



INFLUENCE OF REGIONAL FACTORS ON THE PHYTOCHEMICAL COMPOSITION AND BIOACTIVITY OF INDIAN BORAGE (*COLEUS AMBOINICUS*) COLLECTED ACROSS KERALA

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Abstract: This study investigated the influence of regional factors on the phytochemical composition and the bioactivity of *Coleus amboinicus* (Indian Borage) collected from three distinct regions in Kerala, India - Wayanad, Alappuzha, and Thrissur. The aim of the research is to assess the influence of regional factors like altitude, soil type, climate and rainfall on the medicinal properties of the plant *Coleus amboinicus*. Macroscopical and microscopical studies of leaves were conducted which revealed the differences between the morphological and cellular features of *Coleus amboinicus* in the three different regions Alappuzha, Thrissur and Wayanad. Pharmacognostical and phytochemical investigations identified the constituents responsible for the plant's therapeutic properties. To evaluate the quality standards, physicochemical parameters such as ash values, extractive value, swelling index, foaming index and moisture content were determined. Phytochemical screening of the plant confirmed the presents of alkaloids, glycosides, flavonoids, phenols, tannins, phytosterols, triterpenoids and volatile oils. Aqueous extracts of the leaves were prepared and subjected to bioactivity analysis. Antioxidant activity and antimicrobial activity was determined. The results showed noticable regional variations in phytochemical content and biological activity. The sample collected from Wayanad exhibited the highest total flavonoid content when compared to the samples from Thrissur and Alappuzha. The volatile oil from Thrissur showed the highest phenolic content and strong aroma. All extracts showed significant antioxidant and antimicrobial properties, with the plant aqueous extract which was collected from Wayanad showing the highest zone of inhibition against *Staphylococcus aureus*. These findings indicate that geographical and environmental factors significantly influence the phytochemical composition and therapeutic efficacy of the plant *Coleus amboinicus*. Sample collected from Thrissur may be more suitable for flavouring agent preparations due to their stronger aroma, whereas Wayanad samples are more appropriate for the medicinal formulations because of their higher flavonoid content and enhanced pharmacological activity.

Index Terms - *Coleus amboinicus*, regional factors, bioactivity, comparing.

I. INTRODUCTION

Pharmacognosy may be defined as a branch of bioscience which treats in detail medicinal and related products of crude or primary type obtained from plant, animal and mineral origins[1]. The scope of Pharmacognosy is vast and encompasses various aspects of natural products research and development. It includes: Botanical Identification, Plant Cultivation, Extraction and Isolation, Pharmacological Evaluation, Standardization and Quality Control, Ethnopharmacology, Drug Discovery and Development.[2].

1.1 *Coleus amboinicus*

Indian Borage, also known by its scientific names *Plectranthus amboinicus* or *Coleus amboinicus*, is a versatile, aromatic herb that thrives in tropical climates. It likely traces its roots back to regions in Africa and Asia and is widely found throughout India today. It's commonly known by several names, including Cuban Oregano, Mexican Mint, and Spanish Thyme. Indian Borage features thick, fleshy, and ovate leaves with a distinctive aromatic scent [3]. It is widely used in traditional medicine of India, for treating cough, cold, digestive disorders and skin ailments [4]. The antioxidant, antibacterial, antimicrobial, antiinflammatory and antifungal effects are particularly shown in the volatile essential oil from the extract of the plant leaves. However, their exact composition and effectiveness can differ depending on the plant's variety and the region in which it grows. [4]. The plant is rich in a wide variety of bioactive compounds, with roughly 30 non-volatile and 76 volatile phytochemicals, including flavonoids, esters, alcohols, aldehydes, phenolics, and different groups of terpenoids. Preparations made from its leaves, especially decoctions, have been traditionally used to ease persistent cough and even help manage bacterial infections like tuberculosis. The leaves also

demonstrate antimicrobial action by limiting the growth of harmful bacteria such as *Salmonella typhimurium* and *Escherichia coli*, and they are considered useful against microbes involved in diabetic wound infections.[5]. Phytochemicals that are considered as primary metabolites play a vital role in the growth, development, or reproduction of a plant. In other words, secondary metabolites are compounds produced by plants that are not directly required for basic growth but play important roles in functions like defense and pollination. A variety of these compounds have been identified in *C. amboinicus*, including monoterpenoids, diterpenoids, triterpenoids, sesquiterpenoids, alkaloids, phenolics, and flavonoids.[6]

1.2 Influence of regional factors

One of the most important ways the environment affects phytochemical profiles are through climate, temperature, sunlight, and rainfall. For example, plants growing in warmer climates often produce higher amounts of secondary metabolites, such as phenolics. In addition to climate, altitude also has a significant impact on the phytochemical composition of plants. [7] The primary and secondary metabolites in the plant is decided by the plant growth and available soil nutrients. Soil pH plays an important role in how nutrients are available in the soil, how well plants can absorb them, and overall plant growth. In addition, the amount of phytochemicals present in plants can vary depending on their geographical location, which in turn can affect the medicinal properties we expect from those plants.[8].Regional climate changes can strongly influence how plants produce secondary metabolites such as alkaloids, flavonoids, phenolics, and terpenoids. These compounds are essential for the pharmacological activity of herbal products. Even small changes in climate can alter the therapeutic quality of plants. Understanding how these environmental factors affect plants can help in carrying cultivation practices, ensuring that Indian medicinal plants remain effective and reliable for treatment [9]. Soil science is a broad field that connects areas like environmental science, agriculture, and ecology. The properties of soil is important not just for improving crop production, but also for keeping ecosystems stable and healthy. The balance between the soil's mineral and chemical characteristics plays key role in improving the plant growth .In farming, soil fertility is important, as it affects both the yield and quality of crops.[10]

II. MATERIALS AND METHODOLOGY

2.1 Sample collection

Mature leaves of *Coleus amboinicus* were freshly gathered on 4th November 2025 from Sulthan Bathery (Wayanad), Attoor (Thrissur), Alappuzha (Alappuzha) at the same time. The plant material was authenticated by Dr. Ranjusha A. P., a botanist from the Department of Botany at NSS College, Ottapalam and Herbarium has been deposited at Pharmacognosy Department, Nehru College of Pharmacy, Thrissur, for future reference. *Coleus amboinicus* whole plant were separated from twigs and shade dried for about 20-25 days, powdered with mechanical grinder and stored in an air tight container for further studies.

