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QUALITY AND QUANTITATIVE ANALYSIS OF COLEUS FORSKOHLII BY TLC AND HPLC:

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Abstract:

Coleus forskohlii Briq. is an important plant in Indian system of medicine. The dried and powdered plant parts (*in vitro* root and *in vivo* root) material was extracted with mixture of petroleum ether, chloroform, ethanol and aqueous extracts. The obtained extract was assayed for phytochemical screening. Maximum phytoconstitutents were present in ethanol *in vitro* root extracts revealed the presence of flavonoids, glycosides, tannins, saponins, alkaloids, carbohydrate, steroids, terpenoids and oils. This study undergoes the importance of application of *Coleus forskohlii* in ethno medicine, antioxidant and antimicrobial agents. The present study was aimed to determine the qualitative and quantitative analysis of forskolin by TLC and HPLC methods.

Keywords: Coleus forskohlii, forskolin, TLC and HPLC.

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Introduction:

Coleus forskohlii belongs to Labiatae or Lamiaceae family. *Coleus* is found in all the habitats and altitudes, particularly in the Himalaya, the Southern Ghats, and the Nilgiri region [9]. *Coleus amboinicus*, *Coleus barbatus*, *Coleus caninus*, *Coleus mollis*, *Coleus vetteriveriodes* etc. are the most common species found in India [6]. In Ayurvedic medicine system *Coleus* species have been used to treat heart disease, convulsions, spasmodic pain and painful urination. *Coleus forskohlii* is considered to be an antispasmodic, stimulant and stomachic and is used for the treatment of headache, fever, epilepsy and dyspepsia. It is used to treat conditions such as indigestion, diarrhea, nervous tension, insect bites, toothache, earache, rheumatism, whooping cough and bronchitis [3]. *Coleus barbatus* also known as *Coleus forskohlii* is interesting from a scientific and medicinal standpoint because it produces forskolin, a diterpene used as a vasodilator. The use of indigenous knowledge of traditional medicinal practitioners as leads provides a useful route employed in the search for novel drugs. Numerous investigations have proved that medicinal plants such as tannins, alkaloids and flavonoids, which exhibit various pharmacological, antioxidant, antimicrobial and phytochemical properties. Hence the present study was aimed to investigate the phytochemical studies and qualitative and quantitative study by TLC and HPLC methods.

Materials and method

Plant material:

In vivo and *in vitro* roots of *Coleus forskohlii* plants which are used in this study collected from Sarojini Naidu Girls Govt. P.G. (Autonomous) College, Bhopal (Madhya Pradesh) in the month of July. The plant was authenticated from Laghu Vanupaj Prasannskarn and Anusandhan Kendra Barkheda Pathani, Bhopal (MP). Roots were washed under running tap water twice with distilled water to remove the adhesive contaminants or dust particles and dried under shade. Finally the samples were crushed and converted into powdered form using a mixer grinder and stored in airtight bottles for further analysis.

Preparation of plant extracts: The shade dried and powdered roots were subjected to successive extraction in a soxhlet extractor using petroleum ether, ethanol, chloroform and

distilled water. The extracts were filtered and the filtrates were concentrated under reduced pressure to obtain the extracts as solid residues.

Phytochemical analysis : Preliminary phytochemical tests of various extract of *in vivo* and *in vitro* root powder of *Coleus forskohlii* were performed for phytochemical analysis of alkaloids, glycosides, carbohydrates, tannins, saponins, steroids, and terpenoids according to standard methods.

Test for Alkaloids: Mayer's test: Take 1 ml of the extract, add 1 ml of Mayer's reagent (Potassium mercuric iodide solution). Whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

Test for Glycosides: Baljet test: Take 1 ml of the test extract, add 1ml of sodium picrate solution and the yellow to orange colour reveals the presence of glycosides.

Test for Carbohydrates: Molisch's test: Take 2 ml of the extract, add 1 ml of α- napthol solution and add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet colour at the junction of the two liquids reveals the presence of carbohydrates.

Test for Tannins: Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins**.**

Test for Saponins: Take small quantity of alcoholic and aqueous extract separately and add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. A 1cm layer of foam indicate the presence of saponin.

