Extraction and elemental analysis of *Coleus forskohlii* extract

Haritha Kanne, Narayan Pandurang Burte[1], V. Prasanna, Ravi Gujjula[2]

Department of Pharmacology, Osmania Medical College, Hyderabad, [1]Department of Pharmacology, MNR Medical College and Hospital, Fasalwadi, [2]Department of Chemical Engineering, Indian Institute of Technology, Ordnance Factory Estate, Yeddumailaram, Telangana, India

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ABSTRACT

Background: *Coleus forskohlii* Willd. is a popular traditional medicine used since ancient times for treatment of heart diseases, abdominal colic and respiratory disorders. **Objective:** The aim of this study was to characterize the root extract of the medicinal plant *Coleus forskohlii*. **Materials and Methods:** Dry roots of *C. forskohlii* were used to extract Forskolin using toluene as a solvent. Thus, obtained extract of *C. forskohlii* was standardized to 30% and used for further studies. **Results:** The physical properties of the extract were analyzed through scanning electron microscopy analysis, while the characterization of root extract through X-ray diffraction (XRD) and element analysis. The morphological feature of the *C. forskohlii* extract showed a flake like structure and the XRD showed sulfur trioxide (SO_3) and trimer of sulfur trioxide (S_3O_9). Through element analysis, elements such as carbon, oxygen, magnesium, aluminum, silicon, phosphorous, and sulfur were identified. Carbon showed the highest weight of 75.49% in comparison to all other elements.

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INTRODUCTION

The persistence of traditional medicine depends on the diversity and knowledge on medicinal properties of the plant. India is considered as a hot spot region for the plant diversity and about one fifth of the Indian plants are found to have medicinal properties.[1] It is estimated that about 25,000 plants were used in effective plant formulations and in traditional medicine preparations especially in rural communities of India.[2] *Coleus forskohlii* Briq. (Syn. *Plectranthus barbatus* Andr.) is a common indigenous medicinal plant belongs to Lamiaceae family.[3] In India, the major medicinal Coleus plant species are the tuberous *C. forskohlii*, *C. amboinicus*, *C. blumei,* and *C. malabaricus*, most of these plants were used for digestive disorders and dysentery treatment.[4] Plant derivatives play a vital role in world market for their properties such as fragrances, color, flavor, and its pharmaceutical properties. Phyto-chemicals are used in the medicines such as vinblastine and vincristine (*Catharanthus roseus*), taxol (*Taxus brevifolia*), camptothecin (*Camptotheca acuminate*), and Forskolin (*C. forskohlii*) an Indian Ayurvedic drug.[5]

Address for correspondence:
Dr. Haritha Kanne, Department of Pharmacology, Osmania Medical College, Hyderabad, India.
E-mail: kcharitha9@gmail.com

In modern medicine, *C. forskohlii* gained an importance after the emergence of the therapeutic properties of the Forskolin (FSK, 3) (7-β-acetoxy-8, 13-epoxy-1α, 6 β, 9α-trihydroxy-labd-14-ene-11-one). It is the active ingredient present in *C. forskohlii* which played a major role in stimulating cyclic adenosine monophosphate (cAMP) and other biological activities[6,7] and further exhibits some anti-bacterial activity.[8-10] This monophosphate acts as the second messenger for intracellular signal transduction and also regulates the number of enzymes, hormones, and other biological activities. Therefore, any impairment occurred in this pathway leads to the diseased condition. For instance, asthma and allergic conditions is characterized by decreased cAMP level in bronchial smooth muscles.[11] In general, cAMP regulates the body's thermogenic response to food, increases the body's basal metabolic rate, and further increases the utilization of body fat.

