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Numerical taxonomic studies on *Coleus forskohlii* collected from different geographical regions of India

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Abstract
The present study was carried out with aim to study the microscopic characters of *Coleus forskholii* (Linn.). Various samples of this plant were collected from different phyto-geographical regions (C-1; Chamba, Uttarakhand, C-2; Dhanauti, Uttarakhand, C-3; Agriculture University campus, Jabalpur, M.P, C-4; Tamiya Forest, Madhya Pradesh, C-5; Bilaspur, Chhattisgarh) of country. Most of the samples showed similarities in macroscopic characters, slight variations in microscopic characteristics, but in quantitative microscopy some significant differences were observed. Microscopic evaluation clearly revealed that various diagnostic features like multicellular trichomes, anomocytic stomata were observed. Quantitative microscopical characters like stomatal number, stomatal index and trichome were observed. Here, this study indicated information of taxonomic significance. Leaf trichome formation is significant adaptive feature as it provides resistance against herbivory also.

Keywords: *Coleus forskholii*, numerical taxonomy, identification

1. Introduction
Plectranthus forskohlii (Wild) Briq. (Syn. *C. forskohlii*) that belongs to the family Lamiaceae, commonly known as *Coleus,* Pashanbedi (Sanskrit), Patharchur (Hindi), Manganiperu (Kannada), Marunthu Koorkan (Tamil), which is grown throughout the country. Its tuberous roots are found to be a rich source of forskolin (coleonol) used as a potential drug for hypertension, obesity, bronchitis, asthma, respiratory disorders, painful urination, insomnia and psoriasis., [1]. It is a perennial plant that grows to about 45-60 cm tall and aromatic in nature. *Coleus* is an aromatic perennial, with an erect stem and has tuber like roots, reaching upto 60 cm [11]. It has four angled stems that are branched and nodes are often hairy. Leaves are 7.5 to 12.5 cm in length and 3 to 5cm in width, usually pubescent, narrowed into petioles. Inflorescence is raceme, 15-30cm in length; flowers are stout, 2 to 2.5 cm in size, usually perfect and calyx hairy inside [12]. Upper lip of calyx is broadly ovate. The blue or lilac corolla is bilabiate. Lower lobes are elongated and concave so that they enclose the essential organs. The ovary is four parted, stigma is two lobed and the flower is cross-pollinated by wind or insects [6]. The roots are tuberous, thick, fibrous, brown in colour, orange-red within and is strongly aromatic. It is the only species of the genus to have fasciculate and non- tuberous roots. The leaves and tubers have quite different odours. However, the growth habit of *C. forskohlii* is strikingly variable being erect, procumbent or decumbent; similarly, the root morphology in different populations is also fascinatingly diverse, being tuberous, semi or fibrous (non-tuberous). The *Coleus* group of plants grows in tropical to subtropical situations and in warm temperate climatic zone on mountains of India, Nepal, Burma, Sri Lanka, Thailand and Africa. It comes up well on the sun exposed dry hill slopes from 300m to 1800m altitude. A well-drained medium fertile soil is suitable for its cultivation. It is propagated vegetatively through stem and root cuttings. Indian sub- continent is considered as the place of origin of *C. forskohlii* [15]. It grows wild in the subtropical temperate climates of India, Nepal, Burma, Sri Lanka and Thailand. Apparently, it has been distributed to Egypt, Arabia, Ethiopia, tropical East Africa and Brazil [13]. In India, the plant is found mostly on the dry and barren hills**.** Latitudinal and altitudinal range for the occurrence of the species is between 80 and 310 N^0 and 600 – 800 m, respectively. It is native to India and grows in the subtropical temperate climates of India, Nepal, Thailand and Sri Lanka. *Coleus* is 1 to 2 feet tall having teardrop striking leaves, shimmering green framing a bright purple centre; leaf colour differs with the amount of shade. Flowers are of pale purple or blue colour (Figure 1). The rootstock is thick, fibrous, rapidly spreading and typically golden brown [14]. The whole plant of *Coleus* is important; however, we use normally, root and leaves of this plant.

Fig 1: Morphological view of *Coleus forskholii* (Field pic, Tamiya Forest)

It is propagated by seeds and vegetative method [11]. *Coleus* has been used since ancient times in Ayurvedic medicine [12, 13]. *Coleus* plant have reputed medicinal uses, which includes Antiaggregant, anticancer [7], antidepressant, antidiuretic, anti-glaucomic, antimetastatic, antispasmodic, bronchodilator, broncho spasmolytic, cAMP-genic, Cardiotonic, CNS-depressant, gastro-stimulant, gluconeogenic, glycogenolytic, hypotensive, immunosuppressant, lipolytic, myorelaxant, neurogenic, pancreato-stimulant, positive inotropic, Secretagogue, sialagogue, Thyrotropic and vasodilator [3, 9]. *Coleus* also finds application in the treatment of eczema and psoriasis (Alternative Medicine Review, 2006). The leaf extracts of this plant have significantly high amounts of polyphenols, flavones and flavanols' and high antioxidative activity [15]. This antioxidative activity provides the basis of this plant for cosmetic use.