Test for Steroids: Libermann-Burchard test: 1 gm of the test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride, 3 ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish-green colour show the presence of sterols.

Test for Terpenoids: Noller's test: Dissolve two or three granules or tin metal in 2 ml thionyl chloride solution. Then add 1ml of the extract into test tube and warm, the formation of pink colour indicate the presence of terpenoids.

Test for oil: **Stain test**: Small quantities of extracts were pressed between two filter papers. An oily stain on filter paper indicates the presence of fixed oil.

Qualitative analysis by Thin Layer Chromatography:

The qualitative analysis of alkaloid was done by Thin Layer Chromatography (TLC). In TLC, the qualitative analysis of alkaloids was done on preparative silica gel plates using specific solvent systems for secondary metabolite's group. When the alcoholic extracts of root of *Coleus forskohlii* were subjected to the solvent system chloroform: ethanol (9:1) both the samples showed fluorescent green and blue bands on preparative silica gel plates under ultraviolet light indicating the presence of various alkaloid derivatives.

Detection and Calculation of R_f Value

The R_f value of the spot was calculated using the formula –

$$Rf = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Quantitative characterization through High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography is a separation and isolation technique based on a solid stationary phase and a liquid mobile phase. Separations were achieved by partition, adsorption or ion exchange process depending upon the type of stationary phase used.

4.3 Result and Discussion

4. 3.1 Preliminary Phytochemical Studies of Plant Material

Preliminary phytochemical screening for *in vitro* and *in vivo* root extracts was conducted. The test was carried out for determination of carbohydrate, saponins, alkaloids, tannins and glycosides. Presence or absence of phytochemical components in extract determined by colour change reaction.

For further investigation *in vivo* and *in vitro* root extracts of *Coleus forskohlii* were used in different solvent such as petroleum ether, chloroform, ethanol and aqueous. Particularly ethanol, petroleum ether, aqueous *in vitro* root extract of *Coleus forskohlii* specify good source of different classes of phytochemicals. The result showed that maximum phytoconstituents were present in *in vitro* ethanol root extract. The ethanol root extract was subjected to different qualitative phytochemical tests for detection of different biologically active chemical groups [11].

The presence of secondary metabolites in plant produces biological activity in man and animals and is responsible for their use as herbs in various ailments reported by [10]. Similarly [2] and [1] reported that the qualitative chemical test of *Coleus forskohlii* ethanol root powder. During present phytochemical screening it was observed that *in vitro* ethanol root extract showed positive test for carbohydrate, alkaloid, glycoside, saponin, steroid, terpenoid, tannin, fats and oils. In petroleum ether and in aqueous *in vitro* root extract and no positive test was reported for saponin and steroid. Among all the *in vitro* root extract with different solvent, chloroform extract possess lower number of phyochemicals. The *in vitro* ethanol root extract was found rich source of phytochemicals as compared *in vivo* root extract of different solvents. The findings of present work can be correlated with the study conducted in *Coleus canninus* by [4] and *Coleus forskohlii* by [5].

Qualitative Characterization through TLC

Thin Layer Chromatography (TLC) is based on the adsorption phenomenon. *In vitro* and *in vivo* ethanol root extracts was used for qualitative analysis, initially to estimate the forskolin a major biological compound, number of mobile phase in different ratio. The benzene : ethanol (9:1 v/v) mobile phase was found to be more suitable for extraction and analysis of compound. The R_f value of *in vitro* root and *in vivo* root extracts of *Coleus forskohlii* Briq. was depicted. It revealed

that Rf value of *in vitro* root extract is (0.25) higher as compare to *in vivo* root ethanol extract (0.18) (Plate No.1). [7] documented that TLC is useful for semi quantitative estimation. It is documented that forskolin was found in the cork cells, cortex, medullary rays and xylem in roots and tuber of *Coleus forskohlii*.

4.3.3 Quantitative characterization through HPLC

HPLC method found to be more rapid and less sensitive than GLC (Gas liquid chromatography). It is used to monitor variation in forskolin content. Reversed-phase liquid chromatography with a photo diode array detector at 210 nm was successful for the qualitative and quantitative evaluation of forskolin in plant material. HPLC-ELSD finger print method was used for quality control of *Coleus forskohlii* was suggested by [5] and [7].

