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## Comprehensive review on phytochemistry and *in vitro* biotechnology of *Coleus forskohlii*

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**Abstract**

*Coleus forskohlii* is an important medicinal plant which has a great role in Indian Ayurvedic medicine. It is the only source of the alkaloid forskolin in the plant kingdom. It has excellent potential in the drug trade. It is used as a stomach aid and in treating intestinal disorders. This article reviews the effect of different sources of explants, different agents of sterilization, various media composition on micropropagation of the crop of high medicinal value. The best source of explants for initiation of cultures was the nodal segments of *Coleus*, since they withstood the sterilization process better than apical shoots, exhibiting maximum survival frequency. Simultaneously, when nodal segments were used as explants, shoots multiplied at a rate of 12 fold every six weeks. Sterilization with HgCl<sub>2</sub> shows a good response; however, prolonged sterilization time tends to show browning. In most of the study, Proline was effective for avoiding the browning during *in vitro* regeneration of *Coleus forskohlii*. The maximum percentage of shoot induction from the nodal segment was achieved on the medium containing BA (2 mg/l) and NAA (1 mg/l). Among two cytokinins (BAP and kinetin), BAP (2.0 mg/l) was superior for the regeneration of shoots in *Coleus*. IAA (1.0 mg/l) was a prominent hormone for rooting of regenerated shoots.

**Keywords:** *Coleus forskohlii*, Botany, Phytochemistry, *In vitro* propagation, Benzyl Adenine (BA), Indole Acetic Acid (IAA), Benzyl Amino Purine (BAP), Naphthalene Acetic Acid (NAA), Kinetin (KIN), Proline

**Introduction**

*Coleus forskohlii* Briq. is an essential subtropical, warm temperate medicinal plant. The species is known for its highly valued labdane diterpenoid alkaloid 'forskolin', which is mainly present in the brownish-red tuberous roots of this plant that is the only source of forskolin<sup>[1]</sup>. The root extracts of the plant are rich in forskolin (0.07 to 0.59%) and have many pharmaceutical properties such as anti-inflammatory, antiglaucoma, antithrombotic, antiplatelet aggregation, antidepressant and antidiuretic activities<sup>[2-5]</sup>. The root of this plant is medicinally useful for high blood pressure, spasmodic, obesity and constipation<sup>[6]</sup>. It is evident that due to these ethnomedicinal properties, the plant has a great demand as a potential raw material in the pharmaceutical and food industries. In spite of the tremendous promise it holds in medicinal formulations, very few attempts have been made for its replenishment or cultivation. It has to be noted that the crop is currently registered as one of the plant species vulnerable to extinction in India. Hence this plant has great potential to be tapped. Traditionally, the propagation of *C. Forskohlii* is carried by vegetative propagation. However, it is known that vegetative means of propagation is a time consuming and results in limited number of propagules. *In vitro* plant tissue culture technique has been shown to play an important role in rapid clonal multiplication and reforestation<sup>[7-10]</sup>. Plant tissue culture is a model tool available to rapidly propagate these plants<sup>[6]</sup>. Therefore, this study aims to review various previous studies on micropropagation of the medicinally important plant species, *Coleus forskohlii*.

**Botanical description**

*Coleus forskohlii* Briq. Is aromatic perennial plant and belongs to family Lamiaceae. This plant commonly distributed in Indian subcontinent are, namely, India, Nepal, China, Myanmar and Sri Lanka and other countries, namely, Thailand, Brazil, Ethiopia and Egypt<sup>[11]</sup>. The plant is grown in warm temperate and subtropical climate at 600–1800 m altitude. It grows well in loamy or sandy loam soil with 6.4 – 7.9 pH. The plant has purple leaves from centre and greenish leaf margin. Roots are tuberous and erect stem reaching two feet<sup>[12]</sup>. The colour of the leaf varies due to varying shade condition. The lamina length and inflorescence length varies from 1.5 to 15.5 cm<sup>2</sup> and 3 to 40 cm, respectively.

