



Effect of different hormones on different explants of *Coleus forskohlii* briq

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Abstract

Coleus forskohlii Briq. is a plant that is often used in Ayurvedic medicine and many nutritional supplements. Its pungent roots are the component of the plant cell that is most prescribed to treat diseases of the cardiovascular system, respiratory system, and nervous system. Forskolin is an antidepressant, anticancer, antiglaucoma, antidiuretic, antimetastatic, antispasmodic, and bronchodilator-like drug that is used to treat glaucoma, hypertension, and asthma. There is also evidence that it has anti-inflammatory action. The evidence reviewed of the plant and its forskolin components corroborate these traditional uses while also suggesting that it may be helpful in the treatment of psoriasis, angina, asthma, and cancer metastatic prevention. *forskohlii* is a common traditional medicine that has been used to treat heart disease since ancient times.

Keywords: *forskolin*, traditional medicine, anti-cancer, asthma

Introduction

Plants have been used for medicinal purposes for a prolonged time, dating back to the prehistoric period. Pharmaceutical systems that have been in use for a long time are still widely used for different reasons. Because of the uses of plant-derived therapeutic chemicals over traditional methods of employing natural product chemistry, herbal plants, particularly phytochemistry, have piqued the interest of most academics.

Herbal drugs are chosen over allopathic medicines because of their high efficacy, minimum cost, and ease of accessibility, as well as the fact that they cause fewer or no side effects. Even in the Western world, herbal pharmaceuticals and herbal formulations mentioned in traditional medicines such as Chinese traditional medicines and Indian traditional medicines such as Ayurveda and Siddha literature are being used to treat a variety of illnesses. Apart from traditional medical systems, many Indian cultures use a variety of medicinal plants for therapeutic purposes, and this unmodified approach is known as "Folk Medicine" or "Ethnomedicine."

Although medicinal plants have been utilized for therapeutic purposes since the dawn of time, quality monitoring of herbal medications has received little attention. Herbal medications made by different companies or traditional physicians, as well as batch to batch from the same company or physician, vary in quality in the Ayurveda and Siddha systems of medicine. These differences are attributable to differences in botanical identification of herbal sources, as well as differences in herbal raw medicine collection and processing methods.

It is essential to formulate the originality, quality, efficacy, and safety of herbal remedies in place to ensure their quality. The establishment of botanical identity, cultivation or collecting, harvesting, processing, storage, preservation, formulation, and packaging have all been part of the regulation of medicinal herbs. The biologically active compounds with their quality and quantity have an impact on the therapeutic value of medicinal plants.

Plants' biological active substances differ due to genetic, climatic, soil, and seasonal aspects. (Trease & Evans, 1983) ^[1]. Plants exhibit a wide range of compounds, but the most relate to one of four biochemical classes: alkaloids, glycosides, polyphenols, and terpenes. Alkaloids are bitter-tasting compounds that are available in large quantities and are usually toxic. Anthraquinone and glycosides are present in all laxatives plants, also in senna, rhubarb and aloe. These plants belong to diverse groups that have different modes of action as medications. Cardiac glycosides are potent drugs existing in the plants such as foxglove and lily of the valley. Plants contain polyphenols from a variety of classifications. Colorful anthocyanins, hormone-mimicking phytoestrogens, and astringent tannins are among them. Resinous plants include a variety of terpenes and terpenoids. Plant tissue culture is a novel technology for generating plants rapidly. It can be used to effectively preserve and propagate rare and threatened medicinal herbs in a short period. Tissue culture-derived plants can fulfill the recent surge in sales for herbal raw drugs entirely or completely. As far as the active principle is concerned, the quality of raw medicinal plants contrived by tissue culture is determined to be quite uniform.

Plant biotechnology has provided important growth in recent years as one of the most significant areas of biotechnology, both scientific and commercially. Since White (1934 & 1937) ^[2] pioneering research, there has also been a major innovation in plant tissue culture. Skoog (1944) ^[4] and Murashige & Skoog (1962) ^[5] both contributed a great deal to lay a solid foundation for the application of this multipurpose technology. Due to heavy unscientific and irresponsible exploitation and low multiplication response, important medicinal plants are becoming endangered and vulnerable. Tissue culture technologies are essential for replicating medicinal plants rapidly and generating new types.

The tissue culture technology offers the potential of being suitable for examining secondary product metabolism and, in the perspective, for producing commercially important

natural substances. In opposed to a collection in the natural or production, raw herbal drugs acquired through tissue culture can be supplied in bulk during the year without even being influenced by the weather. *In vitro* propagation of traditional medicines, selection of yielding lines in terms of bulk or active substance is also attainable.