2.2 Preparation of plant extract

The 5 g of powder was macerated with 100 ml of distilled water and 2 drops of chloroform for 7 days with occasional shaking. The extracts were filtered and evaporated to dryness

2.3 Macroscopic characteristics

Macroscopic study of fresh leaves was performed using standard methods, assessing sensory and morphological features visible to the naked eye or with a magnifying lens according to the methods mentioned by Padmaja V et.al [11]

2.4 Microscopic characteristics

Hand sections of the leaf were prepared, stained, and mounted using standard micro techniques. Transverse sections were stained with phloroglucinol-HCl and mounted in glycerine. For powder microscopy, the leaf powder was stained with Sudan red and mounted in glycerine.[12]

2.5 Determination of Physicochemical Constituents

The determination of physicochemical constituents was carried out to evaluate the quality, purity, and authenticity of the crude drug. These parameters are essential for standardization and quality control, as they help detect adulteration, improper processing, and variations arising from environmental and handling conditions. Physicochemical evaluation included the determination of ash values, moisture content, extractive values, swelling index, and foaming index, following standard pharmacopoeial procedures. [13][14]. The results obtained provide baseline data for the identification and quality assessment of the plant material.

2.6 Preliminary phytochemical screening:

The Preliminary phytochemical screening was carried out by methods mentioned in Teresa May et.al [15] and K.V.N Rao et.al [16]

2.7 Quantitative Determination of Phytochemicals

2.7.1 Estimation of Total Phenolic Content

Total phenolic content was determined using the Folin-Ciocalteu method. One millilitre of the extract was mixed with 0.5 ml of Folin-Ciocalteu reagent and 1 ml of 7% sodium carbonate, and the final volume was adjusted to 10 ml with distilled water. The reaction mixture was incubated for 60 minutes with intermittent shaking, and absorbance was measured at 760 nm. A calibration curve was prepared using gallic acid (20–100 µg/ml) as the standard. The reagent blank was used for baseline correction.[17]

2.7.2 Estimation of Total Flavonoid Content

Total flavonoid content was estimated by the aluminium chloride colorimetric method. One millilitre of extract was mixed with 4 ml of distilled water, followed by the addition of 0.3 ml of 5% sodium nitrite. After 5 minutes, 0.3 ml of 10% aluminium chloride was added. Five minutes later, 2 ml of 1 M sodium hydroxide was added, and the volume was made up to 10 ml with distilled water. Absorbance was measured at 510 nm. Quercetin (20–100 µg/ml) was used to generate the standard calibration curve. Results were expressed as µg/mg of extract.[18]

2.8 Bioactivity evaluation

2.8.1 Antioxidant Activity: DPPH Radical Scavenging Assay

The free radical scavenging activity of the extract was evaluated using the DPPH assay according to the method of Brand-Williams *et al.* (1995), with minor modifications. DPPH, a stable free radical, is reduced in the presence of antioxidants, resulting in a color change from deep violet to light yellow, measured spectrophotometrically at 515 nm. Ascorbic acid was used as the reference standard. A 60 µM DPPH solution in methanol was freshly prepared, and 200 µl of this solution was mixed with 50 µl of extract at various concentrations (1.56–800 µg/ml). The reaction mixture was incubated in the dark for 15 minutes at room temperature, and absorbance was recorded at 515 nm. Methanol (95%) served as the blank, and DPPH solution without sample was used as the control.[19]

The percentage of radical scavenging activity was calculated using the formula:

$$\% \text{ Inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100 \quad (2.1)$$

2.8.2 Antimicrobial Activity: Disc Diffusion Method

Antimicrobial activity was evaluated using the disc diffusion method. This method is based on the diffusion of antimicrobial agents from impregnated discs into agar medium inoculated with the test microorganism, resulting in a concentration gradient and subsequent inhibition of microbial growth.

The inoculum was prepared in suitable broth and uniformly spread on agar plates. Sterile discs impregnated with the test sample were placed on the inoculated plates using sterile forceps. Plates were incubated at 30°C (or optimum temperature) for 16–18 hours. Antimicrobial activity was assessed by measuring the diameter of the zone of inhibition (mm) surrounding each disc and compared with standard interpretative criteria.[20]

III. RESULTS

3.1 Plant collection

The plant *Coleus amboinicus* were freshly collected on November 04 2025 at exactly same time, from three different regions across kerala.

- i. Sulthan Bathery, Wayanad, Kerala
- ii. Attoor, Thrissur, Kerala
- iii. Alappuzha, Alappuzha, Kerala

3.2 Identification and authentication

The plant was taxonomically identified and authenticated by the Botanist, Dr. Ranjusha A P, HOD, Department of Botany, NSS college Ottapalam.

3.3 Drying

The plant material was dried under shade for 20-25 days, then powdered using a mixer grinder, and finally stored in an airtight container.



Figure 1: Collection of *Coleus amboinicus* from Wayanad Thrissur and Alappuzha

3.4 Ecogeographical assessment:

The soil of districts Wayanad Alappuzha and Thrissur are Laterite soil, Sandy soil and Fine loamy soil respectively. The average annual rainfall of Wayanad is 1881 mm, Alappuzha is 2804 mm and Thrissur is 2548. Thrissur lies in the mid land, Alappuzha in the low land and Wayanad in high land. The altitude of Wayanad district is 901 m above sea level, Thrissur is 416 m above sea level and Alappuzha is 21 m above sea level.

These leaves were subjected to further evaluation.

3.5 Macroscopic evaluation

Macroscopical evaluation of the leaf samples from Wayanad, Alappuzha, and Thrissur shows clear regional variations in their physical characteristics. The leaf colour ranges from green (Wayanad) to pale green (Alappuzha) and dark green (Thrissur). Differences in size are observed, with Wayanad leaves showing a wider width range, while the others are more uniform. The shape is generally ovate, with Thrissur samples being broadly ovate. Odour and taste intensity increase from Wayanad to Thrissur, indicating stronger aromatic and pungent properties in Thrissur samples.