2. Materials and Methods

2.1 Processing of plant samples

Samples were collected from Uttarakhand, Madhya Pradesh and Chhattisgarh regions of country. The leaves were boiled separately with saturated chloral hydrate solution for determination of different leaf constants. Clearing and staining were done by methods described by Evans & Trease [4] and Kokate [8].

2.2 Quantitative microscopy

The important identifying characteristic of leaf constants like Stomatal Number, Stomatal Index was calculated and tabulated.

2.2.1 Stomatal number

It is the average number of stomata per square mm of the epidermis of the leaf.

2.2.2 Procedure

Cleared the piece of the leaf (middle part) by boiling with chloral hydrate solution or alternatively with chlorinated soda. Upper and lower surface of epidermis was peeled out separately by using forceps. It was kept on slide and mounted in glycerine water. Using camera lucida and drawing board, structure was drawn. Firstly, a square of 1mm was drawn by means of stage micrometre. Leaf epidermis was placed on the slide and the number of stomata/sq. mm of leaf was calculated. Recorded the data for each of the ten fields and average number of stomata per sq. mm of leaf were calculated and recorded accordingly.

2.2.3 Stomatal index

Stomatal index is the percentage which the number of stomata forms to the total number of epidermal cells, each stoma being counted as one cell. Stomatal index can be calculated by using the following formula.

S I= S/S+E x 100

I = Stomatal index, S = Number of stomata per unit area,

E = Number of epidermal cells in the same unit area.

2.2.4 Trichome study

Trichomes are hair-like surface appendages that develop from cells of the aerial epidermis and are produced by most plant species. Leaf trichomes can serve several functions including protection against damage from herbivores. Leaf trichomes contribute to plant resistance against herbivory.

2.2.5 Procedure

Cleared a piece of the leaf (middle part) by boiling with chloral hydrate solution or alternatively with chlorinated soda. Peeled out upper and lower epidermis separately, using forceps. Kept it on slide and mounted it in glycerine water after staining it in saffranine. Using camera lucida and drawing board, structure was drawn. Firstly, a square of 1mm was drawn by means of stage micrometre. Leaf epidermis was placed on the slide. Traced different types of trichomes found on epidermal surface of leaf. Number of trichomes on upper and lower surface (epidermis) of leaf was calculated separately in each field.

3. Results and Discussion

3.1 Table - 1 clearly shows that phytogeographic distribution of plant sample, its altitude, latitude and longitudinal parameters with distinct soil types.

Table 1: Latitudinal and Altitudinal range

S. No.	Phytogeographic zone	Altitude (meters)	Latitude	Longitude	Soil type
C-1	Western Himalayas	4855	$30^0$20'52.93''N	$78^0$23'49.88''E	Sandy- loamy to clayey
C-2	Western Himalayas	6855	$30^0$25'31.17''N	$78^0$14'13.58''E	Sandy- loamy
C-3	Upper Gangetic planes	1385	$23^0$08'37.84''N	$79^0$59'23.07''E	Black soil
C-4	Upper Gangetic planes	3081	$22^0$20'39.80''N	$78^0$40'10.50''E	Black soil
C-5	Upper Gangetic planes	887	$22^0$04'51.81''N	$82^0$08'19.86''E	Sandy

3.2 Microscopic evaluation

On microscopic evaluation of leaf peel/ surface study epidermal cell shape, types of stomata, type of trichome, position of mesophyll cells, type of midrib, presence of calcium oxalate crystal (druces) were observed under microscope that have been depicted in Table 2. In Table 2,

stomatal parameter on both upper & lower surface of leaf were mentioned while in Table- 4, trichomes on both the upper and lower surface of leaf were calculated in per mm square of leaf in different accessions of leaf collected from different phyto-geographic zones. Further, photographs of all five accessions showing stomata and trichome in peel study of *Coleus* plant were visualised in the form of monograph. Monograph - 1 clearly shows the number and distribution pattern of stomata in 1mm^2 area of leaf surface, while monograph - 2 clearly reveals abundance of trichomes on leaf surface in which trichome is 5-6 celled, pointed and non-glandular covering whole leaf surface as well as margins of thick leaf.

Table 2: Microscopic studies of *C. forskohlii* Leaf

Leaf peel surface characters	Types
Epidermal cell shape	Polygonal
Stomatal type	Anomocytic
Trichome type	Peltate
Mesophyll	Dorsiventral
Midrib	Biconvex
Calcium oxalate crystal	Rare druces

In Table-3, the variation in the range of stomatal number and stomatal index on both upper and lower surface of leaf is clearly shown which is an important parameter in depicting its distribution zone as well as also in taxonomic identification of plants.