Within the World Community, the World Health Organisation (WHO) is the guiding and controlling body for global health. WHO experts establish health principles and procedures, as well as support countries concerning public health issues. Health research is also encouraged and supported by WHO. Governments may work together to address global health issues and improve people's lives through the WHO. The Lamiaceae, also known as the mint, deadnettle, or sage family, is a flowering plant family that comprises mint, deadnettle, and sage. The family has a varied population. There are around 236 genera in the wider Lamiaceae, with 6,900 to 7,534 genera documented. The family was again assumed to be closely associated with the Verbenaceae.

Coleus forskohlii Briq is one kind of herbal plant. It was first recorded in Sanskrit literature over 3,000 years ago and is still broadly applied in Ayurvedic therapy. This plant has also been used to treat cardiovascular problems including hypertension and heart palpitations (angina), as well as breathing difficulties like asthma, since prehistoric days. It's an annual herb with perennial rootstock and an annual stem. Depending on the growing conditions, the root material might be tuberous, semi-tuberous, or fibrous. A single plant can yield around 1 and 500g of root material. Forskolin's dry matter content in roots varies between 0.07 and 0.58 percent (Virbala shah and Kalakoti, 1996).

C. forskohlii extracts with high quantities of forskolin are increasingly being produced by herbal product makers. These supplements are being recommended for the same ailments that forskolin has traditionally been used for. Although, there is currently no clear scientific evidence to suggest that oral consumption of *C. Forskohlii* extract is effective.

Materials and Methods

Materials

Coleus forskohlii Briq. (Lamiaceae) is the plant material used in this research. The high- yielding *C. forskohlii* Salem variety was purchased from Ariyalur.

Methods

Extraction of Explant

For the preliminary study, explants from leaf and shoot tips were excised. Initially, explants from first to fifth leaves

from shoot tip were used for extraction of explants as leaf explants. It was found that only leaf explants extracted from the third leaf no. were more proliferative and hence thereafter leaf explants from the third leaf no. as used. All the explants are garden cultivated plants (Fig.1). By utilizing the sterile forceps and blades, the explants were excised. The leaves were divided in the range of 0.5-1.0 cm segments. Moreover, care was taken to incorporate the midrib portion of the explants.

Surface sterilization of the explants

Surface sterilization was performed by utilizing mercury chloride and alcohol.

Explants were subjected to processing with 0.1 percent mercuric chloride for 1-2 minutes. Subsequently, the explants were twice exposed to sterile distilled water and the materials were rinsed with 50% alcohol for 2-3 minutes. Afterward, the explants were carefully rinsed twice with sterile distilled water.



Fig 1

Preparation of medium

Preparation of Hormones

20mg of Auxins such as Indole-3-Acetic Acid (IAA) Indole butyric acid (IBA) has been dissolved in 0,1 N NaOH and diluted with distilled water to 20 ml. 20mg of 2,4-D (Dichlorophenoxy acetic acid) was dissolved in ethanol and diluted with distilled water to make up to 20 ml. All of the above hormones have been stocked in the fridge.

Preparation of stock solution

The composition of the MS basal medium used in this study was similar to the composition of hormones free MS medium used by Murashige and Skoog (1962) [5].

Preparation of stock solution for MS medium

Table 1

Stock solution	Constituents	Concentration of stock solution gm/100ml	Volume of solution in final medium ml/l.
A	NH ₄ NO ₃	8.25	20
B	KNO ₃	9.25	20
C	H ₃ BO ₃ KH ₂ PO ₄ KI NaMoO ₄ 2H ₂ O CoCl ₂ 6H ₂ O	0.124	5
		3.4	
		0.0166	
		0.005	
		0.0005	
D	CaCl ₂ 2H ₂ O	8.8	5
E	MgSo ₄ 7H ₂ O MnSo ₄ 4H ₂ O ZnSo ₄ 5H ₂ O CuSo ₄ 5H ₂ O	7.4	5
		0.446	
		0.172	
		0.0005	

F	Na ₂ EDTA FeSo ₄ 7H ₂ O	0.745	5
		0.5571	
G	Thiamine HCl Nicotinic acid Pyridoxine HCl Glycine Myo-inositol	0.01	1
		0.05	
		0.05	
		0.2	
		1.0	

Na₂EDTA was heated and mixed under constant stirring with FeSO₄.7H₂O solution. Heating and stirring resulted in a more stable FeEDTA complex. All the stock solution was stored in the glass bottles under refrigeration. The bottle which contained the stock solution was shaken gently before use. If any of the solutions showed suspension or precipitate they were immediately discarded. Details about the concentration and date of preparation of all the stock solutions were labeled. The stock solutions were not used for more than four weeks. Appropriate quantities of the various stock solutions sucrose, Myo-inositol and growth regulators were added. The final volume of the medium was obtained employing double-distilled water. After thorough mixing, the pH of the medium was balanced to 6.5 with 0.1 N NaOH or 0.1 N HCl. Subsequently, a suitable amount of agar-agar was supplemented. The medium was heated to the point where the agar liquefied. The medium was then transferred to the growing tubes, subsequently; the tubes were clamped with absorbent cotton. The closed culture tubes were sterilized using a pressure cooker at 121°C for 20 minutes as well as cooled to ambient temperature.

