

Histochemical localization of forskolin and other terpenoids in *Coleus forskohlii*

Coleus forskohlii (Willd.) Briq. [synonym *C. barbatus* (Andr.) Benth.], family Lamiaceae (Labiatae) is an ancient root drug recorded in Ayurvedic materia medica under the Sanskrit name 'Makandi' and 'Mayini'.¹ It is a perennial, aromatic herb, growing wild from Simla eastwards to Nepal, in the hills of Bihar, Gujarat and peninsular India (600–2500 m). It is common on dry, barren hills and is cultivated in Maharashtra, Gujarat and other parts of India for its roots, which are often used for making pickles.^{1,2} Forskolin (7 β -acetoxy-8,13-epoxy-1 α ,6 β ,9 α -trihydroxy-14-en-11-one) [1], a diterpene compound is the active principle.^{3,4} Minor diterpenoids, deacetylforskolin, 9-deoxyforskolin, 1,9-deoxyforskolin, 1,9-dideoxy-7-deacetylforskolin, and four other diterpenoids, have been reported to be present in the roots of *C. forskohlii*.^{4,5}

Forskolin has multifaceted pharmacological effects that have been linked to its role as an activator of adenylate cyclase.⁶ Based on its pharmacological actions, forskolin appears to be well indicated in conditions such as eczema (atopic dermatitis), asthma, psoriasis, cardiovascular disorders and hypertension, where decreased intracellular cAMP level is believed to be a major factor in the development of the disease process.⁷ ForsLean® is an extract prepared from *C. forskohlii* roots and standardized for a minimum of 10% forskolin to promote lean body mass, fat loss and weight loss. Maintaining lean body mass is essential for good health, as lean body mass may have a positive impact on long-term cardiovascular risk and life span. It is a

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Microscopic structure is long established as providing the most useful and reliable criterion in spite of chemical methods of analysis; especially chromatography which now is accepted as a standard technique for the identification of many vegetable materials. Microscopy also has the advantage of requiring only small quantities of the material and once the method is established, a conclusion as to whether or not the sample is genuine can be reached very quickly. Abraham *et al.*⁸ found that the transverse section of the root of *C. forskohlii* shows the presence of cytoplasmic vesicles containing terpenoids; this has been further confirmed by our present study. The objective of our study has been to

develop a histochemical test to locate the terpenoids of *C. forskohlii* and to quantify the percentage of forskolin present in different tissues of the root.

Plants were collected from a cultivator in Maharashtra and grown in our campus garden. Hand-sections of fresh root were cut and observed. Photographs were taken with the help of an Olympus camera-attached microscope.

Transverse sections of the root of *C. forskohlii* were taken and dipped in 75% chloral hydrate solution for 1–2 min and observed under the microscope. The section showed four medullary rays running radially from the centre to the cambium, i.e. the root is tetrarch. The root is divided into cork, cortex, cambium and xylem. Cork consists of polygonal cells. Yellowish to reddish-brown masses are

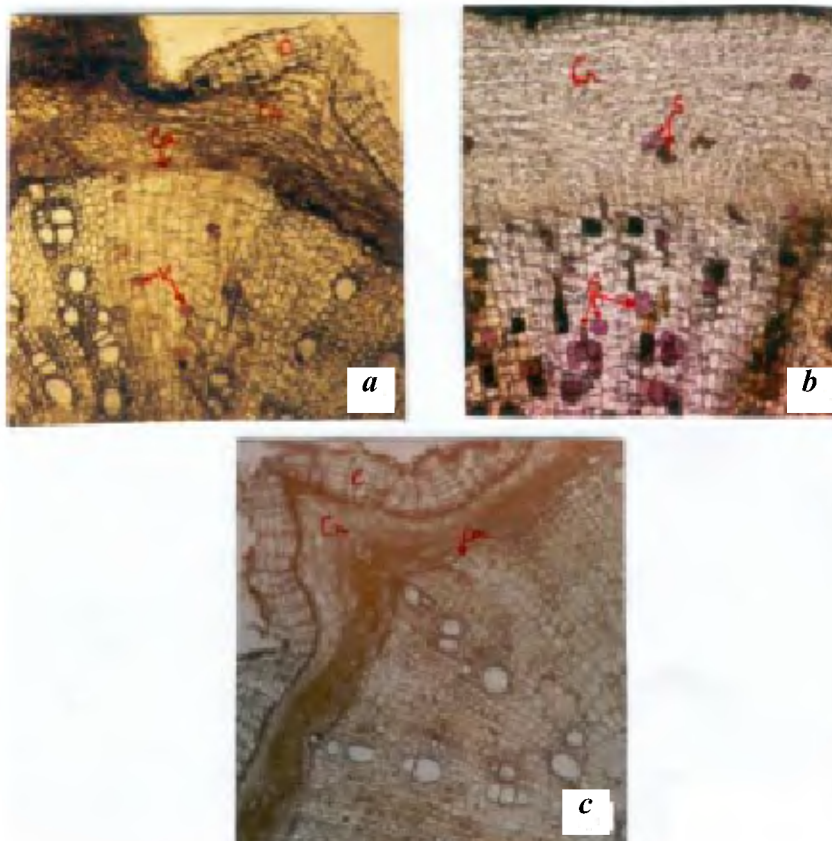
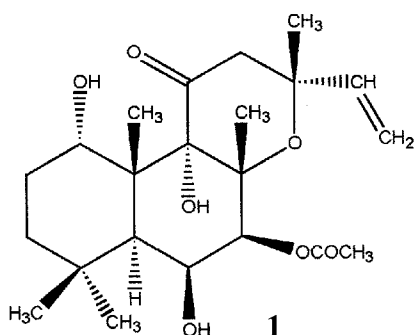