Cluster of stalked blue or pale purple flowers branches off a stem <sup>[13]</sup>. The taxonomical position of *Coleus forskohlii* is as follows:

Kingdom: : Plantae  
 Division : Magnolophyta  
 Class : Magnoliopsida  
 Order : Lamiales  
 Family : Lamiaceae  
 Genus : Coleus  
 Species : Forskohlii

### Phytochemistry

The forskolin (a diterpenic compound) is the key chemical constituent of from the roots of coleus plant <sup>[1]</sup>. The molecular structure of forskolin was presented in the Figure 1. In another study, two diterpenoid quinones were isolated from the chloroform extract of the coleus leaves which are coleon S and coleon <sup>[14]</sup>. Xu *et al.* <sup>[15]</sup> identified six compounds namely demethylcryptojaponol, 14-deoxycoleon U, deoxycoleon U, demethylcryptojaponol, alpha-amyrin, betulinic acid, alpha-cedrol and beta-sitosterol from the *C. forskohlii* roots. Two forskolin derivatives I (1alpha, 6 betadiacetoxy-7 beta, 9alpha-dihydroxy-8,13-epoxylabd-14-en-11-one) and J, (1alpha,9alpha-dihydroxy-6beta,7betadiacetoxy-8,13-epoxylabd-14-en-11-one) were isolated from *C. forskohlii* in Yunnan Province of China <sup>[16]</sup>. Shan *et al.* <sup>[17]</sup>, isolated two labdane diterpene glycosides like forskoditerpenoside A and B from the ethanolic extract of the whole *C. forskohlii* plant. Afterwards, three labdane diterpene glycosides, forskoditerpenoside C, D and E were isolated from the ethanolic extract of whole *C. forskohlii* plant <sup>[17]</sup>. In a recent investigation, GC-MS based studies reported that decanal (9.2%), n-hexadecanoic acid (7.51), cedrol (1.74), and betulin (6.67%) are the predominant compounds in the ethanolic extract of roots <sup>[18]</sup>.

### Effect of explants and surface sterilizants on regeneration of *Coleus forskohlii*

The regeneration of this medicinally important species can be achieved employing the selection of any one of the explants namely, leaf (proximal, middle, distal) <sup>[2, & 8]</sup>, nodal segment <sup>[19]</sup> shoot tip <sup>[20]</sup>. A high frequency shoot organogenesis and plant establishment protocol has been developed for *Coleus forskohlii* from leaf derived callus <sup>[2]</sup>. Nodal segments of *Coleus* species were observed to be the most appropriate explant source for initiation of cultures, since they withstood sterilization process better than apical shoots, exhibiting maximum survival frequency <sup>[21]</sup>. Explants from apical shoot and internode were found higher potential whereas explant from leaf and roots were less efficient for callogenesis response <sup>[20]</sup>.

Crown explants from *C. forskohlii*, when established in MS medium containing 0.5 mg/l Kinetin and 0.25 mg/l BAP showed an average of 91 shoots. High multiplication efficiency, phenotypic stability and yield were ensured <sup>[22]</sup>. Leaf explants that were excised into distal segments i.e., proximal (P), middle (M) and distal (D) when transferred to the nutrient medium, showed regeneration by elongated shoots and profuse rooting <sup>[2]</sup>. Comparison of shoot regeneration response from different leaf segments at 5.0 mg/l BAP showed that the distal end was comparatively higher with 45 shoots than other leaf segments. Culturing of stem tip explants in MS medium contains 0.57 µM IAA and 0.46 µM kinetin showed 100% shooting and rooting <sup>[23]</sup>. When axillary

buds were used as explants, axillary bud break was observed during the second week of incubation in nodal explants in almost all treatments. However, the axillary shoot multiplication was achieved on BA, supplemented MS media only. Cytokinins, especially BA were reported to overcome apical dominance, release lateral buds from dormancy and promote shoot formation <sup>[19]</sup>.

Shoot apex (1-2 cm), internodal (1.5-2.0 cm) segments with a single axillary and leaf lamina with mid-vein (2 cm) were used as excised explants. *In vitro* cultured explants of *Coleus forskohlii* showed various responses viz., swelling of explants, callus induction (callogenesis), multiple shoot differentiation (caulogenesis) and root regeneration (rhizogenesis) which largely depends upon nutrients fortified in the media <sup>[24]</sup>. The per cent survival of shoot apex (93.67%), internodal (85.00%) and leaf lamina (75.33%) were highest with 0.1% Tween-20 for 5 min., 1% bavistin for 1 hour, 0.1% HgCl<sub>2</sub> for 2-3 min. and 0.5% streptomycin for 5 min., respectively <sup>[25]</sup>.

