



## BIOCHEMICAL CONSTITUENTS OF MEDICINAL IMPORTANT *COLEUS FORSKOHLII* BRIQ. (PATHARCHUR), UNDER SOIL-MOISTURE STRESS CONDITION

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### Abstract

In the present study, *Coleus forskohlii* plant was subjected to different water stress conditions under controlled temperature in climate control Greenhouses. The roots of the plant were investigated for its biochemical parameters viz. forskolin, chlorophyll, proline and potassium percentage at variable water stress levels. The results revealed that the forskolin and proline content were found to be maximum in the roots of plants with 80% moisture stress. Similarly, the potassium content was also influenced by the water stress treatment. However, the chlorophyll content was found to be maximum in plants with 20% moisture stress. Consequent upon the interpretation of findings it can be concluded that the soil moisture stress has a significant influence on forskolin and proline content. Hence, the crop may be cultivated in a stress condition for maximum production of pharmaceutically important constituent i.e. forskolin.

### Keywords

Biochemicals, soil-moisture stress, forskolin, proline, potassium.

### Introduction

*Coleus forskohlii* Briq. (Patharchur) belongs to family Lamiaceae, is one of the significant medicinal herb having potential pharmaceutical properties. It is a mountain species occurring between the latitude of 31° N & 30° S in Palaetropic region. In India, it grows over a wide geographical range between the latitude 8° and 31° N in subtropical and warm temperate climate of lower Himalayas from Shimla Eastward to Bhutan and on the hills of central India. The plant grows wild in arid and semi-arid region of India and Thailand (1). It grows in 2 cm to 15 cm deep soil (pH-6.4-7.9) with deficient organic matter. In Egypt and Africa, the leaves of *C. barbatus* are used as an expectorant, emmenagogue and diuretic, while its foliage is employed in treating intestinal disorders.

The plant is cultivated commercially for its roots that are used to prepare drugs for hypertension, glaucoma, congestive heart-failure and certain type of cancer (2). Tuberos roots are succulent but hard, tortuous or straight, short or stout or long and slender. The roots are white or orangish pink flesh with bitter taste and are aromatic. The tuberos roots are found to be a rich source of forskolin. Forskolin is a major

labdane diterpenoid occur in roots (3) and stem (4) along with deacetyl forskolin, 9-deoxyforskolin and 1,9-deoxyforskolin as the minor constituents.

Keeping the above facts in view the studies were conducted to evaluate the effect of various stress levels on *Coleus* and to work out the optimum moisture level for higher forskolin content in *Coleus forskohlii*. Finally, the growth and productivity of *C. forskohlii* at different moisture levels was also quantified.

### Materials and Methods

#### Experimental Site

The present investigation was conducted at the Climate Control Greenhouses research area of medicinal and aromatic plants under Department of Plant Physiology, JNKVV, Jabalpur (M.P.) during the late Kharif season.

#### Experimental Detail

The experiment was carried out in a statically designed C.R.D. (Completely randomized design) with 4 replications

and 5 treatment levels. A total of 20 potted plants were used for the experiment and were planted. The variable treatments consist of five moisture levels i.e. 20% Moisture / 80% water stress, 40% Moisture / 60% water stress, 60% Moisture/ 40% water stress, 80% Moisture/ 20% water stress, 100% Moisture (Control)/ no stress.

### Plant Material

The plants of *Coleus forskohlii* Briq. were transplanted in pot with one plant per pot. FYM, Biofertilizer and Inorganic Fertilizer were applied to each respective pot according to their required doses.

### Sampling

Sampling was done at defined interval of 15 days i.e at 90, 110,130, 150 and 165 Days, and one plant was randomly selected from each treatment for growth analysis and biochemical estimation. The final harvesting was carried out on maturity and the plants were sun dried, roots were cleaned and analyzed for various yield components.

### Determination of biochemical parameters

The effect of moisture stress was determined by evaluating the content of photosynthetic pigment and biochemicals including forskolin, proline and potassium in leaves and roots of plants at different growth stages. Leaf chlorophyll (a, b and total) content was estimated by CCM 200 (chlorophyll content meter). The potassium content was determined by photo-metrically using flame photometer (5). Free proline content in leaves was determined following the method given by Bates (6). The protocol was based on the formation of brick red coloured formazone by proline- ninhydrin complex in acidic medium, which is soluble in organic solvents like toluene and showed absorbance at 520 nm.