Table 1 Macroscopic evaluation of the leaves of *Coleus amboinicus* collected from Wayanad Alappuzha and Thrissur

Sl no	Features	Observation	Observation	Observation
		Wayanad	Alappuzha	Thrissur
1	Colour	Green	Pale green	Dark green
2	Size	Length: 4-8 cm Width: 4-10 cm	Length: 5-9 cm Width: 4-7 cm	Length: 5-9 cm Width: 4-6 cm
3	Shape	Ovate	Ovate	Broadly ovate
4	Odour	Weak aromatic	Aromatic	Strong aromatic
5	Taste	Mild pungent	Pungent	More pungent

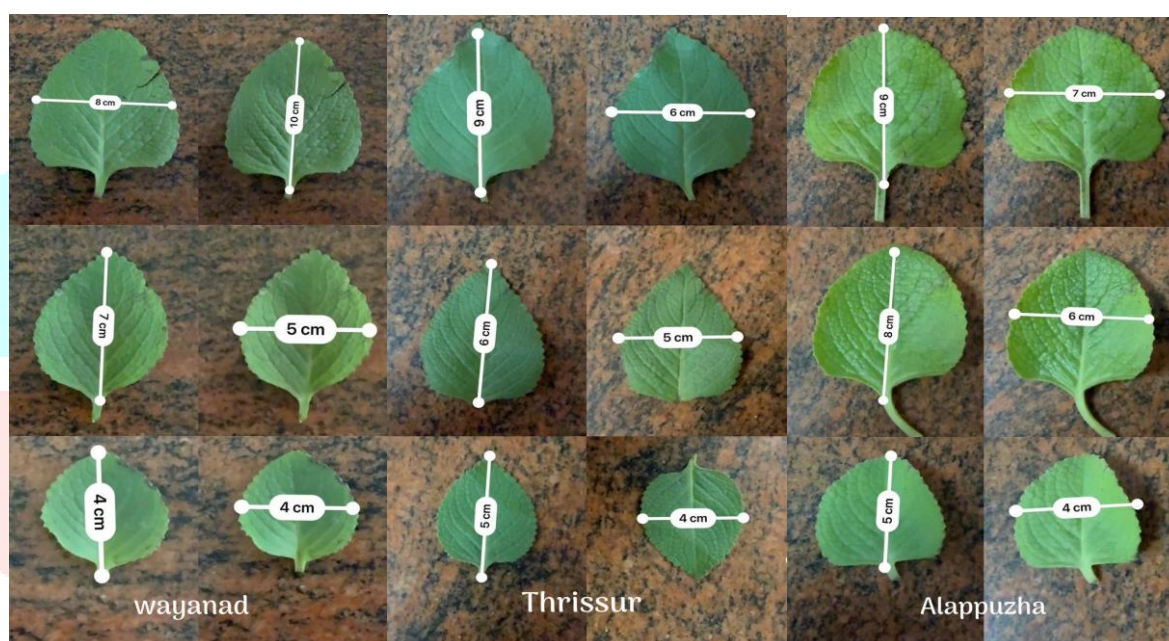


Figure 2: Macroscopical evaluation of *C. amboinicus* leaf

3.6 Microscopic evaluation

3.6.1 Qualitative microscopy

The transverse sections of *Coleus amboinicus* from Wayanad, Alappuzha and Thrissur showed similar basic anatomical features. Yet shows very slight variations. Upper epidermis is made up of single-layered rectangular cells covered with thin cuticle. Trichomes were seen occasionally. Under epidermis cortex consists of parenchyma cells. The central region consists of parenchymatous pith. Samples from Wayanad showed comparatively thicker cortex region and well developed vascular tissues, Alappuzha sample showed larger pith and loosely arranged cortical cells. and Thrissur showed intermediate anatomical features. The powder microscopy of Thrissur, Alappuzha and Wayanad showed similar structures in powder microscopy. The quantitative microscopy was also performed and it showed slight variations in the stomatal index, stomatal number, vein islet number and vein termination number. Microscopically, Wayanad samples showed stronger mechanical and conductive tissues; Alappuzha samples indicated adaptation to humid, low-stress conditions; and Thrissur samples exhibited intermediate features.

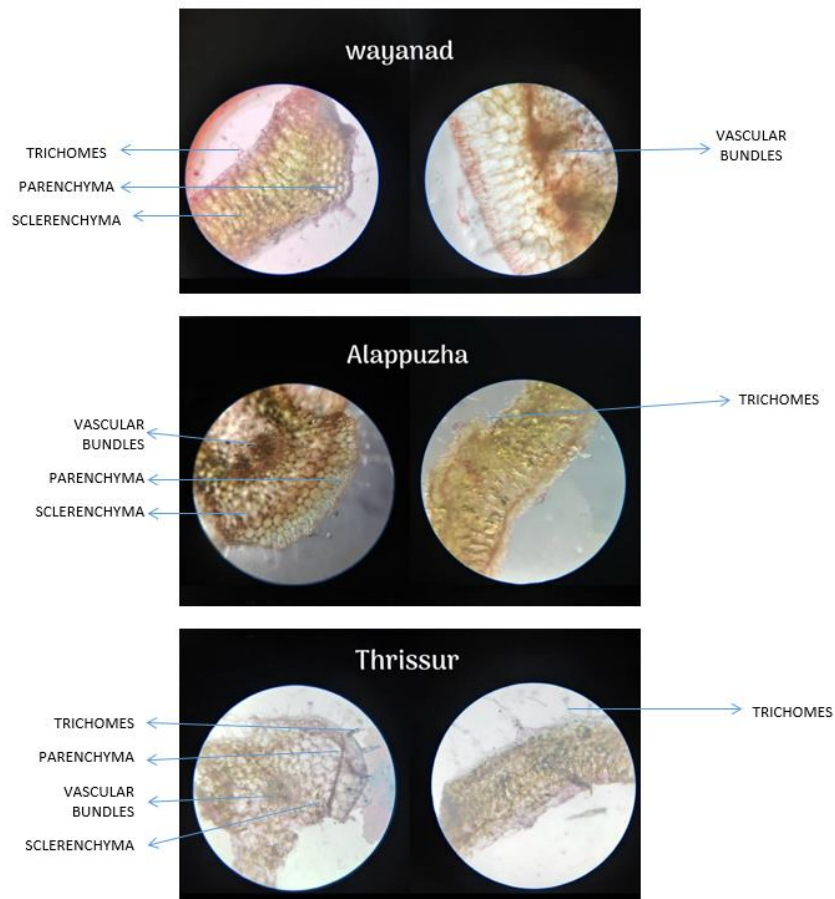


Figure 3: Transverse section showing lamina and midrib of three *Coleus amboinicus* samples