Inoculation

Before starting inoculation, culture tubes containing media, instruments like spirit lamp, sterilized forceps, scissors, Petri dishes and sterilized distilled water were transferred to a UV chamber and exposed under UV light for 30 minutes. After that, the surface-sterilized explants were inoculated. The leaf explants were planted on the medium with the abaxial surface in contact with the nutrient medium.

Culture room

The culture room has been sustained at a temperature of 25±2°C as well as employing the white fluorescent lamps with the light intensity of 2,000 Lux, the cultures tubes were maintained. A photoperiod of 12hrs per day was maintained. The room has been supported by 70% of the relative humidity. The experiments were subjected to ten replicates as well as it was repeated at a minimum of one for the verification of the result.

Growth Measurement

Fresh and dry weights of a few explants before and after the culture period were measured. At an interval of two days, the periodic inspection was carried out to investigate the origination of callus along with a change of colour as well as initiation of the root or shoot. Explants of the shoot tip, leaf lamina, and leaf base of *C. forskohlii* (Lam) (Plate-1) were propagated on several hormone doses. Growth responses such as enlargement, callus development, and shoot development were reported in all explants, especially leaf lamina, leaf base, and shoot tip explants. Therefore, all of the mentioned *C. forskohlii* explants were applied in this study. The impacts of auxin and cytokinin on the development of multiple *C. forskohlii* explants are shown in Tables I, II and III. After 4 weeks of culture after using different concentrations of IAA (0.5-2.0 mg/l), the highest increase of all explants was reported on 1 mg/l, followed by 0.5 mg/l (Table-I). Of all the explants, shoot tip explants registered the highest growth (978±58.75mg fr.wt.)

followed by leaf base, at 1mg/l concentration of IAA. On MS media treated with 1mg/l IAA (Fig.2) morphogenesis were not observed in shoot tip and leaf base explants, which expanded more than twenty-fourfold in fresh weight and dried weight.

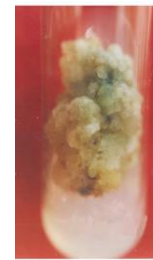


Fig 2

When IBA was added to MS basal medium in various concentrations, all explants demonstrated the greatest growth augmentation at 1mg/l concentration (Table II). Shoot tip explants grew the fastest (898±88.76mg fr.wt.) at 1 mg/l IBA concentration, (Fig.-3) followed by leaf base explants (712±69.91mg fr.wt.). After 4 weeks of culture, shoot tip and leaf base explants at IBA 1mg/l concentration revealed a more than twenty-fourfold growth augmentation, with no callus or morphogenesis observed in all of the IBA concentrations.

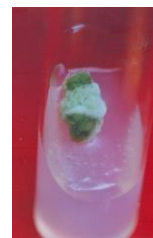


Fig 3

In practically all of the explants, 2, 4-D at various doses generated the greatest growth increase and formation of callus (Table III). After two weeks in culture, all of the explants developed callus. At 1.5 mg/l 2, 4-D concentration, shoot tip explants were more proliferative and had the maximum growth (19519±1850.21mg fr. Wt.). (Fig.-4) With this concentration, all the additional explants have the significant capability to grow as well as all of the explants grew well in MS media supplemented with 1 mg/l 2,4-D.

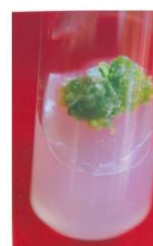


Fig 4

Table 2: Effect of different concentrations of IAA on the growth of *C. forskohlii* explants

Conc. of IAA (mg/l)	Leaf base		Leaf lamina		Shoot tip	
	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)
Basal	133.29±12.11	1.33±0.06	127.37±11.29	1.26±0.07	141.08±13.4	1.40±0.04
0.5	847 ±82.91	8.47±1.46	755±70.41	7.55±1.56	907±50.16	9.15±1.09
1.0	828±85.76	9.08±1.65	882±86.56	8.82±1.49	978±58.75	9.23±1.18
1.5	742±73.57	7.40±0.59	639±61.07	6.34±1.41	776±36.71	7.75±1.94
2.0	147±12.09	1.46±0.07	130±9.06	1.30±0.06	153±13.49	1.52±0.08

Medium: MS Basal

Age of culture: 4 Weeks

Table 3

	Leaf base (mg)	Leaf lamina (mg)	Node (mg)	Shoot tip (mg)
Initial fresh weight	38.50 ± 3.25	22.0 ± 2.45	12.0 ± 0.9	37.9 ± 2.85
Initial dry weight	6.3 ± 0.05	1.1 ± 0.04	1.0 ± 0.03	22.0 ± 2.45