### Analytical Method

#### Standard preparation

Weigh accurately 4 mg of forskolin reference standard was accurately weighed in a 10 ml volumetric flask and dissolved in methanol (stock solution).

#### Sample preparation

5 g of powdered root with methanol was taken in soxhlet unit and extraction was done at 50°C for about six hours. The resulting solution was filtered through a 0.45 µm nylon filter to remove all remaining undissolved material. The filtered solution was dried under vacuum and the resulting mass was further diluted in methanol.

#### HPTLC Method

5 µl each of test solution and reference solution were applied on five different tracks on pre-coated silica gel plate (10 x 10 cm) of uniform thickness (0.2 mm). The plate was developed using the Mobile phase Benzene: Ethyl Acetate (8.5 : 1.5) to a distance of 8 cm. The densitometry scanning wavelength was 210 nm for both reference and test solution track. Quantification of forskolin in the test solution was done by

comparing their peak areas and Rf (0.27) with those present in the reference solution track. The post scanning visualization of spots was done by spraying the plate with p-anisaldehyde sulphuric acid reagent and heated at 110°C for 5 minutes.

### Statistical analysis

Analysis of observation taken on different variables was carried out to know the degree of variation among all the treatments. The pooled data were statistically analyzed through Completely Randomized Design (8).

### Results and Discussion

#### Chlorophyll content (cm<sup>2</sup>)

The finding of the study clearly reveals the influence of moisture stress on plant biochemicals. The leaf chlorophyll was recorded from 90 days to 150 days of growth stages. The data for Chlorophyll content (g/m<sup>2</sup>) were statistically analyzed. It was found that the total leaf chlorophyll increases with the days of maturity and maximum content of chlorophyll was observed at 130 days of growth stages. It was further observed that the water-stress condition has a great influence on the leaf chlorophyll. The minimum Chlorophyll content was found in 80% moisture stress (9.198%) followed by 60 % (13.450%) and 40% moisture stress (16.100%) conditions. The maximum content was recorded in 20 % (18.119%) moisture stress condition. The results are given in Figure 1. Thus, the chlorophyll percentage in the leaves is directly proportional with the moisture content which clearly indicates the dependence of physiological phenomenon on moisture availability.

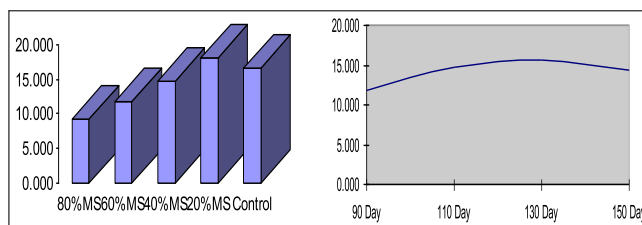


Figure 1: Chlorophyll content of *C.forskohlii* at different moisture stress condition at different interval of days.

The potassium, proline and forskolin content in the roots of plant were very much influenced by growth stages and moisture stress conditions. The content of all the three biochemicals were recorded at different development stages and it was found that the content increases from 90 -150 days of growth and thereafter it decreases. The maximum content of potassium, proline and forskolin was recorded in the roots of plants at 150 days of growth stages.

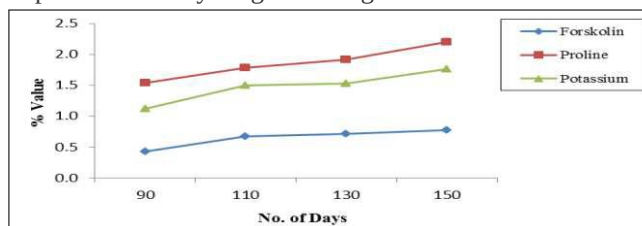
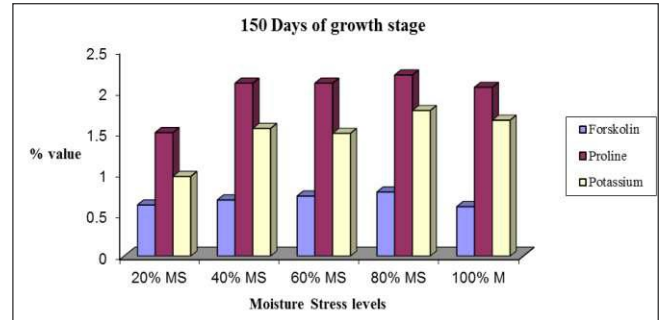


