Research Article

Preliminary Phytochemical Evaluation of Leaf Extracts of *Aristolachea bractiata* Lam

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ABSTRACT

Aristolocheabracteata Lam is plant distributed in tropical Africa, Arabia, Srilanka, Pakistan and India. The present paper deals with preliminary phytochemical evaluation of leaf *Aristolochea bracteata* Lam to establish authenticity and possibly to help to distinguish drug from other plants. The study includes preparation of different extracts by successive solvent extraction for detailed analysis. Fluorescence analysis of different successive extracts and powder were noted under UV light and normal ordinary light, which signifies there characteristics. Different physicochemical parameters such as ash value, extractive value, were carried out as per WHO recommended physicochemical determinations and authentic phytochemical procedures. Preliminary qualitative chemical test for different extract shows the presence of Alkaloids, Glycosides, Phytosterols/triterpenoids, Flavonoids, Saponins, Fixed oils & Fats and phenolics/tannins.

Keywords: *Aristolocheabracteata* Lam, phytochemical evaluation, successive solvent extraction.

INTRODUCTION

Plant derived substances has obtained greater attention in the recent years to prevent and curehuman diseases as they are considered to be more bio-friendly. The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years. While 25 to 50% of current pharmaceuticals are derived from plants, though the traditional Indian system of medicine has a long history of use, they lacked adequate scientific documentation, particularly in light modern scientific knowledge. *Aristolochea bracteata lam* is an important medicinal plant. This species is globally distributed in Tropical Africa, Arabia, Sri Lanka, Pakistan and India. Within India, it is found in northern and central India from Haryana to West Bengal and southwards to Tamil Nadu and Kerala. It is common in dry areas, particularly on black cotton soil, usually growing as a weed. It is used in the treatment of microbial infections, fever, snake bite, allergy, tedious labour, helminthiasis, constipation, inflammation, amenorrhea dysmenorrhea. It contains flavonoids, alkaloids, glycosides, phytosterols/triterphenoids, saponins, phenolics and tanins. The present study is designed to explore the preliminary phytochemical and physicochemical analysis of *Aristolochea bracteata* leaf.

MATERIALS AND METHODS

Collection and identification of Plant Material

Fresh plant leaves were collected from Nellore, A.P. The taxonomic identities of this plant were confirmed by Dr.S.M.Khasim, department of botany, ANU, Guntur, A.P. Fresh plant material was washed, air dried and then homogenized to fine powder and stored in air tight container.

Extraction of plant leaf material

The powdered plant leaf material was subjected to successive solvent extraction taking from non-polar to polar solvents like petroleum ether, benzene, chloroform, ethyl acetate, ethanol and water. Powdered plant material was subjected to soxhlet extraction for 8 hrs with 250ml of the various solvents. The extracts obtained were later kept for evaporation to remove the excessive solvents. These extracts were stored in a cool dry place and was subjected for identification of various plant constituents

Analysis of primary and secondary metabolites in the extracts

The physicochemical parameters like extractive values, florescence characteristics of powdered leaf and leaf extract, preliminary phyto-profiling and phytochemical analysis were determined as per WHO guidelines[1]. The average percentage

w/w of the ash content and the extractive values were determined. The Fluorescence analysis was carried out according to the reported method[2,3]wherein the color of the powdered leaf and leaf extract were also studied under ordinary and ultra-violet light at 366nm. The primary metabolites like proteins, carbohydrates and fixed oils and fats, were analyzed for their presence as per the standard procedures[4,5.]. Similarly, the secondary metabolites like, alkaloids, flavonoids, saponins, phenolics, tannins volatile oils, terpeniods and glycosides were also assessed in the leaf extracts of *Aristolocheabracteata lam.*