### Effect of hormones on regeneration of *Coleus forskohlii*

When nodal segments were used as explants, shoots multiplied at a rate of 12 fold every six weeks. Rooting was achieved upon transfer of shoots onto MS medium containing IAA (1.0 mg/l) <sup>[26]</sup>. The maximum percentage of shoot induction from nodal segment and shoot tip was achieved on the medium containing BA (2 mg/l) and NAA (1 mg/l<sup>3</sup>) <sup>[27]</sup>. Terminal cutting of coleus were cultured *in vitro* MS supplemented with 500ppm of IBA recorded the highest level of rooting percentage <sup>[28]</sup>. Optimal callus was developed from mature leaves on MS medium supplemented with 2.4µM kinetin alone. Shoots were regenerated from the callus on MS medium supplemented with 4.6 µM kinetin and 0.54 µM 1-naphthalene acetic acid <sup>[2]</sup>. Within 35–40 days by culturing stem tip explants in MS medium containing through direct multiplication at the rate of 12.5 shoots per explant. 100% shoots rooted and micropropagated plants were successfully established in soil after hardening with a high survival rate when developed in MS + 0.57 µM indole-3-acetic acid and 0.46 µM kinetin. Regeneration achieved was 12.5 shoots per explants <sup>[23]</sup>.

The best adventitious shoots were developed in solid MS medium supplemented with 1.5 mg/l BAP, while best rhizogenesis was achieved without growth regulator <sup>[29]</sup>. Shoot multiplication has been achieved through axillary bud development and direct adventitious shoot formation in nodal explants on MS medium containing 6-benzyladenine (BA) (5 µM). Further shoot multiplication was recorded up to third subculture on MS medium containing BA (5 µM) in combination with NAA (0.1 µM). Callus formation from leaf explants occurred in MS medium supplemented with 1-2 mg/l each of NAA and BAP. From such 50-60 days old greenish calli, a large number of shoots were formed when they were transferred to a medium containing MS + 1 mg/l BAP. Rooting occurred on MS + 0.1-0.4 mg/l NAA <sup>[30]</sup>. Morphogenic callus was induced from young leaves on MS medium augmented with NAA and BA. These calli, when subcultured on MS with KN alone gave rise to shoots.

Shoot tip and nodal segments of *Coleus forskohlii* were cultured *in vitro* on MS medium supplemented with 8.87 µM BAP along with 0.54µM NAA, resulted in direct regeneration to avoid callus phase and get true to type with a success rate of 85%. These rooted cultures conserved at 10 degree celsius for 6 months without sub culturing <sup>[31]</sup>. Auxins (2,4-D) alone and in combination with cytokinines (BAP) induced green colour, light green colour, cremish, green fragile, brown

colour was formed. The best shoot induction was achieved from the callus using BAP (0.5 mg/l) and NAA (0.1 mg/l) after 2 weeks of inoculation with highest no. of shoot induced and with maximum frequency was regenerated [32].

MS medium supplemented 2,4-D alone or both with (BAP), were superior for somatic embryogenesis. Embryogenic callus transferred on 2,4-D and BAP-supplemented medium favoured induction of embryos in advance as compared to that on 2,4-D alone supplemented medium. Eighty percent of the embryos underwent maturation and conversion to plantlets upon transfer to half-strength MS medium having  $\alpha$ -naphthaleneacetic acid (NAA), with BAP. Embryo-derived plantlets were transferred onto specially made plastic cup containing soilrite followed by their transfer to the garden soil [33]. *Coleus* was developed from callus using MS medium supplemented with BAP 2mg/l + NAA 0.5mg/l increases the shoot multiplication [34]. *Coleus* was developed from callus using MS medium supplemented with BAP 2mg/l + NAA 0.5mg/l increases the shoot multiplication [34]. Development of complete plants of *Coleus forskohlii* in MS medium containing 2.0 mg/l BAP through direct multiplication at the rate of 15-20 shoots per explant. The rooting occurred in all the plants and survival rate of full grown plants is 100% [35].