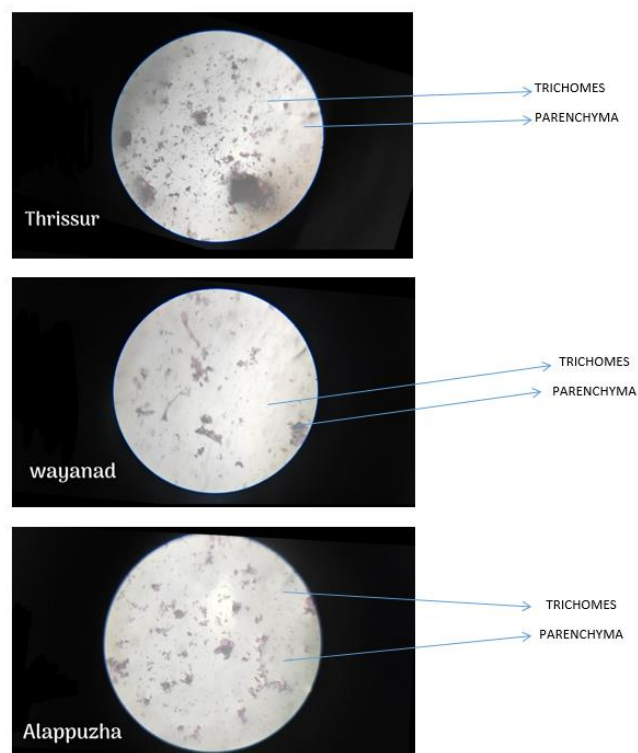


Figure 4: Powder microscopy of *Coleus amboinicus* leaves from Thrissur, Wayanad, and Alappuzha

3.6.2 Quantitative microscopy

Quantitative microscopical evaluation of the leaf samples from Wayanad, Alappuzha, and Thrissur reveals variations in quantitative anatomical parameters. The vein termination number is highest in Thrissur (10), followed by Wayanad and Alappuzha (8 each), while the vein islet number is highest in Alappuzha (11). Stomatal number is slightly higher in Wayanad (4) compared to Alappuzha and Thrissur (3 each). The stomatal index decreases from Wayanad (22) to Alappuzha (20) and Thrissur (18). These differences indicate that environmental factors influence the internal leaf structure and physiological characteristics.

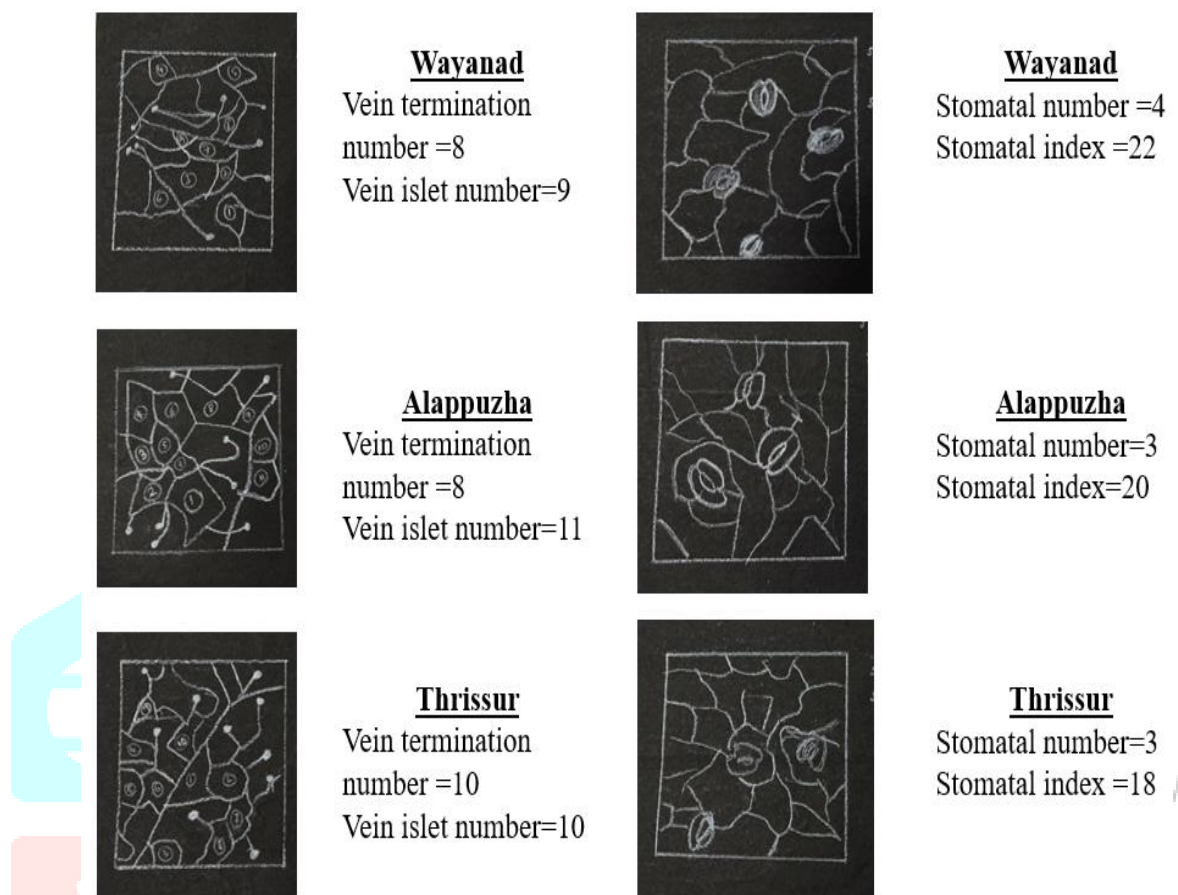


Figure 5: Vein islet number, vein termination number, Stomatal number and stomatal index of *C. amboinicus* leaf

3.7 Determination of physicochemical constants

The determination of physicochemical constants shows that the values among samples from Wayanad, Alappuzha, and Thrissur are almost similar, with only slight variations. Minor differences are observed in ash values, extractive values, and moisture content, but they do not indicate any major deviation. Swelling and foaming indices are also comparable across the regions.