For quantitative analysis, in HPLC, temperature was held constant at 30^0C or room temperature and flow rate employed for analysis was 1.0 ml/min. The analysis of sample completed within 30 min. The HPLC chromatogram of ethanol *in vitro* and *in vivo* root extract of *Coleus forskohlii* showed 12.498 ± 0.3 min.A stock solution of forskolin with a concentration of 1 mg/ml was prepared in benzene and further diluted with the solvent and final concentration was made up to 100 mg/ml. The calibration curve was linear over the concentration range between 5 - 25 µg/ml. The standard curve plotted between concentrations against area. In the ethanol *in vitro* root extract maximum forskolin a major chemical compound was identified with respect to *in vivo* ethanol root extract. Identification of chemical constituents was based on the peak areas which represent the percentage of forskolin compound. Graph 4.1 and 4.2.

The HPLC chromatogram exhibited 0.54% quantity of forskolin in *in vitro* ethanol root extract and 0.48% of forskolin in *in vivo* ethanol root extract. The present data showed that percentage of estimation of *in vitro* ethanol root extract is higher as compare *in vivo* ethanol root extract. Similar report of forskolin quantification by reverse phase liquid chromatography was showed by [8]. Differentiation capacity of *in vitro* ethanol root extract was found to be greater when compared to *in vivo* ethanol root extracts.

Conclusion

The present result reveal that the *in vitro* ethanol root extract of *Coleus forskohlii* have a maximum number of chemical constituent, which may be responsible for many therapeutically activities. Further work will emphasize the isolation and characterization of active principles which are responsible for bio efficacy and bioactivity

Table 4.1 Preliminary phytochemical screening for selection of *in vivo* root and *in vitro* root extract of *Coleus forskohlii* with different solvent

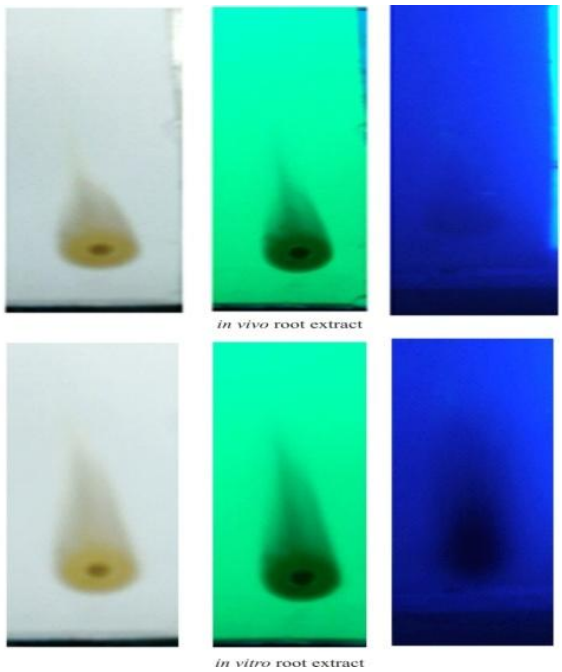
Chemical test	***In vitro* root explants**				***In vivo* root explants**			
	Pt	Et	Chl	Aq	Pt	Et	Chl	Aq
Alkaloids	+	+	-	+	+	+	-	+
Carbohydrate	+	+	-	+	+	+	-	+
Saponins	-	+	+	-	-	-	+	-
Steroids	-	+	-	-	-	+	-	-
Glycosides	+	+	-	+	+	-	-	+
Tannins	+	+	-	+	+	+	-	+
Terpenoids	+	+	-	+	+	+	-	+
oil	+	+	-	+	+	+	-	+

+=Present, - = Absent, Pt - petroleum ether, Chl- Chloroform, Et- ethanol, Aq- aqueous