The highest percentage of callus induction and biomass production was observed leaf explants were developed in MS + 3 mg/l NAA + 1 mg/l BAP [36]. Nodal segments as explants on MS medium supplemented with kinetin (2.0mg/l) and IAA (1.0mg/l) are rooted well in their plantlets were established successfully under field conditions [13]. Shoot multiplication was obtained *in vitro* within 20-25 days from shoot tip explants, using BAP (2 mg/l). Shoot multiplication was further enhanced with the gradual decrease in the level of BAP, and its final omission after 4 months. It was observed that shoot tip elicited maximum callusing, shooting and rooting response than that of leaf and node. Multiple shoots were obtained from shoot tip explants and callus from leaf explants [6]. Sterilized explants were cultured onto MS medium augmented with different concentrations and combinations of auxin (IAA) and cytokinin (BAP) can be used for large-scale commercial cultivation, *in situ* conservation, and genetic characterization of this endangered medicinal plant [25]. According to Saravanan *et al.*, [37] this plant leaves were extracted with various solvents and observed multiple shoot induction, 2.0 mg/l BAP and 0.5 mg/l TDZ hormone concentration have high percentage of multiple shoots formation.

### Hairy root culture

Hairy root culture, also called transformed root culture is used to produce valuable secondary metabolites with plant genetic engineering. A naturally occurring soil bacterium *Agrobacterium rhizogenes* that contains root inducing plasmids (also called Ri plasmids) can infect plant roots and cause them to produce a food source for the bacterium and make them to grow abnormally. The abnormal roots are particularly easy to culture in artificial media because hormones are not needed [38]. The advantage of hairy root culture is that they often exhibit the same or greater biosynthetic capacity for secondary metabolite production compared to their intact plants [39], coupled with genetic stability [38]. Root cultures from primary callus were

established by infecting surface sterilized leaves with *Agrobacterium rhizogenes* strain 15832. The forskolin synthesis was found to be 4-5 mg/l [40].

Hairy roots of *Coleus forskohlii* were induced by the infection of *Agrobacterium rhizogenes* MAFF 03-01724 strain. The highest yield obtained was 106 mg/100 ml flask [41]. Good growth of hairy root culture of *Coleus forskohlii* was found on hormone free MS medium with 3% sucrose and Gamborg medium with 2% sucrose [42]. Then surface sterilization is done with 2% sodium hypochlorite for 10 minutes and rinsed sterile double distilled water. The hairy root cultures were successfully induced from leaf explants inoculated with A4 strain of *Agrobacterium rhizogenes* on MS media. The frequency of hairy root induction was found to be enhanced with the addition of acetosyringone 10  $\mu$ M to bacterial culture. After appearance of hairy roots, the roots were maintained in media contains antibiotic cefatoxime (500 mg/l). The bacterial strains are eliminated after 3 subcultures. For the roots to grow, it was transferred to liquid media containing IBA (1.0 mg/l), casein hydrosylate (600 mg/l). The roots showed 90% initiation and the hairy roots were white in colour and highly branched [43].

*Agrobacterium* mediated hairy root culture was done on hormone free semi solid MS medium supplemented with B5 vitamins. The explants used were stem and leaf. The explants were sterilized using 0.1% Bavistin and 0.1% HgCl<sub>2</sub>. The experiment resulted in the emergence of hairy roots after 20<sup>th</sup> day of infection [44]. Hairy root cultures established from leaf explants by infecting with *Agrobacterium rhizogenes* strain A4 on MS medium was investigated for forskolin production. Abiotic elicitors like salicylic acid (100  $\mu$ M and 500  $\mu$ M), Methyl jasmonate (100  $\mu$ M and 500  $\mu$ M) and precursors such as  $\alpha$ -ketoglutaric acid (0.2  $\mu$ M and 1.0 mM), L-phenylalanine (0.2 mM and 1.0 mM) were added to improve the yield. Methyl jasmonate (500  $\mu$ M) and L-phenylalanine (1 mM) enhanced the production [43]. The hairy root with the highest amount of forskolin was obtained from with nodal stem part. From the nodal part first 3 to 4cm long shoot emergence took place and after that within 12 days roots started emerging from base of node. If the node portion was cut and transferred to MS medium supplemented with 3% sugar, it started producing root within 5 days [45].

