

PRELIMINARY PHYTOCHEMICAL ANALYSIS OF FRESH JUICE AND AQUEOUS
EXTRACT OF *COLEUS AMBOINICUS* LINN LEAVES.

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575018, Karnataka, India.**ABSTRACT**

Background: Plants are used as medicine universally and recently their use has increased because of their easy availability. The compounds present in the plants responsible for its therapeutic effects are called phytochemicals. *Coleus amboinicus* (*Plectranthus amboinicus*) is used for its different medicinal properties worldwide. In view of this, we performed a preliminary phytochemical analysis of fresh juice and aqueous extract of *Coleus amboinicus* leaves.


Materials and Methods: The extract from fresh leaves was prepared by crushing, squeezing the leaves. The test solution A was the filtrate obtained of the above leaf juice. The test solution B was the extract obtained from the Soxhlet apparatus of the shade dried leaves of *Coleus amboinicus*. The test samples were subjected to phytochemical analysis in order to find out the presence of phytochemical constituents.

Result: The results showed the presence of identical chemical constituents in both the test solution, except for the absence of alkaloid and fixed oil and fatty acids in fresh leaf extract. The fresh leaf extract also showed a weak response to Benedict's test.

Conclusion: The variation in the chemical composition may be due to the difference in the process of collection and extraction.

Key words: *Coleus amboinicus*, *Plectranthus amboinicus*, Phytochemical tests, fresh leaves, aqueous extract.

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INTRODUCTION

Plants are the rich source of medicine. The use of herbs for the medicinal properties is almost universal because of their easy availability and affordability compared to the expensive pharmaceuticals (Sharma R K, 2006).

The World Health Organization (WHO) traditional medicine strategy 2002-2005 estimates that 80% of the population of some Asian and African countries use herbal medicine for some aspect of primary health care. Studies in the United States and Europe have shown that their use is less common in clinical settings, but has become increasingly more common in recent years as scientific evidence about the effectiveness of herbal medicine has become more widely available. The annual global export value of pharmaceutical plants accounted for over US\$2.2 billion (Sharrock S et al, 2014).

Phytochemicals are chemical compounds that occur naturally in plants (phyto means "plant" in Greek). These chemicals are responsible for color and other organoleptic properties. They are bioactive non-nutrient plant compounds (Khare C P, 2007).

Coleus amboinicus (Synonyms: *Plectranthus amboinicus*, *Plectranthus aromaticus*, *Coleus aromaticus*) also known as Indian-borage (English), Indian-mint, French-thyme (Lukhoba C W et al, 2006) is used for its different medicinal properties worldwide. The coleus group of plants grows in tropical to subtropical situations and in warm temperature climate zone. It comes up well on sun-exposed areas too (Warrier P K et al 2007).

Coleus amboinicus is a large succulent aromatic perennial herb with hispidly villous or tomentose fleshy stem. The leaves are simple, opposite, broadly ovate, crenate and fleshy. Flowers are pale purplish in dense whorls at distant intervals in a long slender raceme and fruits are orbicular nutlets (V V M Gogte, 2009).

It is used in folk medicine for conditions like abdominal colic, diarrhoea due to cholera (Nadkarni A K, 1996), malarial fever, epilepsy and renal calculi (Rashmi S K et al, 2011). The juice prepared from its leaves is mixed with sugar and given to children for treating asthma, chronic cough, gonorrhoea and piles (Nadkarni A K, 1996). Previous studies have reported antioxidant (Kumaran A et al, 2006), anticlastogenic and radioprotective (Satish B S R et al, 2006) effect, Mast cell stabilization property (Kumar A et al, 2007), antimicrobial and ant-helminthic (Prameela T S et al, 2011), analgesic activity (Megha Rani et al, 2013) of leaves of this plant. Arunkumaran and Karthikeyan (2011) reported that methanolic extract of *Coleus amboinicus* leaves contains alkaloids, flavanoids, terpenoids, cardiac glycosides, saponin, tannin.

