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PRELIMINARY PHARMACOGNOSTIC EVALUATION AND PHYTOCHEMICAL STUDIES ON LEAF OF *ASYSTASIA DALZELLIANA* (NEELKANTH)

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ABSTRACT

The leaves of *Asystasia dalzelliana* (Acanthaceae) are reported to have great medicinal value in folk system of medicine. However, very limited work has been carried out on the drugs used in folk system toward documenting its ethno medicinal uses and establishing its phytochemical and pharmacognostic fingerprints. Studies were therefore carried out to determine the phytochemical and pharmacognostic profile of *Asystasia dalzelliana*. Pharmacognostic evaluation including examinations of morphological and microscopic characters, determination of leaf constant, moisture content, ash value, powder analysis, and extractive values. The phytochemical analysis of the various extract of powdered leaf revealed the presence of alkaloids, tannins, steroids, terpenes and flavonoids.

Key words: *Asystasia dalzelliana*, phytochemical, soxhlet extraction, phytoconstituents.

INTRODUCTION

Asystasia dalzelliana commonly known as violet Asystasia (Marathi: Neelkanth) belongs to Family Acanthaceae. It is a perennial branched herb, about 60-100 m. Stem quadrangular, swollen at nodes. Leaves are opposite, elliptic-oblanceolate acute apex truncate at base; petiole 2cm long. Flowers purplish violet and trumpet shaped, 5-18 cm long, unbranched, 2-lipped. Upper lip is 4-lobed and Lower lip is dark violet, spotted, projecting out and calyx lobe 5, each 7-8mm long, narrow oblong hairy. Corolla 1.5-1.7cm long blue, stamens 4, didynamous; filament 6-8mm long and flowering is during August-November [1]. The whole plant is used in Indian folk medicine such as antioxidant, anti-inflammatory, antivenom a novel whose pharmacology yet to be proved [2]. The preliminary phytochemical [3],[4] and pharmacognostic fingerprints [5] ,[6] of this

potential drug plant toward monograph development and for quality control purposes are reported here for the first time.

MATERIALS AND METHODS

All chemicals used were of analytical reagent grade (supplied by either Merck) and used as supplied.

Plant material and processing

The fresh leaves were collected from Bidar (district of Karnataka state, India) and authenticated by Dr. Shiddamallay Regional Research Institute, Bangalore. The voucher specimen (RRR/BNG/SMP/2009-10/717) was deposited in the same institute. Prior to use, it was ensured that the leaves were free from contamination, sand and had no microbial growth.

Physico-Chemical Analysis of Asystasia dalzelliana

To check the quality, parameters as mentioned in "Quality Standards of Indian Medicinal Plants" by Indian Council of Medical Research were followed. The parameters and methodology are mentioned as below:

Determination of moisture content (Loss on Drying):

2gm of powdered drug was taken in tarred china dish. Dried in the oven at 100ºC or 105ºC, cooled in a desiccator and watch. After that the loss was recorded as moisture. The procedure was continued for at least two common readings.

Physico-chemical parameters [7] [8] [9]:

Total Ash: 2gm of powdered drug was taken in tarred china dish. After than it was subjected to muffle furness at 450ºC temp. The weight was taken after red hot and cooling at each two hours constant readings.

Acid Insoluble Ash: 2gm of powdered drug was taken and mixed 25 ml of hydrochloric acid (HCL). Total ash was boiled for 5 min. and diluted was 25 ml of hydrochloric acid (HCL). Insoluble matter was collected on ash less filter paper (Grade 4T SD'S clear drop, 90mm code- F0401C10, Circuler-100). Filter paper washed with hot water. Crucible was ignited, cooled and kept in dessicator. Residue was weighed and calculated acid insoluble ash of drug.

Powder characteristics [13] [14] [15]:

Procedure:

The plant was morphologically examined for shape of leaves, apex, base, margin etc. A separate section was prepared and examined for the identification of starch grains by staining with iodine solution. Powder (# 60) of the dried leaf was used for the observation of powder microscopic characters. The powder drug was separately treated with phloroglucinol –HCL solution, glycerin and iodine solution to determine the presence of lignified cells, calcium oxalate crystals and starch grains as a part of quantitative microscopy, stomatal number, stomatal index, vein islet and veinlet termination number were determined by using fresh leaves of plant Moisture content, Total ash, acid insoluble ash, water and alcohol soluble ash was also determined. Alcohol and water-soluble extractive values were determined.

Preliminary Phytochemical studies [10] [11] [12]

The powder of dried leaves was subjected to continuous soxhlet extraction with various organic solvents such as petroleum ether (60-80^{o}C), chloroform, benzene, methanol, ethanol and water respectively. After concentration and drying of each extract in vacuum dessicator identification of phytoconstituents was carried out by standard chemical test and also by using thin layer chromatography method by different detecting reagents.