Table 2: Physicochemical analysis of *Coleus amboinicus*

Parameter	Result Wayanad	Result Alappuzha	Result Thrissur
Total ash	11± 0.21 %w/w	13± 0.32%w/w	12± 0.22% w/w
Acid insoluble ash	3± 0.19 %w/w	3± 0.18%w/w	2± 0.17%w/w
Water soluble ash	3± 0.23 %w/w	3± 0.24%w/w	5±0.25 %w/w
Sulphated ash	12± 0.72%w/w	14± 0.76%w/w	11± 0.74%w/w
Ethanol soluble extractive value	11±0.34 %w/w	12±0.33 %w/w	13± 0.35%w/w
Water soluble extractive value	19±0.53 %w/w	21± 0.54%w/w	20±0.52 %w/w
Moisture content	0.40%	0.42%	0.38%
Swelling index	1	2	2
Foaming index	100	111.11	111.11

Values are expressed as mean ± sd of the triplicate experiment

3.8 Phytochemical studies

3.8.1 Preparation of extract

Table 3: nature, colour, yield of *Coleus amboinicus* extracts

Extract	Nature of extract	Color	Percentage yield
Wayanad	Semisolid and aromatic	Brownish green	73.33%
Alappuzha	Semisolid and aromatic	Brownish green	73.33%
Thrissur	Semisolid and aromatic	Brownish green	80%

The phytochemical extract preparation shows that samples from Wayanad, Alappuzha, and Thrissur have almost similar characteristics. All extracts are semisolid in nature, aromatic, and brownish green in colour. The percentage yield is also comparable, with Wayanad and Alappuzha showing the same yield (73.33%) and Thrissur showing a slightly higher yield (80%).

3.8.2 Phytochemical screening

Phytochemical screening of *Coleus amboinicus* extracts from Wayanad, Alappuzha, and Thrissur showed the presence of major bioactive constituents. Alkaloids, carbohydrates, phenolic compounds, tannins, saponins, phytosterols, triterpenoids, proteins, amino acids, and volatile oils were present in all extracts. Glycosides were largely absent, with detection observed only in the Thrissur extract. Flavonoids were present in the Wayanad and Thrissur extracts, while limited presence was observed in the Alappuzha extract. Resins were absent in all samples.

Table 4: Phytochemical screening of *Coleus amboinicus*

Phytoconstituents	Chemical test	<i>Coleus amboinicus</i> extract Wayanad	<i>Coleus amboinicus</i> extract Alappuzha	<i>Coleus amboinicus</i> extract Thrissur
Alkaloids	Dragendorff's test	+	+	+
	Mayer's test	+	+	+
	Hager's test	+	+	+
	Wagner's test	+	+	+
Carbohydrate	Molisch's test	+	+	+
	Iodine test	+	+	+
	Fehling's test	+	+	+
	Benedict's test	+	+	+
	Barfoed's test	+	+	+
Glycosides	Baljet's test	+	+	+
	Bontrager's test	+	+	+
	Modified bontrager's test	+	+	+
	Keller-killiani test	+	-	-
Phenolic compounds and tannins	Ferric chloride test	+	+	+
	Lead acetate test	+	+	+
	Decolourisation test	+	+	+
Flavanoids	Shinoda test	+	+	+
	Alkali reagent test	+	-	-
	Zinc hydrochloride solution test	-	-	-
Saponins	Foam test	+	+	+
Phytosterols and triterpenoids	Salkowski's test	+	+	+
	Liebermann-burchard's test	+	+	+
Proteins and amino acids	Biuret test	+	+	+
	Ninhydrin test	+	+	+
	Xanthoproteic test	+	+	+
	Millon's test	+	+	+
	Test for protein containing sugar	+	+	+
Volatile oil	Test for volatile oils	+	+	+
Resins	Test for resins	-	-	-

3.9 Isolation and extraction of volatile oil

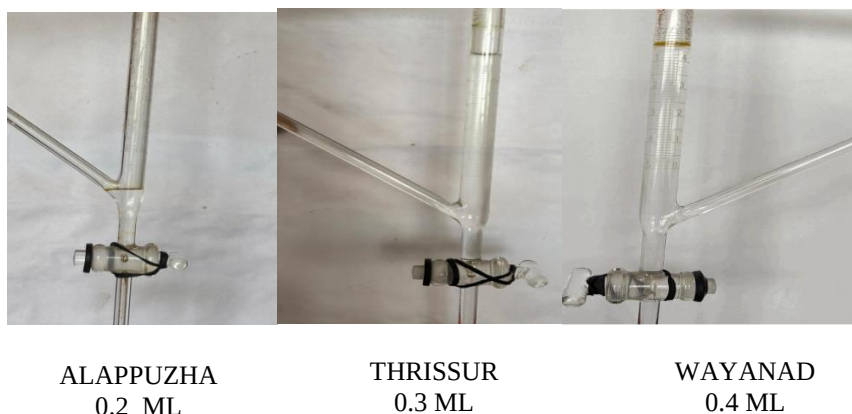


Figure 6: Isolation of volatile oil of *Coleus amboinicus* from Alappuzha, Thrissur and Wayanad

The volatile oil content of *Coleus amboinicus* showed variation. The Wayanad sample yielded the highest amount of volatile oil (0.4 ml), followed by Alappuzha (0.3 ml) and Thrissur (0.2 ml)

3.10 Quantitative estimation of phytoconstituents

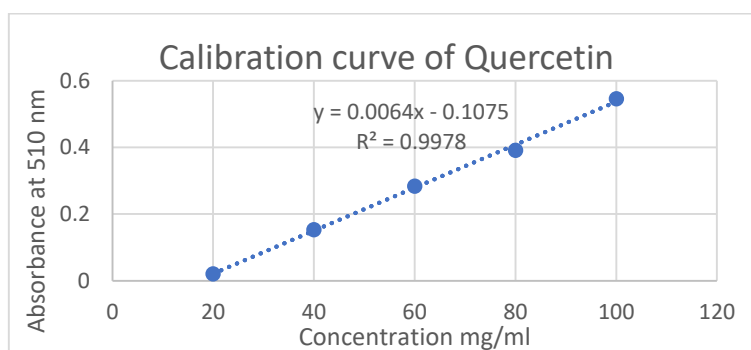
3.10.1 Estimation of total flavanoid content

The total flavonoid content of the aqueous extract of *Coleus amboinicus* was estimated using quercetin as the standard. A calibration curve was prepared using different concentrations of quercetin (20–100 mg/ml), and absorbance was measured at 510 nm. A gradual increase in absorbance was observed with increasing quercetin concentration, indicating good linearity.