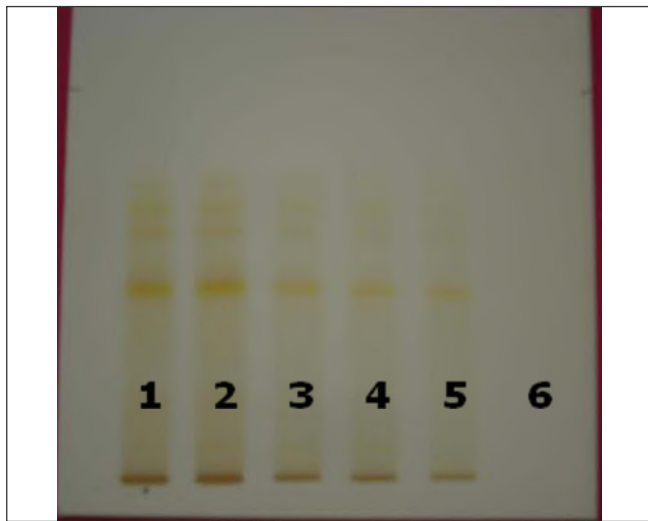
Figure 2: Forskolin, Proline and Potassium content of *C.forskohlii* at different days of interval.

**Potassium (%)**

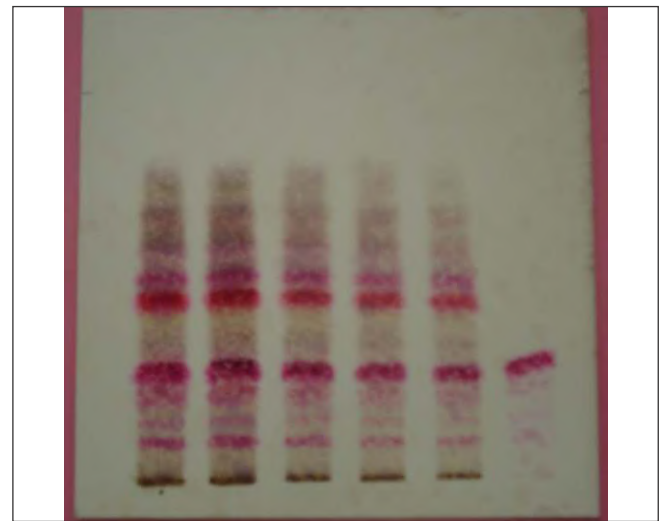
The data for potassium were analyzed and it was found that the water stress treatments have influenced on the potassium content. The maximum potassium content was found in 80% (1.7675%) moisture stress followed by 40% (1.55%) and 60% (1.49%) moisture stress conditions whereas minimum content was noted in 20 % (0.9675%) moisture condition (Figure 3). Potassium ion plays a central role in stomatal closure which is directly related with water stress tolerance mechanism. Therefore, it is one of the important biochemical parameter that is to be evaluated for water stress as it has signal responses in guard cells.



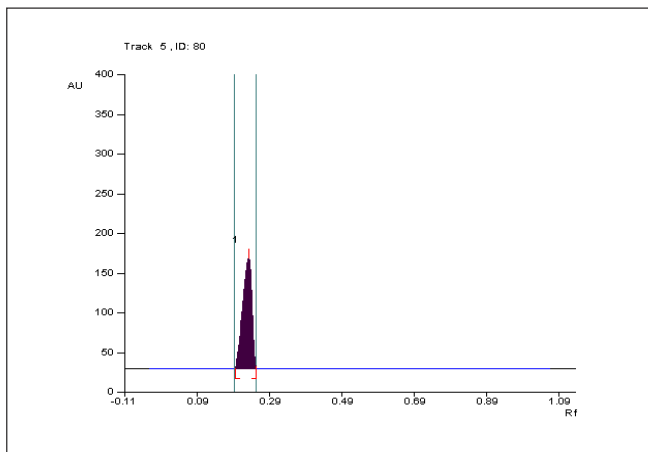
**Figure 3: Forskolin, Proline and Potassium content of *C. forskholii* at different moisture stress condition.**