Forskolin a diterpene compound has been evaluated qualitatively and quantitatively from root and stem of *C. forskohlii* by using reversed-phase liquid chromatography with a photodiode array detector

at 210 nm.[12] The morphology, phytochemistry and pharmacological activities of the root and stem of this species have been reported. Morphologically the root of *C. forskohlii* is thick, fibrous and radially spread, golden brown in color, and their size is 20 cm long and 0.5–2.5 cm in diameter. While the stem is four-angled branches and nodes are hair-like in nature.[13] The root extract of this species have a wide variety of phytochemical constituents, deactylforskolin, 9-deoxyforskolin, 1, 9-deoxyforskolin, forskoditerpenoside C, D, and E, labdane diterpene glycosides, labdane diterpene forskoditerpene A, 1, 9-dideoxy-7– deacetylforskolin, forskolin (7-acetoxy-8, 13-epoxy-1, 6, 9-trihydroxylabd-14-en-11-one).[14,15] Forskolin is the chief chemical constituent and it have various pharmacological properties.[16] This plant is used to treat eczema, cardiovascular disorder, respiratory disorders, congestive heart failure, hypertension, painful urination, asthma, convulsions, insomnia, and prevention of cancer metastases, where, the decreased level of activated cAMP is believed to play a major role in the disease development.[16-18] The chemical study of alcoholic extract of *C. forskohlii* is a major research tool for identifying the cAMPs role in cellular physiology.[19,20]

The Forskolin production is high in roots of *C. forskohlii* and this production has been increased (ca. 1.6 mg/100 ml flask at week 5) by inducing the infection with *Agrobacterium rhizogenes* artificially and cultured in different medium with insertion of T-DNA in the plant genome.[21] Previous studies have focused to identify the new compounds from the root extract of *C. forskohlii*., Xu *et al.*[22] identified the structure of six new compounds like 14-deoxycoleon U, betulic acid, beta-sitosterol, alpha-amyrin and alpha-cedrol in the root of *C. forskohlii*. Likewise, Shan *et al.*[23,24] identified two more new compounds such as eudesmane sesquiterpenes and diterpene glycosides. Harde and Singhal[25] extracted 30.38% Forskolin from *C. forskohlii* root by using three phase partitioning. The chemical composition of root extract of *C. forskohlii* showed six major components as two labdane derivatives, β-cadinene, ß-citronellol, α-cedrene, and citronellal.[26] However, previous studies failed to focus on the physical properties and the characterization of the root extract. This study aimed to focus on the element composition and characterization of root extract of *C. forskohlii*. Keeping in view the importance of the inorganic constituents of the herbal medicines and therapeutic properties, elemental analysis was undertaken in this study. As the element composition of the extract can be used to identify the adulterants in the medicinally important products of *C. forskohlii* available in the market.

MATERIALS AND METHODS

Preparation of root extract

Dry Coleus roots were collected from Tiruvannamalai District of Tamil Nadu State. The dried roots of *C. forskohlii* were made into coarse powder by using pulverizer for the purpose of decreasing the moisture content. At 600C, the pulverized material was subjected to the Soxhlet apparatus for the continuous percolation extraction process. Using standard protocols given by Sofowora,[27] Harborne,[28] and Trease and Evans,[29] the roots of C. forskohli was treated with toluene, which was used as a solvent for extraction in 1:4 (raw materials to solvent) ratio, till spent assay was nil. After concentrating the toluene extract, hexane was added to the concentrate followed by adding equal volume of water to the separating funnel and was shaken well. After sometimes, the mixture was allowed to settle and the hexane layer was separated. Until, the free flowing wet powder was obtained, the process of washing with Hexane was repeated. The wet cake was dried followed by milling and sieving. Thus, obtained product was used to analyze the quality and the solubility properties.

Sample preparation for scanning electron microscope

The dried samples were grounded and fixed on an adhesive tape and then coated with a thin gold layer by a sputter coater. A high range of X-ray beams was made to fall under a high vacuum mode to perform the scanning of the specimen. The voltage and magnifications in the test set up were altered until a more refined picture was observed. Scanning electron microscope (SEM) images and compounds present were observed and identified based on their shapes and colors in comparison with standards of those particular materials.

Scanning electron microscopy analysis

A SEM is a type of electron microscope used to analyze the sample images with a focused beam of electrons. The electrons interact with atoms in the sample and produced various signals that can be detected and surface topography and its composition information can also be obtained. In this study, microstructural characterization was carried out by using field emission SEM (FESEM, Carl Zeiss, and Supra 40).