The aqueous extract of *Coleus amboinicus* sample from Wayanad showed an absorbance value of 0.539 at 510 nm, which was comparable to the higher concentrations of the quercetin standard. This indicates the presence of a significant amount of flavonoids in the plant extract. Among the samples collected from different regions, the Wayanad extract showed comparatively higher flavonoid activity. This suggests that leaves from the Wayanad region contain a higher concentration of flavonoids, possibly due to favorable climatic and geographical conditions.

Table 5 : Absorbance of standard quercetin and extract at 510nm

Sl.no	Concentration of quercetin(mg/ml)	Absorbance at 510nm
1.	20	0.021
2.	40	0.153
3.	60	0.284
4.	80	0.392
5.	100	0.546
6.	Aqueous extract of <i>Coleus amboinicus</i> - Wayanad	0.539
7.	Aqueous extract of <i>Coleus amboinicus</i> - Alappuzha	0.507
8.	Aqueous extract of <i>Coleus amboinicus</i> - Thrissur	0.527



Graph 01. Calibration curve of standard quercetin

Table 6 : Total flavonoid content aqueous extract of *Coleus amboinicus*

Sl no.	Extract(100µg/ml)	Concentration of flavanoid content in µg/ml of sample [mg qe/g]
1.	Aqueous extract- Wayanad	99.09
2.	Aqueous extract- Alappuzha	94.94
3.	Aqueous extract- Thrissur	97.53

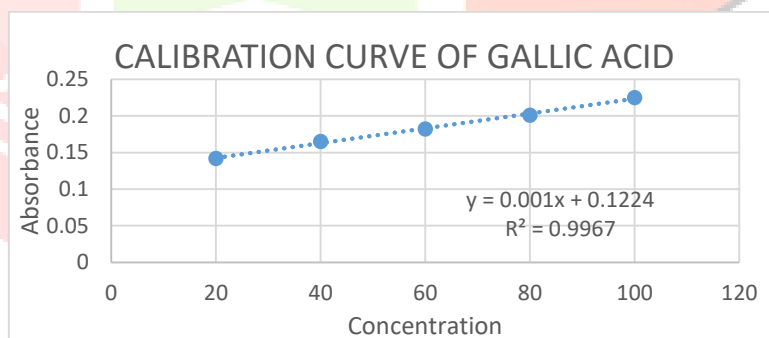
3.10.2 Estimation of total phenolic content

Total phenolic content was determined using the folin–ciocalteu method, with gallic acid as the standard. Standard gallic acid solutions (20–100 mg/ml) showed a steady increase in absorbance at 510 nm, confirming the reliability of the calibration curve. The aqueous extracts of *Coleus amboinicus* from different regions showed variation in absorbance values.

The Thrissur extract showed higher absorbance (0.24) compared to Wayanad (0.17) and Alappuzha (0.19), indicating a higher phenolic content in the Thrissur sample. Similarly, the volatile oil samples also showed regional variation, with the Thrissur volatile oil exhibiting the highest absorbance (0.23), suggesting a higher concentration of phenolic compounds in the volatile fraction. These results clearly demonstrate that geographical location influences the phenolic composition of *Coleus amboinicus*

Table 7: Absorbance of standard gallic acid and extract at 510nm

Sl.no	Concentration of gallic acid (mg/ml)	Absorbance at 760nm
1.	20	0.142
2.	40	0.165
3.	60	0.182
4.	80	0.201
5.	100	0.225
6.	Aqueous extract of <i>Coleus amboinicus</i> – Wayanad	0.17
7.	Aqueous extract of <i>Coleus amboinicus</i> - Alappuzha	0.19
8.	Aqueous extract of <i>Coleus amboinicus</i> - Thrissur	0.24
9.	Volatile oil of <i>Coleus amboinicus</i> - Wayanad	0.15
10.	Volatile oil of <i>Coleus amboinicus</i> - Alappuzha	0.21
11.	Volatile oil of <i>Coleus amboinicus</i> - Thrissur	0.23



Graph 02. Calibration curve of standard gallic acid

Table 8. Total phenolic content aqueous extract of *Coleus amboinicus*

Sl no.	Extract(100µg/ml)	Concentration of total phenolic content in µg/ml of sample (gae/g)
1.	Aqueous extract- Wayanad	46.06
2.	Aqueous extract- Alappuzha	67.6
3.	Aqueous extract- Thrissur	117.6
4.	Volatile oil - Wayanad	27.6
5.	Volatile oil-Alappuzha	87.6
6.	Volatile oil- Thrissur	107.6

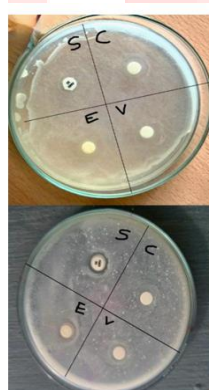
3.11 Bioactivity evaluation

3.11.1 Antimicrobial activity

The aqueous extract and volatile oil of *Coleus amboinicus* were evaluated for antimicrobial and antioxidant activities. Antibacterial activity was tested against gram-positive *staphylococcus aureus* and gram-negative *escherichia coli* using the disc diffusion method. Extracts from Thrissur, Wayanad, and Alappuzha showed clear zones of inhibition compared to the control (distilled water), with amoxicillin used as the standard drug. Gram-positive bacteria were found to be more sensitive than gram-negative bacteria.