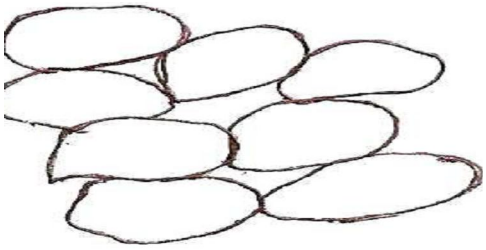
RESULTS AND DISCUSSION

Macroscopy

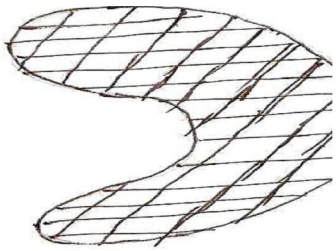
The morphological studies revealed the shape of leaves of Asystasia dalzelliana simple, rhomboid, deltoid to lanceolate, upper entire, lower toothed or irregularly lobed, extremely variable in cultivated forms, 11-14 cm long, petioles often as long as thick blade, lanceolate to oblong and length varies from 5 cm to 5.5 cm broad with dentate margin. It has been found in dark green color with smooth under surface. The petioles were of 1 to 1.3 cm in length.

Microscopy

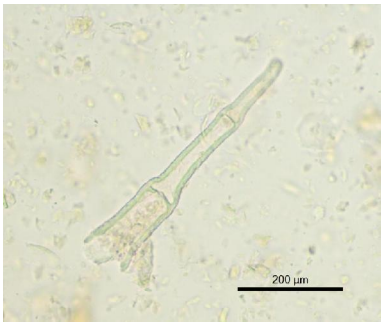
In powder microscopy straight walled polygonal collenchyma and yellow colored bean shaped mass with mesh like striations were observed prominently. Multicellular covering types of trichome distinctly observed with fragments of Epidermis were, acicular and prismatic calcium crystals, Spiral Vessels was observed (Fig 1).



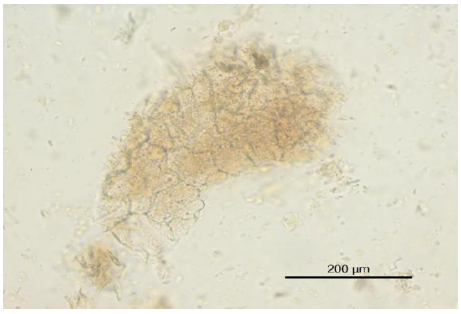
Straight polygonal cells



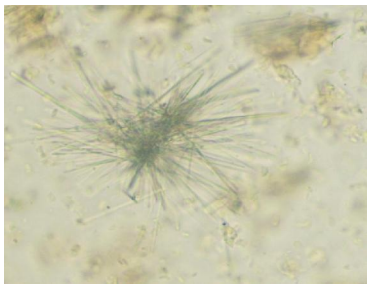
yellow coloured mass of the cells



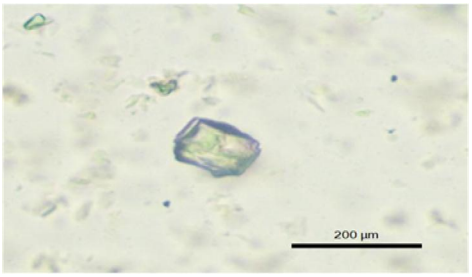
Multi cellular covering trichomes



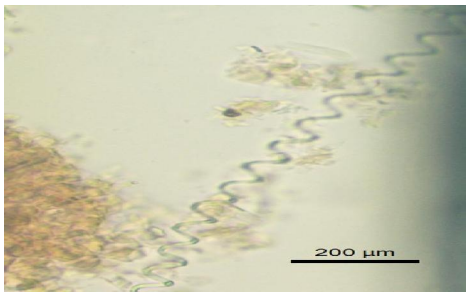
Fragaments of Epidermis



Acicular crystals



Calcium Oxalate Crystals (Prismatic)



Spiral Vessels

Stomata

Figure 1
Powder microscopy characters of *Asystasia dalzelliana*

Physiochemical analysis

Successive solvent extraction values in various organic solvent were observed as petroleum ether0.89%, chloroform 0.76%, methanol 2.4%,ethanol 1.4% and water 3.6, Colour & Consistency (Table-1).

Table 1: Successive solvent Extraction of leaves of *Asystasia dalzelliana*

S.I No.	Solvents used	Colour & Consistency	Average extractive values in % w/w on dry weight basis
1	Petroleum Ether	Greenish yellow sticky mass	0.89
2	Chloroform	Light green sticky mass	0.76
3	Methanol	Greenish brown sticky mass	2.4
4	Ethanol	Green sticky mass	1.4
5	water	Brown dry mass	3.6

The proximate analysis revealed that total ash value 9%, acid insoluble ash 3%, alcohol soluble extractive16%, water soluble extractive18.4%,stomatal no 160-224, stomatal index 26.31-35.89, vein islet no 7-9, vein islet termination no 6-7 and palisade ratio 6-8 values were observed in fresh leaves (table 2).

