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# Monitoring of forskolin production from roots and callus by HPTLC in *Coleus forskohlii* Briq.

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## Abstract

High Performance Thin Layer Chromatography was a sensitive and accurate method for detection and estimation of forskolin in roots and by tissue culture of *Coleus forskohlii.* Maximum absorption was observed at 315 nm using fluorodensitometric analyis. Forskolin production was observed in callus cultures from leaf, stem and root origin as well as roots of *in vitro* grown plants.

**Key words** : *Coleus forskohlii,* forskolin, tissue culture.

### Abbreviations

BA : Benzyl adenine
2,4-D : 2,4-Dichlorophenoxyacetic acid
HPTLC : High Performance Thin Layer Chromatography
IAA : Indole-3-acetic acid
IBA : Indole-3-butyric acid
Kn : Kinetin
MS : Murashige and Skoog's medium
NAA : α-Naphthaleneacetic acid
TLC : Thin Layer Chromatography

## Introduction

*Coleus forskohlii* Briq. (Labiateae), an aromatic herb found in many parts of India, is the only known source of forskolin (Shah *et al.* 1980) which is used in the treatment of glaucoma, congestive cardiomyopathy and asthma. The plant is generally propagated by planting terminal stem cuttings from rapidly growing plants during November-December. Protocols for rapid micropropagation of the plant are available. A comprehensive review on the *in vitro* culture of the plant has been published (Petersen 1994). Production of forskolin has been reported in

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suspension cultures by Mersinger *et al.* (1988) who recorded an yield of 0.031% and later by Sen & Sharma (1991) with 0.009% yield. Forskolin production in root callus cultures giving an yield of 0.075% has been reported by Tripathi *et al.* (1995). Forskolin was detected in *Agrobacterium* mediated tumorous callus (0.002%), and in rhizogenic (0.011%) and root cultures (0.014%) (Mukherjee *et al.* 1996). While TLC has been reported to be useful for semiquantitative estimation (Inamdar *et al.* 1984), HPLC and GC have been used for accurate quantification of forskolin (Petersen 1994). In a recent report, forskolin has been estimated by dual wave length transmode Shimadzu CS-1910 TLC scanner at 212 nm with hexane-ethyl acetate (78:32) on silica gel $60F_{254}$ plates (Tripathi *et al.* 1995). HPTLC offers a sensitive, accurate and reliable method for routine detection and quantification of compounds in a mixture such as in plant material. This paper reports the quantification of forskolin in roots as well as callus of *C. forskohlii* by fluorescence mode using HPTLC.

## Materials and methods

*Chemicals and reagents*

Authentic sample of forskolin was obtained from Hoechst-Marion Roussel (India). Chromatographic grade reagents were obtained from Sisco Research Labs, Mumbai and precoated silica gel plates $60F_{254}$ from E Merck (India) Ltd.

*Instrumentation*

Standard forskolin and other samples were spotted on precoated silica gel plates as narrow bands of 4 mm width at a constant rate of 8 sec/µl using a Camag Linomat IV model applicator under a nitrogen atmosphere. A mixture of benzene and ethyl acetate (85:15) was used as the mobile phase. The length of the chromatogram was 90 mm and 15 min were required for each run. The plates were sprayed with anisaldehyde-sulphuric acid reagent (0.5 ml anisaldehyde + 1 ml $H_2SO_4$ + 50 ml acetic acid) and heated at 110°C for 5 min. Orange fluorescence was observed at 366 nm which was optimally detected and quantified at 315 nm using the Camag TLC scanner with CATS 3.17 software for quantification of the separated compounds on the chromatogram.

*Callus initiation from the plant*

Terminal stem pieces about 10 cm in length and young roots were excised from an actively growing 1 year old plant. The stem and root pieces were washed in tap water and surface sterilized by immersing in 98% ethanol for 30 sec. After rinsing with sterile distilled water, the explants were treated with 0.1% $HgCl_2$ for 4 min, washed thrice with sterile distilled water and dried on sterile filter paper. The nodal segments, leaves and root pieces were cut into 1 cm segments and placed on MS medium supplemented with auxins NAA, 2,4-D, IBA, IAA and cytokinins BA and Kn. The cultures were grown at 25°C and 65% RH with a 12 h photo period.

*Extraction of forskolin from samples*

Samples of roots (tissue cultured roots and roots from market) and callus (1 month old) were dried at 50°C and extracted with benzene in a soxhlet apparatus for 8 h. The solvent was evaporated and the residue was dissolved in 1 ml of benzene. A fixed amount (5 ml) was then spotted on the precoated TLC plates using the Camag Linomat applicator.

*Standard curve of forskolin in benezene*

A stock solution of forskolin with a concentration of 1 mg/ml was prepared in benzene and further diluted with the solvent to obtain a final concentration of 100 ng/ml. Appropriate quantities of this solution were applied to obtain spots in the range of 50-500 ng. The standard curve was plotted with concentration against area.