Table 4: Effect of different concentrations of IBA on the growth of *C. forskohlii* explants

Conc. of IBA (mg/l)	Leaf base		Leaf lamina		Shoot tip	
	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)
Basal	133.29±12.11	1.33±0.06	127.37±11.29	1.26±0.07	141.08±13.41	1.40±0.04
0.5	658±59.49	6.57±5.56	536±52.01	5.35±4.77	769±75.81	7.68±6.47
1.0	712±69.91	7.12±6.94	575±56.71	5.75±4.81	898±88.76	8.97±7.45
1.5	206±19.67	2.06±1.15	198±15.92	1.98±0.09	290±20.49	2.19±1.10
2.0	101±6.34	1.01±0.09	76±6.04	7.6±6.30	179±15.21	1.79±0.09

Medium: MS Basal

Age of culture: 4 Weeks

Table 5

	Leaf base (mg)	Leaf lamina (mg)	Node (mg)	Shoot tip (mg)
Initial fresh weight:	38.50 ± 3.25	22.0 ± 2.45	12.0 ± 0.9	37.9 ± 2.85
Initial dry weight :	6.3 ± 0.05	1.1 ± 0.04	1.0 ± 0.03	22.0 ± 2.45

Table 6: Effect of different concentrations of 2, 4-D on the growth of *C. forskohlii* explants

Conc. of 2,4-D (mg/l)	Leaf base		Leaf lamina		Shoot tip	
	Freshweight (mg)	Dryweight (mg)	Freshweight (mg)	Dryweight (mg)	Freshweight (mg)	Dryweight (mg)
Basal	133.29±12.11	1.33±0.06	127.37±11.29	1.26±0.07	141.08±13.41	1.40±0.04
0.5	2106±201.77	21.06±2.17	1140±110.84	1140±101.86	2121±209.18	21.20±1.10
1.0	5326±527.39	53.26±5.61	3400±336.43	34.00±3.10	10070±996.18	100.58±19.70
1.5	8678±855.19	86.78±8.31	4698±456.21	46.98±5.04	19519±1850.21	195.10±19.93
2.0	746±47.35	7.46±1.02	541±14.02	5.41±0.72	840±82.71	7.40±7.05

Medium: MS Basal

Age of Culture: 4 Weeks

Table 7

	Leaf base (mg)	Leaf lamina (mg)	Shoot tip (mg)
Initial fresh weight	38.50 ± 3.25	22.0 ± 2.45	37.9 ± 2.85
Initial dry weight	6.3 ± 0.05	1.1 ± 0.04	22.0 ± 2.45

Discussion

Leaf and shoot tip explants of *C. forskohlii* Briq. (Lamiaceae) demonstrated the greatest growth response in culture. *C. forskohlii* leaves are succulent, with more meristematic tissues in immature leaves than mature leaves. Although auxiliary bud-containing function explants proliferated well *in vitro*, shoot tip explants are better for inducing callus and numerous shoots.

Explants of *C. forskohlii* favored 2, 4-D for profuse callusing out of three auxins utilized in the study (IAA, IBA, and 2, 4-D). On *C. forskohlii* explants, the three auxins could induce callus in the following order: 2, 4-D > IAA > IBA. A significant callusing potential has been explored by the shoot tip explants of *C. forskohlii* with medium supplemented with 2, 4-D 1.5mg/l + IAA 0.5mg/l. In this

study, it is evident that the ratio of auxin to cytokinin regulates differentiation in culture tissues and influences varying growth responses in utilizing the explants of the shoot, as well as leaf tip with various factorial combinations of hormones.

The exogenous need for plant hormones is altered by the endogenous hormone level of explants or organs. After Skoog and Miller's (1957) [7] pioneering work, it was established that growth regulators play a role in the growth of plant tissue cultivated *in vitro*. The morphogenetic potential of tissue explants is also influenced by the mother plant's genetic and physiological age. Shoot tip explants of *C. forskohlii* induced shoots through callus (IAA 0.5mg/l + 2, 4-D 0.5mg/l and IAA 1mg/l + IBA 0.5mg/l). The

maximum number of shoots (5) was formed from callus derived from shoot tip explants.

When shoots created from numerous shot mediums were subcultured on the same medium, they developed and proliferated new shoots, however, shoots formed from other media did not develop well after several subcultures on the same medium. It may, however, be feasible to trigger the proliferation of shoots further by altering the sequence of plant hormones. By altering the sequence of growth regulators, Chandra (1981)^[8] was able to induce embryogenic potential in *Daucus carota*. *Syzygium travancoricum* has lately produced a large number of numerous shoots from seedling explants on the third subculture on multiplication media by Anand *et al.* (1999)^[9].

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