Table 2: Determination of Proximate Analysis for *Asystasia dalzelliana*

Tests	Results (in %)		Inference (in %)
Tests for extraneous material	T1	T2	Average
Foreign matter	0.67	0.69	0.68
Sand & Silica	Absent	Absent	Absent
Insect infestation	Absent	Absent	Absent
Rodent contamination	Absent	Absent	Absent
Physico-chemical analysis			
Moisture content	5	6	5.5
Total Ash content	8	10	9
Acid insoluble ash	2	4	3
Tests for extractive value			
Alcohol soluble extractive	15	17	16
Water soluble extractive	17.4	19.4	18.4

Preliminary Phytochemical studies

The preliminary phyto chemical studies revealed that presence of alkaloid in chloroform, ethanol, methanol and aqueous extract prominently. The flavonoid was present in Ethanol, methanol and aqueous extract respectively. The steroid was observed

in petroleum ether methanol and aqueous extract clearly. The tannins were observed methanol and aqueous extract. (Table 3).

Table 3: Phytochemical screening of successive solvent extracts of selected medicinal plants

SI NO	Name of the test	pet ether	Chloroform	Ethanol	Methanol	Water
1	Steroids	+	-	-	+	+
2	Alkaloids	-	+	+	+	+
3	Saponins	-	-	-	-	-
4	Tannins	-	-	-	+	+
5	Flavonoids	-	-	-	+	+
6	Carbohydrates	-	-	-	-	-
7	Proteins	-	-	-	-	-
8	Fixed oil	-	-	-	-	-
9	Resins	-	-	-	-	-
10	Glycosides	-	-	-	-	-

Thin Layer Chromatography

Test Solution: 0.5g of powdered drug was extracted with methanol (3 x 15 ml) under reflux on a water bath. Methanolic extract was filtered and concentrated and made up the volume to 25ml with methanol.

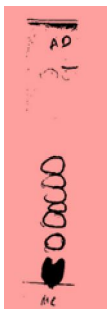
Solvent System: Toluene: Ethyl acetate: Formic Acid: water (100:11:11:27)

Procedure: Applied 10ml each of test solution and standard solution on pre coated Silica Gel 60 F254 plate of uniform thickness of 0.5mm. The plates were developed in the solvent system.

Visualization: The plates were examined under ultraviolet light at 254nm.

Evaluation: The seven spots separated and evaluated under UV (table 4).

Table 4: TLC Screening of *Asystasia dalzelliana*



Solvent system	Detection	Color of spots	Rf value
Toluene: Ethyl acetate: n-butanol: Formic Acid:water (100:11:11:27)	Ultraviolet light at 254nm	Purplish green	0.2
			0.3
			0.35
			0.425
			0.475.
			0.525

UV Analysis:

Powder drug under ultraviolet and ordinary light when treated with different reagent emitted various color radiations which helps in identifying the drug in powder form (table 5).

Table 5: UV analysis of leaf extract of *Asystasia dalzelliana*

Treatment	Visible ligth	Ultra-violet light	
		Short wave (254)	Long wave (365)
Powder as such	Greenish yellow	Green	No fiuorescence
In methanol	Green	Green	No fiuorescence
In meth.NaoH	Green	Green	No fiuorescence
In ethanol	Green	Green	No fiuorescence
In etha.NaoH	Green	Green	No fiuorescence
In 1N HCL	Greenish black	Green	No fiuorescence
In 50% H_2SO_4	Greenish black	Green	No fiuorescence
In 50% HNO_3	Greenish black	Green	No fiuorescence
In 5% KOH	Green	Green	No fiuorescence

CONCLUSION

In conclusion it may stated that the approach given for standardization of any new herbal or medicinal plant including physical, chemical and evaluation and comparison should be developed systematically for completion of database of newer plants. This shall help to obtain monograph of the future medicinally active plant. For developing analytical method pure active chemical constituent should be isolated in further study and identification on basis of reference standard shall be made. This also helps in setting in-house standards of the medicinal plants used extensively by herbal manufacturers. Asystasia dalzelliana is rich in secondary metabolites and has numerous uses in traditional medicine to treat several ailments, ethno medicinally reputable as antiveenom. It has potential for development into a phytomedicine. More work should carry out to isolate and characterize the chemical constituents to ascertain its antiveenom properties. The phytochemical and pharmacognostic fingerprints reported here for this potential drug plant could be useful for monograph development and for quality control purposes.

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