Table 9: Antibacterial activity of *Coleus amboinicus*

Organism	Sample	Zone of inhibition[mm]
<i>Staphylococcus aureus</i> Gram +ve	Aqueous extract of <i>Coleus amboinicus</i> - Wayanad	15 mm
	Volatile oil of <i>Coleus amboinicus</i> - Wayanad	12 mm
	Standard amoxicillin 30 mg	10 mm
	Control	2 mm
	Aqueous extract of <i>Coleus amboinicus</i> Alappuzha	10 mm
	Volatile oil of <i>Coleus amboinicus</i> - Alappuzha	12 mm
	Standard amoxicillin 30 mg	11 mm
	Control	1 mm
	Aqueous extract of <i>Coleus amboinicus</i> Thrissur	10 mm
	Volatile oil of <i>Coleus amboinicus</i> -Thrissur	12 mm
	Standard amoxicillin 30 mg	14 mm
	Control	0 mm
<i>Escherichia coli</i> Gram -ve	Aqueous extract of <i>Coleus amboinicus</i> - Wayanad	9 mm
	Volatile oil of <i>Coleus amboinicus</i> - Wayanad	11 mm
	Standard amoxicillin 30 mg	11 mm
	Control	1 mm
	Aqueous extract of <i>Coleus amboinicus</i> Alappuzha	13 mm
	Volatile oil of <i>Coleus amboinicus</i> -Alappuzha	13 mm
	Standard amoxicillin 30 mg	14 mm
	Control	0 mm
	Aqueous extract of <i>Coleus amboinicus</i> Thrissur	8 mm
	Volatile oil of <i>Coleus amboinicus</i> - Thrissur	9 mm
	Standard amoxicillin 30 mg	13 mm
	Control	1 mm



WAYANAD GRAM +VE

Standard: 10 mm
Extract: 15 mm
Volatile oil: 12 mm
Control: 2 mm



ALAPPUZHA GRAM +VE

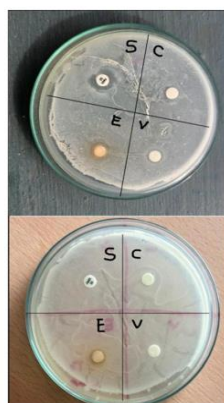
Standard: 11 mm
Extract: 10 mm
Volatile oil: 12 mm
Control: 1 mm

WAYANAD GRAM -VE

Standard: 11 mm
Extract: 9 mm
Volatile oil: 11 mm
Control: 1 mm

ALAPPUZHA GRAM -VE

Standard: 14 mm
Extract: 13 mm
Volatile oil: 13 mm
Control: 0 mm



THRISSUR: GRAM +VE

Standard: 14 mm
Extract: 10 mm
Volatile oil: 12 mm
Control: 0 mm

THRISSUR: GRAM -VE

Standard: 13 mm
Extract: 8 mm
Volatile oil: 9 mm
Control: 1 mm

Figure 7 : Antibacterial activity of *Coleus amboinicus* of Wayanad, Thrissur and Alappuzha

3.11.2 Antioxidant activity

Antioxidant activity was assessed using the DPPH radical scavenging assay. All regional samples demonstrated significant free radical scavenging activity, indicated by decolorization of DPPH solution. Variations in activity among regions may be due to differences in phytochemical and phenolic content. Overall, the results confirm the presence of bioactive compounds responsible for antibacterial and antioxidant properties.

3.11.2.1 DPPH assay: Anti-oxidant activity of aqueous extract of *Coleus amboinicus* leaves was determined by DPPH assay and percentage scavenging activity of test samples Thrissur, Alappuzha and Wayanad was compared with standard ascorbic acid.

Standard	Concentration	Absorbance	% inhibition
	100	0.024	96.63
	200	0.046	93.55
	300	0.067	87.54
	400	0.125	82.49
	500	0.195	72.68
Alappuzha	Concentration	Absorbance	% inhibition
	100	0.041	92.37
	200	0.100	81.41
	300	0.110	79.5
	400	0.132	71.0
	500	0.205	59.8
Thrissur	Concentration	Absorbance	% inhibition
	100	0.044	91.82
	200	0.103	80
	300	0.168	68.7
	400	0.206	61.7
	500	0.431	19.8
Wayanad	Concentration	Absorbance	% inhibition
	100	0.039	92.7
	200	0.061	88.66
	300	0.110	84.59
	400	0.156	75.4
	500	0.216	61.8

Table 9: Comparison of percentage inhibition of ascorbic acid and extracts of *Coleus amboinicus*

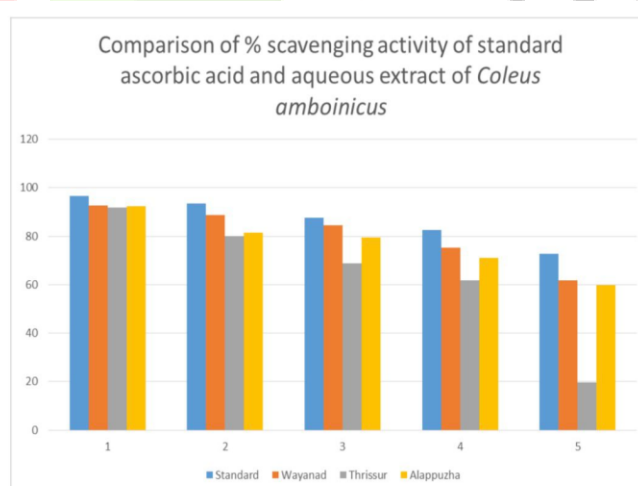


Figure 8: comparison of scavenging activity of standard ascorbic acid and *Coleus amboinicus* extract of Alappuzha, Wayanad and Thrissur

The samples show clear variation in antioxidant activity. The Wayanad sample exhibits the highest scavenging activity among the extracts. The Alappuzha sample shows moderate antioxidant potential. The Thrissur sample demonstrates the lowest activity, especially at higher concentrations. All samples show a decrease in % inhibition with increasing concentration. These differences indicate the influence of geographical origin on antioxidant activity.

