic compounds, saponins, and tannins.

CONCLUSION: The plant *A. bracteolate* Lam., was screened for its macroscopic, microscopic, physiochemical parameter, fluorescence analysis, general and microchemical analysis for crude

powder and plant cell inclusions. Extraction was carried out by using a soxhlet apparatus. The presence showed the alkaloids, flavonoids, carbohydrates, phenolic compounds, saponins, and tannins. Developing countries like India having the percentage of poor people more, to meet with the demand of the poor public, the *Aristolochia bracteolate* Lam., may serve the purpose once the evaluation and detailed studies may over. This work is valuable for further continue the research doing persons.

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CONFLICT OF INTEREST: Nil

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