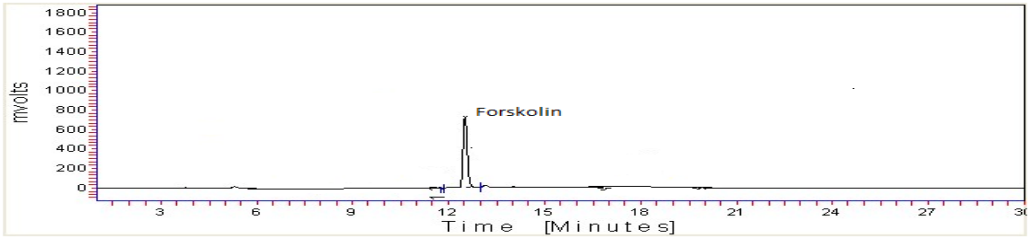
Table 4.3: Qualitative Characterization through TLC

S. No.	Explant	Extract (mobile phase)	Rf Value
1.	*In vivo* root	Benzene : Methanol (9:1 v/v)	0.18
2.	*In vitro* root		0.25

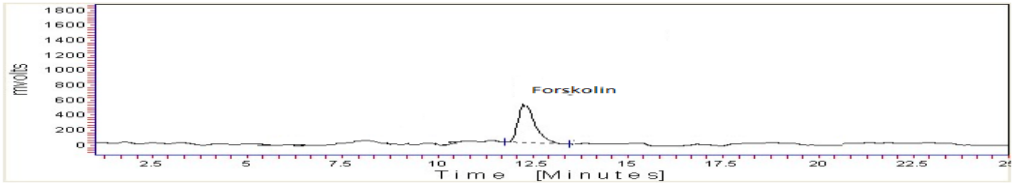
Plate:1.



Graph 4.1: Chromatogram of sample spectra of *in vitro* root extract



Graph 4.2: Chromatogram of sample spectra of *in vivo* root extract



Bibliography:

1. Atulkar, Poornima; Thakur, Ritu and Singh, Pratibha (2015). Preliminary Phytochemical analysis of root extracts of *Coleus forskohlii* Briq: *International Journal of Advanced Research*. 3(12) : 1145 – 1150.

2. Baskaran, C.A.; Rathabaia, V.; Sivamanib, P. and Thiagarajanc, V. (2011). Antimicrobial activity of various root extracts of *Coleus forskohlii*. *Int J Curr Sci*. 1 : 78-84.

3. Boily, Y. and Van Puyvelde, L. (1986). Screening of medicinal plants of Rwanda (Central Africa) for antimicrobial activity. *J. Ethnophar.* 16 : 1-13.

4. Chithrashree and Srinivas, C. (2014). Antimicrobial, Phytochemical and Antioxidant study of hydroalcoholic extracts of *Coleus caninus* (roth) vatke. *Int. J. Pharm. Sci. Rev. Res*. 52 : 288-291.

5. Inamdar, P.K.; Kanitkar, P.V.; Reden, J., and De Souza, N.J. (1984). Quantitative determination of forskolin by TLC and HPLC. *Planta Med*. 50: 30-34.

6. Kavitha, C.; Rajamani K. and Vadivel E. (2010). *Coleus forskohlii*: A comprehensive review on morphology, phytochemistry and pharmacological aspects. *Journal of Medicinal Plants Research*. 4(4) : 278-285.

7. Kumar, Hemanth and Spandana, M. (2013). Method of extraction and determination of forskolin from *Coleus forskolii* of Nepal, *Journal of Pharmacognosy and Phytochemistry*. 2 (4): 37-39.

8. Schaneberg, Brian T. and Khan, A. Ikhlas (2003). Quantitative Analysis of forskolin in *Coleus forskohlii* (Lamiaceae) by Reversed - Phase Liquid Chromatography. *Journal of AOAC International*. 86 (3): 467-471.

9. Sharma, N.; Chandel, K.P. and Srivastava, V.K. (1991). *In vitro* propagation of *Coleus forskohlii* Briq. a threatened medicinal plant. *Plant Cell Rep*. 10(2) : 67-70.

10. Sofowara, A. (1982). Medicinal plants and traditional medicines in Africa. John Wiley and Sons, New York. 256-259.

11. Wang, G.X. (2010). *In vivo* anthelmintic activity of five alkaloids from *Macleaya microcarpa* (Maxim) Fedde against Dactylogyrus intermedius in *Carassius auratus*, *Veterinary Parasitology*. 171 : 305–313.