## Results and discussion

*Optimization of chromatogram*

A mixture of benzene and ethyl acetate (85:15) was used as the mobile phase and optimum resolution was obtained at Rf 0.33 + 0.01. The chromatogram was scanned at different wave lengths from 300-400 nm for λmax. It was observed that though at 365 nm and 315 nm, the peak areas were similar for a particular concentration of forskolin, at 315 nm other peak areas were minimum and therefore forskolin was the predominant compound that was estimated. Along with forskolin, at Rf = 0.7 another similar spot was observed with an orange floresecence. This could be 1,9-dideoxforskolin (DDF), which was observed in all samples. No other spot gave orange fluorescence and therefore was not detected at 315 nm. Production of DDF in quantities 1.6 to 2.5 times less than forskolin was earlier reported in suspension cultures (Mersinger *et al.* 1988). A linear regression was obtained for standard forskolin with regression coefficient = 0.9929 and the equation y = 7126 x. The lowest concentration of forskolin that was accurately detected was 25 ng.

*Organogenesis in callus cultures*

Organogenesis was observed from callus of leaf nodal segments and roots though callus of stem origin exhibited a greater potential for organogenesis (Table 1). In the medium where regeneration was observed, the callus was green with dark green spots of growth which later gave rise to shoots and roots. Cline (1996) observed that in *Coleus* spp. exogenous auxin did not repress lateral bud outgrowth as compared to many other genus where the Thimann - Skoog experiment was confirmed. Auxin treatments were carried out on 10 different species of plants including *Coleus*. Exogenous auxin did repress lateral bud outgrowth in most species but not in *Coleus* and *Arabidopsis* spp. In our experiments shoot induction was observed from callus cultures with a combination of IAA and IBA without the presence of cytokinins.

*Forskolin production in roots and callus cultures*

Forskolin content of stem callus was higher than leaf callus. Callus from roots were slow growing as can be seen from the low dry weight. It has been reported that forskolin production could only be observed after induction of differentiation into roots (Mersinger *et al.* 1988). Differentiation capacity of stem callus into shoots and roots was observed to be greater as compared to leaf callus (Table 1). After growing in the soil for 1 year, the roots of tissue cultured plants produced 0.02% forskolin. In comparison, 0.01% forskolin was detected in roots of 1 month old *in vitro* grown plants (Sen & Sharma 1991). Variation in forskolin content has been reported from 0.01 - 0.4% in roots, 0.1% being most common in roots grown at higher altitudes (Shah 1996). The higher forskolin content of market roots which are generally cultivated in hill slopes could be due to this difference

**Table 1.** Organogenesis in callus cultures of *Coleus forskohlii*

| Explant | MS basal media+ Hormone ($mgl^{-1}$) | No. of days | No. of shoots | No. of roots |
|---|---|---|---|---|
| Leaf callus | NAA (1.0) + BA (0.5) | 20 | 5 | 2 |
| Leaf callus | IAA (1.0) + IBA (1.0) | 20 | 0 | 7 |
| Root callus | NAA (1.0) + BA (0.5) | 30 | 4 | 0 |
| Stem callus | NAA (1.0) + BA (0.5) | 20 | 2 | 0 |
| Stem callus | NAA (2.0) + BA (0.5) | 25 | 5 | 0 |
| Stem callus | IAA (1.0) + IBA (1.0) | 25 | 3 | 12 |

in habitat (Table 2). The forskolin content of callus from stem and leaf origin increased steadily with time as the dry weight of the callus also increased (Fig. 1). Production of forskolin was detected only after 21 and 18 days of growth in leaf and stem callus, respectively. Differentiation of callus into shoots or roots was visible after 20 days of growth. Thus induction of organogenesis was necessary for the initiation of forskolin production. Production of forskolin was maximum after 40 days of growth in concentrations similar to market roots. Mature roots of *C. forskohlii* showed presence of cytoplasmic vesicles in which terpenoids and other secondary metabolites were found to be accumulated (Abraham *et al.* 1988). Since callus cultures are less complex than differentiated tissues, the formation of vesicles and subsequent

**Table 2.** Forskolin content in roots and callus of *Coleus forskohlii*

| Sample | MS basal media+ Hormone ($mgl^{-1}$) | Dry weight (g) | Forskolin content (%) |
|---|---|---|---|
| Leaf callus | NAA (1.0) + BA (0.5) | 0.267 | 0.010 |
| Leaf callus | IAA (1.0) + IBA (1.0) | 0.100 | 0.007 |
| Stem callus | NAA (1.0) + BA (0.5) | 0.060 | 0.009 |
| Stem callus | IAA (1.0) + IBA (1.0) | 0.133 | 0.010 |
| Root callus | NAA (1.0) + BA (0.5) | 0.069 | 0.002 |
| Tissue cultured roots | - | 1.793 | 0.020 |
| Market roots | - | 2.000 | 0.040 |

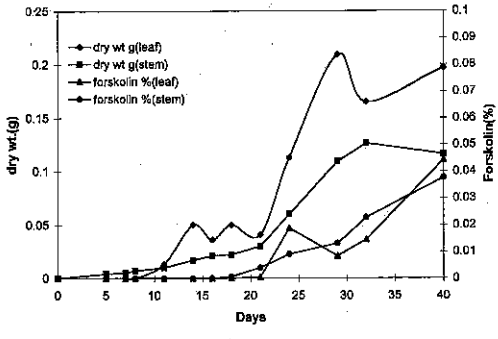


Fig. 1. Production of forskolin in callus cultures of *Coleus forskohlii*

translocation and segregation of the secondary metabolite as seen in the roots are not essential. Thus callus cultures could be a continuous and alternative source of the compound. HPTLC is a simple, sensitive, accurate and quick method for estimation of forskolin from roots and tissue culture of *C. forskohlii* and can be used as a routine method of analysis.

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