X-ray diffraction analysis

The particle size and the nature of extract were determined using X-ray diffraction (XRD) analysis. This data were collected using a PANalytical X'Pert Pro MPD diffractometer in a θ-θ configuration employing Cu Kα radiation (λ=1.54 Å) with a fixed divergence slit size 0.5° and a rotating sample stage. The samples

were scanned between 5° and 100° with an X'Celerator detector. The ground powders were manually frontloaded into a standard circular sample holder. Powdered sample of the mix were subjected to an intense X-ray beam and diffracted beam was detected. The peaks obtained were analyzed according to the intensities using Joint Committee on Powder Diffraction Standards data and the peaks were matched with the minerals present in the database.

ELEMENTAL ANALYSIS

The extract obtained from *C. forskohlii* root was subjected to the elemental analysis using SEM along with an energy dispersive X-ray spectrometer (EDX).

RESULTS AND DISCUSSION

Plants still constitute one of the major sources of drugs in modern as well as traditional medicine throughout the world. It has been used to cure diseases since antiquity. In this study, active ingredient forskolin was extracted from the roots of *C. forskohlii* in powder form. The purity of Forskolin content was quantified using high performance liquid chromatography and it was found to be 30% w/w. The extract thus obtained was devoid of heavy metals and microbial contamination. The physical character of this powder includes the brown colored with characteristic odor and hygroscopic in nature. The ash content obtained was 6.4% (normal limits <15%), which implies that the extract has low inorganic components. The total ash value can be used to detect foreign organic matter and adulteration with sand and earth, which indicates the care needed in preparing the plant for medicinal use. The bulk density was 3.53% (normal limits 0.4–0.6 g/ml), at 1050C loss on drying was calculated as 3.53% (normal limits >6% at 1050C) and <20 ppm heavy metals were present. Solubility test highlighted that 25% of the extract was soluble in water whereas 50% of the extract was soluble in alcohol. The aim of washing with hexane was to remove all traces of organic matter.[30] SEM analysis showed that [Figure 1] the powdered extract had a flake like morphological feature.

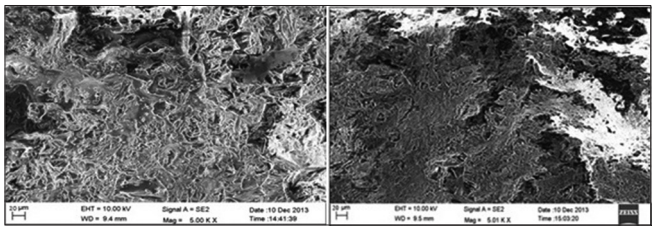

Figure 1: Scanning electron microscope images of *Coleus forskolii* extract

The XRD pattern of the *C. forskohlii* was analyzed using the root extract. A Bragg reflections with 2 θ values on X-axis was observed and identified as sulfur trioxide (SO_3) (SO_3-10.84 [110], 14.27 [002], 16.46 [102], 21.78 [022], 23.95 [112]), which is having Orthorhombic structure and trimer of sulfur trioxide (S_3O_9) or sulfur trioxide trimer (S_3O_9-18.5 [101]) as shown in Figure 2. The slight shift in the peak positions indicated the presence of strain in the crystal structure.

In the root extract of *C. forskohlii* include various distinct chemical elements with different weights. Therefore, the weight percentage of different elements such as carbon, oxygen, magnesium, aluminum, silicon, phosphorous, and sulfur was carried out through energy dispersive spectroscopy (EDX). Figure 3 depicts the area where the EDX was analyzed along with the sample. The EDX spectrum of element analysis was tabulated in Table 1. $CaCO_3$, SiO_2, MgO, Al_2O_3, SiO_2, GaP, and FeS_2 were used as the standards. Carbon showed the highest weight of 75.49% among the different elements in the extract; followed by oxygen (24.30%), while magnesium, phosphorus, silicon, sulfur, and aluminum were present in trace quantities. Silicon is considered as major element to prevent the arteries and veins hardening,[31] while phosphorous plays a crucial role in enzymatic reactions in relation to the phosphorylation. In addition, magnesium (Mg) also plays an important role in regulating muscular activity of heart rhythm and acts as an important cofactor in conversion of blood glucose into energy. De Souza[32] reported that methanolic extracts of *P. barbatus* root tubers that lowered the blood pressure and had positive inotropic activity in animal models. Hence, the presence of these elements even in small quantities might serve to impart therapeutic value to the preparation.