IV. DISCUSSION

The leaves were collected from Wayanad, Thrissur, and Alappuzha districts, Kerala and authenticated by DR. Ranjusha A P, a botanist from the Department of Botany at NSS College. The leaves were subjected for pharmacognostical investigation which includes determination of physico chemical constants such as ash values and extractive values. Macroscopic and microscopic characteristics of the leaves were studied.

In microscopical studies, Wayanad sample indicates stronger mechanical and conductive tissues, Alappuzha sample indicates adaptation to humid, low stress environment, Thrissur samples shows intermediate anatomical adaptations. The macroscopical evaluation showed noticeable regional variations in *Coleus amboinicus*. Leaves from Wayanad were green with moderate size, weak aroma, and mildly pungent taste, whereas Alappuzha samples were pale green with larger leaves and moderate pungency. Thrissur samples exhibited dark green, broadly ovate leaves with strong aroma and higher pungency. Leaves of *Coleus amboinicus* collected from three different regions Wayanad, Thrissur and Alappuzha showed noticeable variation in drying behaviour.

Leaves collected from Wayanad dried rapidly, while those from Thrissur exhibited moderate drying. In contrast, leaves from Alappuzha required a longer time to dry. These differences may be attributed to variations in climatic conditions, altitude and environmental factors influencing plant growth and secondary metabolite production. Wayanad, being a high-altitude region with relatively lower humidity, facilitates faster moisture loss from plant material. Thrissur, with moderate climatic conditions showed intermediate drying behaviour. Alappuzha, a low-lying coastal region with high humidity and moisture content, resulted in slower drying of leaves. These differences in drying characteristics can influence the preservation, phytochemical composition, and bioactivity of the plant material, highlighting the importance of geographical location in pharmacognostic studies.

The sample collected from Wayanad exhibited a higher rate of wilting under identical conditions, whereas the sample from Alappuzha demonstrated greater stability and resistance to wilting. Whereas Thrissur shows intermediate behaviour.

The extraction of *Coleus amboinicus* plant using distilled water was carried out and the percentage yield was found to be: Wayanad-73.33%, Alappuzha -73.33%, Thrissur – 80%. Phytochemical screening of *Coleus amboinicus* extract was done and it contains phytoconstituents such as flavonoids, alkaloids, glycosides, carbohydrates, volatile oils, saponins, protein and aminoacids, phenolic compounds and tannins.

Quantitative estimations of phytoconstituents was done by using total flavonoid content and total phenolic content method. Quantitative analysis of extracts has Wayanad -99.09 mcg/ml, Alappuzha -94.94 mcg/ml, Thrissur -97.53 mcg/ml of flavonoid and Wayanad -46.06 mcg/ml, Alappuzha -67.6 mcg/ml, Thrissur -117.6 mcg/ml (volatile oil) Wayanad -27.6 mcg/ml, Alappuzha -87.6 mcg/ml, Thrissur -107.6 mcg/ml (Aqueous extract) of phenol.

The antimicrobial activity of *Coleus amboinicus* was evaluated against Gram – positive *Staphylococcus aureus* and Gram – negative *Escherichia coli* using disc plate method. Plant extracts and volatile oils from Thrissur, Wayanad and Alappuzha showed noticeable zones of inhibition when compared with the control (distilled water), while amoxicillin served as the standard drug. The results indicated that both extracts and volatile oils possessed antibacterial activity with variations observed based on the geographical region. Overall Gram-positive bacteria were more sensitive than Gram – negative bacteria.

Antioxidant activity was assessed using the DPPH radical scavenging assay. Extracts from Wayanad, Alappuzha and Thrissur demonstrated significant free radical scavenging activity, evidenced by a reduction in the purple colour of DPPH. All samples exhibited antioxidant potential, though the extent varied among regions, which may be attributed to differences in phytochemical and phenolic content. The results confirm the presence of bioactive compounds contributing to antioxidant activity.

V. CONCLUSION

The pharmacognostical and physico-chemical analysis of the plant was performed. The plant was extracted using distilled water by hydro distillation method and phytochemical analysis was performed in which it was found that plant extract contains flavonoids, alkaloids, glycosides, carbohydrates, volatile oils, saponins, protein and aminoacids, phenolic compounds and tannins. Quantitative estimation of phytoconstituents was done by using total flavonoid content and total phenolic content. It shows good quantity of flavonoid and phenol. It may be responsible for antioxidant property. All the samples were found to have antioxidant activity, even though it varied from region to region. This difference may be due to variations in phytochemical and phenolic content.

Phytochemical screening confirmed the presence of flavonoids in extracts of leaves of *Coleus amboinicus* collected from all three regions namely, Wayanad, Thrissur, and Alappuzha. The extract of *Coleus amboinicus* collected from Wayanad showed positive results for three flavonoid tests, while the samples from Alappuzha and Thrissur showed only two. This indicates that the Wayanad sample has a comparatively higher flavonoid content than the other two regions.

Flavonoids are well known for their antimicrobial properties, as they can inhibit bacterial growth by disrupting cell walls and interfering with microbial enzymes. Therefore, the higher flavonoid content in the Wayanad extract may be the reason for its stronger antimicrobial activity observed during the assay. This relationship suggests that geographical factors affecting phytochemical composition can directly influence the biological activity of the plant. During macroscopic evaluation, the sample from Thrissur showed a stronger aroma and more distinct morphological features compared to the samples collected from Wayanad and Alappuzha. However, despite these noticeable sensory characteristics, the sample collected from Thrissur exhibited relatively lower antioxidant and antibacterial activity.

In contrast, the Wayanad sample demonstrated higher flavonoid content and showed better antimicrobial activity, along with notable antioxidant potential. This indicates that sensory characteristics such as aroma and appearance do not necessarily correlate with bioactive potential. The biological activity of the plant is mainly influenced by its phytochemical composition, particularly flavonoids and phenolic compounds, rather than macroscopic features alone.

Thus, even though the Thrissur sample showed superior external characteristics, the Wayanad sample proved to be more pharmacologically active.

The results show that geographical and environmental conditions play an important role in determining the phytochemical composition and medicinal effectiveness of the plant. Samples obtained from Thrissur may be more appropriate for flavouring purposes because of their stronger aroma, whereas samples from Wayanad are more suitable for medicinal preparations due to their higher flavonoid levels and better pharmacological activity.

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