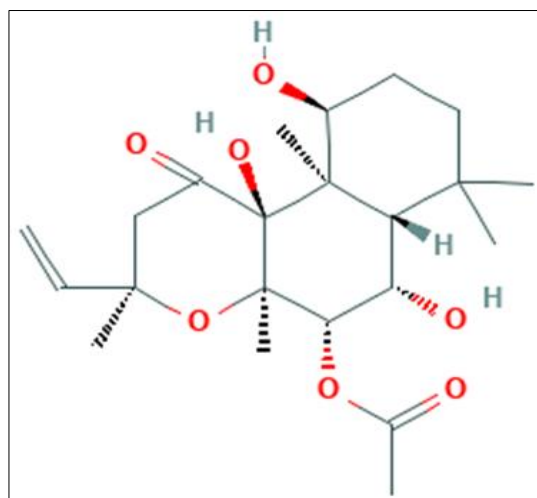


Fig 1: Chemical structure of forskolin

**Table 1:** *In vitro* regeneration responses of *Coleus forskohlii*

Sl. No	Explant	Media composition	Shoot regeneration (%)	Reference
1.	Leaf	B5 + 2,4-D(2.3 µM) + Kn(0.93 µM)	-	46
2.	Nodal segments	MS + Kn (2.0mg/l) + IAA (1.0mg/l)	99.9	26
3.	Shoot tip	MS + BAP (2mg/l) + NAA(1 mg/l)	100	47
4.	Nodal segment and shoot tip	MS+ BAP (2 mg/l) +NAA(1mg/l)	-	27
5.	Shoot tips	MS+ IAA(1mg/l) + BAP(1.5mg/l) MS+IAA+Kn(2 mg/l)	100%	48
6.	Terminal cutting	MS + 500 ppm IBA	82	28
7.	Shoot tip	MS + 0.57 µM+ IAA + 0.46 µM Kinetin	100	23
8.	Leaf	MS + Kn(4.6 µM) + NAA(0.54 µM)	-	8
9.	Callus	MS+ Kn(4.6 µM)+ NAA(0.54 µM)	-	8
10.	Shoot tip	½ MS (No PGR)	-	8
11.	Leaf	MS + BAP(1mg/l) + NAA (2 mg/l) MS+ BAP(1mg/l)	100	49
12.	Shoot tip	MS+ NAA(0.54 µM)+ BA(8.87 µM)	-	31
13.	Leaf	MS+ BA(1 mg/l)+ NAA(2 mg/l)	-	49
14.	Leaf	MS+monuron+diuron	-	50
15.	Leaf	MS + BAP (1-2 mg/l) + NAA (0.1-0.4 mg/l) +	-	30
16.	Nodal segments and Shoot tip	MS + BAP (2mg/l) + NAA(1 mg/l)	-	51
17.	Leaf	MS + BAP (5.0mg/l) + IAA (0.1mg/l)	98	2
18.	Microshoots	MS + BAP(2mg/l)	99	9
19.	Shoot tip and nodal segments	MS + 8.87 µM BAP + 0.54 µM	85	31
20.	Nodal segments	MS+1.5 mg/l BAP	-	29
21.	Shoot meristem	MS + BAP(2 mg/l) + Kn(0.5 mg/L)	75	11
22.	Shoot	MS+ BAP(0.5 mg/l)+NAA(0.1 mg/l)	80	32
23.	Nodal segments	MS+BAP(5µM)+NAA(0.1 µM)	70	19
24.	Leaf	MS + 2,4-D(0.5mg/l)+BAP(1 mg/l)	80	33
25.	Shoot tip	MS + BAP(2 mg/l)	100	35
26.	Leaf	MS + 3 mg/l NAA + 1 mg/l BAP	-	36
27.	Nodal segments	MS + 2.0 mg/l Kinetin + 1.0 mg/l IAA	-	13
28.	Shoot tip	MS + BAP (2 mg/l)	-	6
29.	Nodal segments	MS + 2.0 mg/l BAP + 0.5 mg/l TDZ	-	37
30.	Shoot tip	MS + IAA (1 mg/l) + BAP (5 mg/l)	84.7	25

Not reported

## Conclusion

The various parts of *Coleus forskohlii* have significant role in traditional medicine. A bioactive alkaloid forskolin was only found in this plant. Due to the presence of forskolin, this plant has various therapeutic potential like anti-inflammatory, antiglaucoma, antithrombotic, antidepressant and antidiuretic activities. Traditional propagation of this plant take time consuming and limited number of propagates. Hence, *in vitro* propagation method is the only option for multiplication of higher number of plantlets. This review was remarked that several previous study reports are suggested proline was effective against browning in *in vitro* regeneration. BAB and IAA were superior for regeneration of shoots and roots, respectively in *Coleus forskohlii*.

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