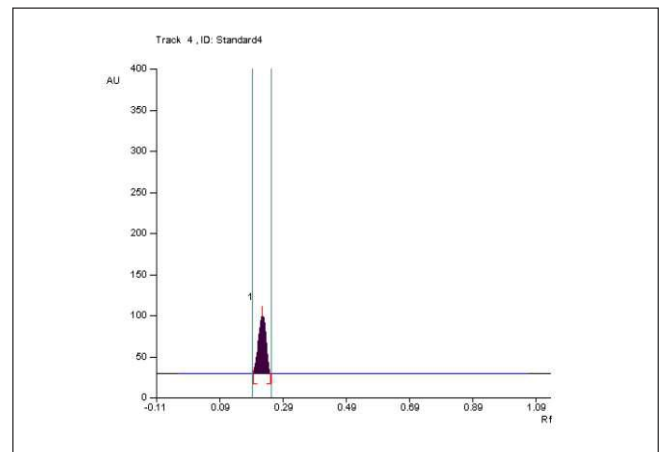
**Figure 4 (a): TLC plate just after development. Lanes 1: 80% MS, 2: 60% MS, 3:40% MS, 4: 20% MS, 5-Control, 6: Standard.**



**Figure 4 (b): Plate developed by *p*-anisaldehyde –sulphuric acid reagent.**



**Standard**



**Sample**

**Figure 4(c): HPTLC graphs of forskolin content in *C. forskholii***

**Proline content (%)**

Proline is an important amino acid produced under stress condition and is mainly related with the defense mechanism of plant. Proline content in relation to growth and water stress was noted up to 150 days. The data for proline content of roots for different water stress treatments were statistically

analyzed. The results reveal the significant influence of all the water stress treatments on proline content. The maximum proline content was found in 80% moisture stress (2.2 %) followed by 60% and 40% moisture stress (2.1%) conditions. Whereas, minimum was recorded in 20 % (1.5%) moisture stress condition.

Proline content in relation to growth and water stress was noted up to 150 days. The results were in accordance with the reports given on the factors responsible for the accumulation of proline. The proline content of the leaf was determined at two development stages [7]. Study also revealed that the simulated accumulation of free proline in germinated wheat seeds and this metabolic manifestation may be used as a good biochemical index of moisture stress tolerance in plants [8]. Lab experiment which was carried out to assess the effect of PEG – 6000 induced short term moisture stress on drought tolerance of ten rice genotypes (9). The experiment was based on change in some important physico-chemical parameters like proline contents, RWC and NR activity in germinated seedling. Study also revealed that water stress induces accumulation of proline in the leaf [10]. After screening of a large number of sunflower genotypes, the results revealed that genotypes accumulating more proline did not show tolerance to moisture stress. In fact proline is considered as a water stress tolerance gene product, which may act to maintain cellular function through protection of cellular process by osmotic adjustments. Hence, understanding of proline is of great value in evaluating water stress tolerance mechanism and also to develop water stress tolerance varieties.

#### **Forskolin content (%)**

Forskolin, a labdane diterpenoid produced under stress condition. It is well related with growth and water stress mechanism of plant. The results of forskolin content in roots are depicted in Figure 3. The data for forskolin content in leaf was quantified through HPTLC (Figure 4a,b,c) and it was found that lowest forskolin was recorded in 20% moisture stress (0.621%) and control conditions, whereas 80% (0.778%), 60% (0.729%) and 40% moisture stress (0.681%) had the relatively high content.

The results were in accordance with the reports available for other plants. Andrographolide is the major and bitter constituent of *A. paniculata* Nees, extracted from the leaves of the plant (11). It is bicyclic diterpene lactones, increase under stress condition. The average forskolin content varied from 0.254% to 1.464% for dried Roots (12). Similarly, the date of planting as well as harvest dates has great influence on forskolin concentration of roots. Research reveals that the best planting period is during September/October. The plant is ready for harvest 4 1/2 to 5 months after planting. The plants are uprooted, tubers are separated, clean and sun dried for further use (13). *Coleus* plants raised in presence of arbuscular mycorrhizal fungi *Glomus bagyarajii*, showed an increase in plant growth and forskolin content over those grown in the absence of AM fungi (14). Forskolin and Proline content increases with increase in water stress condition. Potassium content is directly related to water stress treatment, thereby regulating the water stress tolerance mechanism in plants. Hence, the crop may be cultivated in a stress condition for maximum production of pharmaceutically important constituent i.e. forskolin.

#### **Conclusion**

On the basis of above interpretation of findings it can be concluded that the soil moisture stress has a significant influence on forskolin, proline and potassium content. Chlorophyll content of the leaves was greatly influenced by water stress condition. Forskolin and Proline content increases with increase in water stress condition. Potassium content is directly related to water stress treatment, thereby regulating the water stress tolerance mechanism in plants. Hence, the crop may be cultivated in a stress condition for maximum production of pharmaceutically important constituent i.e. forskolin.

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