In view of the above uses, properties and studies done on this plant, we performed a preliminary phytochemical analysis of fresh juice and aqueous extract of *Coleus amboinicus* leaves.

MATERIALS AND METHODS

The study was conducted in the Department of Pharmacology, Yenepoya Medical College, Mangaluru. The plant was authenticated by Plant Taxonomist, Mangalore University and was used for the study.

Preparation of fresh juice (Test solution A):

The fresh leaves were collected, washed and cleaned. These leaves were crushed using mortar and pestle and the juice was squeezed from the leaves using a muslin cloth. The obtained greenish brown extract was then filtered using Whatmann filter paper and the light brown juice obtained from the above procedure was used for chemical analysis. The test was performed on the same day of collection of leaves.

Preparation of aqueous extract (Test solution B):

The leaves were collected, cleaned, shade dried, powdered and stored at 4°C until extraction. The powdered leaf material was wrapped in a muslin cloth and extracted using distilled water (1000ml) as solvent in Soxhlet apparatus (Agrawal S S et al, 2007) maintained at 90°C for 23 hours (till transparent liquid was observed in siphon tube of Soxhlet apparatus). The aqueous extract of *Coleus amboinicus* was kept in water bath for evaporation of solvent and solid extract was obtained. The yield (Agrawal S S et al, 2007) was calculated by using the formula:

$$\frac{\text{Weight of the final dry extract}}{\text{Total weight of the fresh leaves}} \times 100$$

The yield obtained was 23.7% w/w.

The extract was dissolved in distilled water and used for chemical analysis.

Chemicals for Phytochemical analysis: The following analytical grade chemicals obtained from Rajesh chemicals, Mumbai were used for the study- Mayers reagent, Dragendroffs reagent, Ferric chloride, Glacial acetic acid, Dil. ammonia solution, Conc. Sulphuric acid, Chloroform, Fehling's A and B solution, Sodium picrate solution, Biuret reagent.

Phytochemical analysis (Harbone J B 1998, Sofowara A E, 1993, Rajesh H et al, 2013)

The test sample was subjected to phytochemical analysis in order to find out the presence of phytochemical constituents.

Test for Alkaloids

- a) **Mayer's test:** To 2ml of test solutions, few drops of Mayers reagent (potassium mercuric iodide solution) is added and observed for turbidity or precipitation. The appearance of cream colored precipitate signifies the presence of alkaloid.
- b) **Dragendroff's test:** To 1ml of test solutions, few drops of chloroform is added mixed and kept over water bath to facilitate evaporation of chloroform. The solution is then acidified with Dragendroff's reagent (potassium bismuth iodide solution). The appearance of orange red precipitate signifies the presence of alkaloids.
1. **Test for Cardiac Glycosides (Keller-Killiani test):** To 2ml of test solutions, 1ml of glacial acetic acid and 1-2 drops of ferric chloride solution is added. Then 0.5ml of concentrated sulphuric acid is slowly added along the sides of the test tube avoiding shaking of test tube. A reddish brown ring at the interface of two liquids indicates the presence of deoxysugar characteristic of cardenolides.
2. **Test for Tannins and Phenolic compounds (Lead test):** To 2 ml of test solutions, 1-3 drops of Ferric chloride is added and the mixture is observed for blue or green color.
3. **Test for Saponins (Foam test):** The test solutions are taken in a test tube and shaken vigorously till a stable persistent froth is obtained. The persistence of froth for 10mins signifies the presence of saponins.
4. **Test for Terpenoids and Phytosterols (Salkowski test):** To 0.5ml of test solution, add equal quantity of chloroform and then add sulphuric acid along the sides of the test tube avoiding shaking. The appearance of reddish brown color in the chloroform layer signifies presence of phytosterols.
5. **Test for Flavonoids:**
 - a) **Lead acetate test:** To 1 ml of test solutions, add 1 ml of 10% lead acetate solution. The appearance of yellow colored precipitate signifies the presence of flavanoids.
 - b) **FeCl₃ test:** To 2 ml of test solutions, add ferric chloride solutions drop by drop. The formation of greenish black color indicates presence of flavonoids.
6. **Test for Carbohydrates:**
 - a) **Fehling's test:** Few drops of extract heated with Fehling's A and B solution. Appearance of orange red precipitate indicates presence of carbohydrates.
 - b) **Benedict's test:** To 5 ml of Benedicts reagent, 8 drops of test solution is added, mixed well and boiled for 2-3mins. The test tube is cooled and color change is observed.
7. **Test for Lactones (Baljet's test):** Treat the test solutions with sodium picrate solution. Appearance of yellow to orange color indicates presence of lactone ring.
8. **Test for Proteins (Biuret test):** Add 2ml of Biuret reagent to 2ml of test solutions. Shake well and warm it on water bath. Appearance of red or violet color indicates presence of proteins.
9. **Fixed oils and fatty acid (Spot test):** A drop of test solutions are added to the filter paper and observed. Oil staining on the filter paper indicates the presence of fixed oil and fats.