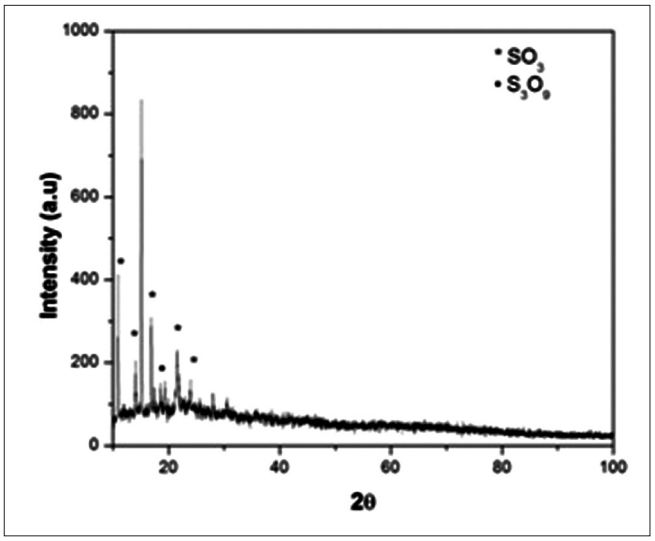

Figure 2: X-ray diffraction peaks of *Coleus forskohlii* extract

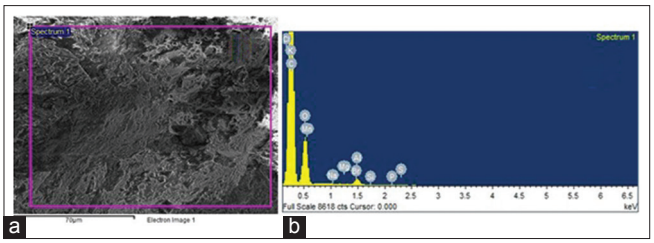

Figure 3: Elemental analysis of *Coleus forskohlii* extract, (a) shows the area from where energy dispersive X-ray has been taken and (b) is the energy dispersive X-ray spectroscopic spectrum of the sample

Table 1: Element analysis of *Coleus forskohlii*

Element	Weight (%)	Atomic (%)
Carbon	4.01	75.49
Oxygen	1.72	24.30
Magnesium	0.00	0.02
Aluminum	0.009	0.72
Silicon	0.00	0.02
Phosphorous	0.00	0.02
Sulfur	0.01	0.04

CONCLUSION

The present study characterizes the root extract of *C. forskohlii*, Forskolin is the major compound having high therapeutic value. The morphological features of the extract were examined and showed a flake like structure. Elements such as carbon, oxygen, magnesium, aluminum, silicon, phosphorous, and sulfur were identified by elemental analysis. These elements play a key role in secondary metabolite production which decides the quality of herbal raw material. Further no harmful heavy metals were detected in this study. The presence of the heavy metals depends on the locations from which plants were collected. Accurate quantitative analysis of the elemental content of plants is very important both in basic and applied plant studies, as a contribution to their potential effects on human health. Thus, the morphology and chemical properties of the plant extract perceives knowledge in relation to the herbal medicines. Further, the spectroscopic profiles of the present study act as pharmacognostic marker to identify the *C. forskohlii* from the adulterants in the market through simple spectroscopic examination in pharmaceutical industries. The result generated from this study would be useful in identification and standardization of the plant material toward quality assurance and also for preparation of a monograph of the plant.

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