RESULTS AND DISCUSSION

All the results generated from the present study are represented in the respective tables. The powdered leaf of *Aristolocheabracteata* subjected to preliminary physicochemical and phytochemical analyses which were found to be very promising. Physicochemical values and Fluorescence characters of the plant powder under ordinary light and UV light (UV 366 nm) were determined and are tabulated in Table 1,2(a,b) The determination of ash value was carried out which gives an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The percentage of total ash, acid insoluble ash, sulphated ash and water soluble ash are carried out and results are as tabulated in the Table 1. Extractive values were also determined which are primarily useful for the determination of exhausted or adulterated drugs. The water soluble and alcohol soluble extractive values were also determined. The fluorescence characteristics was also studied under ordinary and UV light (366nm), wherein the powdered leaf sample and leaf extracts showed the visibility of varying colors which are as tabulated in the Table no. 2(a) and 2(b). The preliminary phyto–profiling for the leaves extracts of *Aristolochea bracteata lam.* was carried out wherein the consistency was found to be sticky in the non-polar to not so polar solvent extracts whereas the polar solvent extracts were found to be non-sticky. The percentage yield w/w of the extracts was also analysed wherein the highest yield was found to be in the methanol extract-7.5%. (Table no. 3) The preliminary phytochemical screening revealed the presence of flavonoids, alkaloids, glycosides, phytosterols/triterphenoids, saponins, phenolics and tanins. (Table 4).The Plant *Aristolocheabracteata lam.*was subjected for preliminary phytochemical analysis viz; Physico-chemical parameters, primary metabolites like, Protein, Carbohydrate, Fixed oils, fats and secondary metabolites like; alkaloids, flavonoides, volatile oils, phenols and tannins, glycosides, terpenoides were tested. Physio-chemical parameters of the leaf extract of *Aristolochea bracteata lam* are tabulated in Table 1. Deterioration time of the plant material depends upon the amount of water present in plant material. If the water content is high, the plant can be easily deteriorated due to contamination by fungal colonies. The loss on drying at 105°C in leaf was found to be 4.6 %. The total ash value of plant material indicated the amount of minerals and earthy materials attached to the plant material. The analytical results showed that; total ash value content was 4.9 %. Similarly; negligible amount of acid insoluble siliceous matter present in the plant 1.08% was observed. The water soluble extractive value was indicating the presence of sugar, acids and inorganic compounds and the alcohol soluble extractive values indicate the presence of polar constituents like phenols, steroids, glycosides, flavonoids are represented in the Table 1. Preliminary phytochemical results showed the presence and absence of certain phytochemicals in the extract. The tests were performed using different organic solvents; Petroleum ether, Benzene, Chloroform, ethyl acetate, Methanol, and Aqueous extracts respectively. The presence of phyto-chemicals in *Aristolochea bracteata lam.*extract revealed that, tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention and anticancer[6,7] Flavonoids have been shown to exhibit their actions through effects on membrane permeability, and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase A2[8.] Flavonoids serve as health promoting compound as a results of its anion radicals[9]. These observations support the usefulness of this plant in folklore remedies in the treatment of stress related ailments and as dressings for wounds normally encountered in bruises, cuts and sores[10-12] The plant extract was also positive for steroids which are very importand compounds especially due to their relationship with compounds such as sex hormones [13] The presence of these phenolic compounds in this plant contributed to their anti-oxidative properties and thus the usefulness of these plants in herbal medicament. Alkaloid was detected in this plant study. Alkaloids have been

associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity[14].

CONCLUTION

The present study on preliminary phytochemical and physicochemical evaluation of *Aristolochea bracteata* leaf could be used as diagnostic tool for the standardization of medicinal plant. WHO parameters as per WHO guidelines discussed here can be considered as the identifying parameters to substantiate and authenticate the drug.

ACKNOWLEDGEMENT

The authors sincerely thank the authority of southern institute of medical science for permitting us to carryout research work.

Table 1: Physicochemical characterization of leaf of *Aristolochea bracteata*

WHO Parameters	Average values %w/w Leaves
Total ash	4.9
Acid insoluble ash	1.08
Water soluble ash	1.31
Sulphated ash	7.12
Alcohol extractive value	7.25
Water extractive value	5.12
Loss on drying	4.6

Table 2(a): Florescence characteristic of leaf extract of *Aristolochea bracteata*

S.no.	Extract	Under ordinary light	Under uv light
1.	Pet.ether	Yellowish green	Yellowish black
2.	Benzene	Yellowish black	Yellowish green
3.	Chloroform	Yellowish brown	Dark brown
4.	Ethyl acetate	black	Greenish black
5.	Methanol	Yellowish green	Dark green
6.	water	black	Blackish Green

Table 2(b): Florescence characteristic of leaf powder of *Aristolochea bracteata*

S. no.	Particulars of the treatment	Under ordinary light	Under UV light (366 nm)
1.	Powder as such	Green	Dark green
2.	Powder + 1N NaOH (aqueous)	GreenishBrown	Black
3.	Powder +1N NaOH (alcoholic)	Dark brown	Black
4.	Powder + 1N HCL	GreenishBrown	Dark green
5.	Powder + H2SO4 (1:1)	green	Dark green
6.	Powder + HNO_3 (1:1)	green	Dark green
7.	Powder + Ammonia	Brown	Black
8.	Powder + Iodine	Dark brown	Dark green
9.	Powder + 5% $FeCl_3$	Brownish green	Black
10.	Powder + Acetic acid	green	GreenishBrown