Figure 1. TS of root of *C. forskohlii* after **a**, Dipping in chloral hydrate for 1–2 min; **b**, Treatment with 10% vanillin in acetic acid and perchloric acid; and **c**, Clearing with chloral hydrate, followed by treatment with 10% vanillin in acetic acid and perchloric acid. C, cork; Cr, cortex; Ca, cambium; V, cells containing yellowish-red mass; S, cells containing yellowish-red mass stained violet, ($\times 50$).



found in the cells of cork, cortex, medullary rays and xylem, as seen in Figure 1 a. These are identified as cytoplasmic vesicles containing secondary metabolites/terpenoids⁸.

Forskolin is reported to give violet colouration with vanillin in acetic acid and perchloric acid, which has been used as a spectrometric method for detection and quantification⁹. This colour reaction was tried directly on the transverse sections and powder of the root drug. Sections of the root and the powder of the drug were first placed in 2 ml of 10% vanillin in acetic acid to which 2–3 drops of perchloric acid (70%) was added and placed on water bath (70°C) for 2–3 min. Then these sections and the powder were observed under a microscope and it was found that the yellowish-red masses were stained violet, as shown in Figure 1 b. In another study the sections and powder of root of *C. forskohlii* were cleared with 75% chloral hydrate solution for 2 h. These sections and powder were then stained with the reagent (10% vanillin in acetic acid and perchloric acid) as above. They did not get stained, indicating that the terpenoids have been washed away by chloral hydrate, as shown in Figure 1 c. TLC of the chloral hydrate washings showed presence of forskolin (TLC comparison with standard forskolin) and other terpenoids. This confirms that the yellowish-red masses seen in the sections and powder contain the terpenoids.

The cytoplasmic vesicles are present in both the bark and wood regions. This was further confirmed by separating the bark and wood from fresh roots. Each part was dried separately and powdered, and the powder obtained was studied for its characteristics. One gram each of bark and wood were extracted exhaustively

with benzene and the extract was subjected to quantitative analysis as follows. The extract was concentrated under reduced pressure and the residue was dissolved in chloroform. The volume was made up to 10 ml in a volumetric flask and HPTLC of these extracts was performed. Standard forskolin obtained as a gift sample from Hoechst, India (1 mg/ml) was prepared in chloroform. Fifty µl each of standard and extracts were spotted on a HPTLC plate. Development conditions, benzene:ethyl acetate (80:20); development distance, 12 cm. After developing, the plate was sprayed with anisaldehyde sulphuric acid reagent (1 ml concentrated H₂SO₄ is added to 0.5 ml anisaldehyde in 50 ml acetic acid) and heated at 100–105°C. Violet coloured spots were obtained and the R_f value of forskolin was 0.45. The plate was then scanned at its maximum λ_{max} of 560 nm. Bark was found to contain 0.073% of forskolin, whereas wood contained 0.40% of forskolin.

Epidermis of the leaf of *C. forskohlii* also shows presence of yellowish to reddish-brown glands, which are a characteristic feature of this plant. These structures are more predominant on the lower surface and can be seen under a dissecting microscope. Surface peels of lower epidermis on treatment with the reagent (10% vanillin in acetic acid and perchloric acid) showed that the reddish-brown gland contains terpenoids.

These yellowish and reddish-brown masses are of diagnostic importance for this drug plant and can be used for its characterization. Quantification of forskolin in different tissues indicates that terpenoids are more concentrated in the woody layer.

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