RESULTS AND DISCUSSION

The use of plants as medicine dates back from many centuries or can be said from the time humans existed on earth. Then, Man did not require the modern methods of investigation to authenticate the use of plants for therapeutic purpose, which he often used in conjunction with the magical and other ritual practices. These folk medicines differed with the plant available to the particular area, the practices and beliefs followed. The use of such medicine must have started with trial and error method over centuries and their continuing use now is not without any valid results. With increase interest in herbal medicine worldwide, a study authenticating the use of these for medicinal purpose is very helpful mainly because of their easy availability and affordability by many (Ewans W C 2009).

Traditional medicines invariably involve crude plant extracts containing multiple chemical constituents. Identification of these chemical constituents is necessary for further analysis of the plant material. Hence a preliminary phytochemical analysis of fresh leaves and aqueous extract of *Coleus amboinicus* was performed and compared.

The results showed the presence of identical chemical constituents ie glycosides, tannins and phenolic compounds, saponins, terpenoids and phytosterols, flavonoids, lactones and proteins in both the test solution, except for absence of alkaloids and fixed oil and fatty acids in fresh leaf extract. The fresh leaf extract also showed a weak response to Benedict's test. This may be due to the inability of the chemical test performed to identify the chemical because of their scanty presence. The difference in the climatic conditions have different chemical constituents and therapeutic effect, similarly the process of collection like fresh, shade or sun dried, extraction process etc effect the chemical constituent of the plant extracts (Agrawal S S et al, 2007).

Table 1: Phytochemical analysis of leaves of *Coleus amboinicus*.

Phytochemicals	Test	Test solution A		Test solution B	
		Observation	Inference	Observation	Inference
Alkaloids	Mayer's test	NC	-	Red ppt	+
	Dragendroff's test	NC	-	NC	-
Glycosides	Keller Killiani test	Brown ring	+	Brown ring	+
Tannins and Phenolic compounds	Lead test	Green color	+	Green color	+
Saponins	Foam test	Foam persists for 10 mins	+	Foam persists for 10 mins	+
Terpenoids and Phytosterols	Salkowaski's test	Reddish -brown ring	+	Reddish-brown ring	+
Flavonoids	Lead acetate test	Yellow ppt	+	Yellow ppt	+
	FeCl ₃	Greenish -black color	+	Greenish - black color	+
Carbohydrates	Fehling's test	Green ppt	Weak	Orange ppt	+
	Benedict's test	Green ppt	Weak	Orange ppt	
Lactones	Baljet's test	NC	-	NC	-
Proteins	Biuret's test	Violet color	+	Violet color	+
Fixed oil and fatty acids	Spot test	No staining	-	No staining	-

(+) : indicates presence, (-) : indicates absence, NC: no appreciable change, ppt: precipitate.

CONCLUSION

It can be concluded from the above study that for preparation of herbal medicine it is important to follow the traditional method of collection, extraction process strictly in order to avoid any phytochemical alteration of the particular medicinal preparation.

Conflict of interest: Nil

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