Table 3: Preliminary Phyto-profile for leaves of *Aristolochea bracteata*

S. No.	Solvent used	Color	Consistency	% Yield w/w
1.	Petroleum ether (40-60°C)	Green	Sticky	4.55
2.	Benzene	Dark green	Sticky	1.7
3.	Chloroform	Dark green	Sticky	0.2
4.	Ethyl Acetate	Green	Non -sticky	1
5.	Methanol	Dark green	Non -sticky	7.5
7.	Water	Brown	Non -sticky	5.25

Table 4: Phytochemical analysis of different extracts of *Aristolochea bracteata*

S. no.	Name of the test	Procedure	Observation	P*	B*	C*	Ea*	M*	aq*
1.	Alkaloids	Drug + Dragondroffs reagent Mayer's reagent Hager's reagent	 Orange color White ppt. Yellow ppt.	-	-	-	-	+	-
2.	Glycosides	Anthrone + H_2SO_4+ Heat	Purple or green	-	+	-	+	-	-
3.	Carbohydrates	Drug + Molishs reagent+ conc. H_2SO_4 Fehling's solution A&B	 Purple color Brick red colour	-	-	-	-	-	-
4.	Phytosterols/tri terpenoids	Liebermann Test Salkowski Test Noller's test	Bluish green Red & fluorescent Pink color	-	+	-	+	+	-
5.	Proteins & amino acids	Biuret test Xanthoprotein test Millon's reagent test Lead acetate test Ninhydrin test	Violet color Orange color White ppt White ppt	-	-	-	-	-	-
6.	Saponins	Drug + water + shaking	Formation of honey comb like froth	-	-	-	-	-	+
7.	Flavonoids	Shinodaw's Test Zn-HCl acid reduction Test	Red color Magenta color	-	-	-	-	-	+
8.	Fixed oils & fats	Spot test	Stains appear after drying	+	-	-	-	-	-
9.	Gums/mucilage	Drug + water	No thickening of the substance	-	-	-	-	-	-
10.	Volatile oil			+	-	-	-	-	-
11.	Phenolics/tannins	$FeCl_3$ Drug + lead acetate + water	Intense color Formation of white ppt.	-	-	-	-	+	+

REFERENCES

1. World Health Organization, Quality Control Methods for Medicinal Plant Materials, WHO, Geneva, 1998.
2. Chase CR and Pratt RJ, Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification, Journal of American Pharmacology Association, 1949, 38, 32.
3. Kokate CK, Practical Pharmacognosy, 1st ed., VallabhPrakashan, New Delhi, 1986, 111.
4. Harborne JB, Methods of extraction and isolation. In: Phytochemical Methods. Chapman & Hall, London, 1998, 60-66.
5. Brain KR and Turner TD, The Practical Evaluation of Phytopharmaceuticals, Wright-Scientechnica. Bristol, 1975, 36-45.
6. Ruch RJ, Cheng SJ, Klaunig JE, Prevention of cyto toxicity and inhibition of intra cellular communication by anti oxidant catechins isolated from chineese green tea, carcinogens1998, 10,1003-1008.
7. Motar MLR, Thomas G, Barbosa Fillo JM, Effects of Anacardoum occidentale Stem bark extract on in vivo inflammatory models. Journal of Ethnopharmacology, 1985, 95(2-3), 139-142.
8. Li H, Wang Z, Liu Y, Review in the studies on tannins activity of cancer prevention and anticancer. Zhong yao cai Zhongyaocai Journal of Chinese medicinal materials, 2003, 26(6), 444-448.
9. Hausteen B, Flavonoids, a class of natural products of high pharmacological potency. Biochemical Pharmacology, 1983, 32, 1141-1148.
10. Lourens ACU, Reddy D, Baser KHC, Viljoen AM, Van Vuuren SF, In vitro Biological activity and essential oil composition of four indigenous South African Helichrysum species. Journal of Ethnopharmacology, 2004, 95, 253-58.
11. Fergusion LR, Role of plant polyphenols in genomic stability. Mutation Research, 2001, 475, 89-111.
12. Grierson DS, Afolayan AJ, Antibacterial activity of some indigenous plants used for the treatment of wounds in the Eastern Cape. Journal of Ethnopharmacology,1999, 66, 103-106.
13. Okwu DE, Evaluation of the chemical composition of medicinal plants

belonging to Euphorbiaceae. Pakistan Veterinary Journal, 2001, 14, 160-162.

14. Nobori T, Miurak K, Wu DJ, Takabayashik LA, Carson DA, Deletion of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. Nature, 1994, 368 (6473), 753-756.