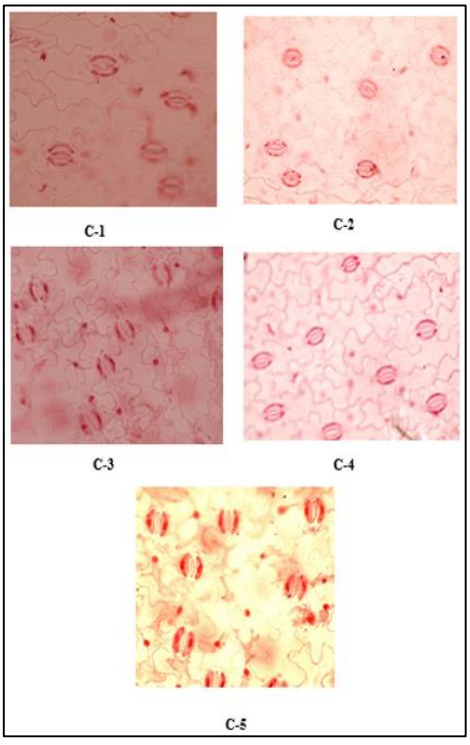
Table 3: Quantitative microscopic studies of *C. forskohlii* Leaf

Sample	S I-lower	S I-upper	SN-lower	SN-upper
C-1	31.16-33.24	24.87-26.16	12.13-13.64	8-10.14
C-2	33.12-35.10	29.76-31.34	14.28-16.52	11.33-13.24
C-3	28.15-29.46	27.99-30.20	15.13-16.18	13.33- 15.26
C-4	34.13-35.16	30.77-32.28	15- 18.12	13- 15.68
C-5	30.12- 32.98	28.85- 29.02	14.22-17.16	12.46-16.12

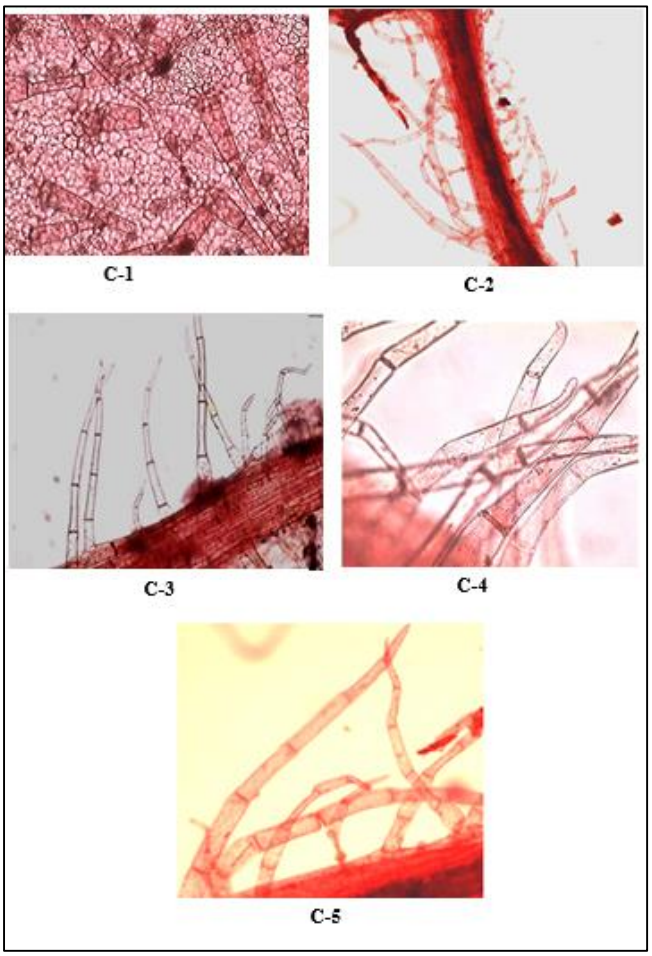
Below drawn Table shows variation on number of peltate type of trichome on both upper and lower surface of leaf in 1mm^2 which ultimately depicts the density of trichome with changing Agro-climatic zone. High density of trichome on leaf surface is an important adaptation of plant to prevent herbivory [2].

Table 4: Trichome studies on *C. forskohlii* Leaf

Sample	No of trichome/mm^2 of leaf	
	Upper surface (Peltate)	Lower surface (Peltate)
C-1	9.2	7.3
C-2	8.42	6
C-3	13.6	10.4
C-4	10.24	7.66
C-5	12.32	9.24



Monograph 1: Quantitative microscopic studies on stomata



Monograph 2: Quantitative microscopic studies on trichome

4. Conclusion

This result supports the finding of Irshad *et al*, 2014 [5] and Dalin *et al*, 2008 [2]. The macroscopical & microscopical parameters reported here can be considered as a distinctive enough to identify and decide the authenticity of reported *Coleus forskholii* species that can further be used in industrial production of drugs after proper authentication. Microscopic evaluation showed diagnostic characters of *C. forskohlii*. As

we move from northern to southern part of country stomatal number and stomatal index both were increased. It clearly showed that with increase in temperature, stomatal density is increased. Accordingly, transpiration rate also enhanced which is required for providing cooling effect to plant to survive in warmer region. Peltate types of trichomes were observed more in warmer (Sub-tropical) region. It can be used in taxonomic identification of this particular genus. Dense trichome is a defence tool of this plant against herbivory.

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