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A Review on Forskolin: a Cyclic AMP modulator from Tissue Cultures of *Coleus forskohlii*

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Abstract: Forskolin has the unique property of activating all hormone sensitive adenylate cyclase enzymes in a biological system. *Coleus forskohlii* is the only source for forskolin among the plant kingdom. This article reviews about the need of systematic studies on cell cultures of *C. forskohlii* for the production and to enhance the production of forskolin by employing various strategies.

Keywords: *Coleus forskohlii*, Forskolin, Tissue culture, Chemistry, Biogenesis.

Introduction


Fig 1

Coleus forskohlii Briq. (Lamiaceae) is an important plant (Fig. 1) in Indian Ayurvedic medicine. The tuberous roots (Fig. 2) of the plant produces labdane diterpenoid forskolin (1). It is distributed over the subtropical warm temperate climatic zone on mountains of India, Nepal, Myanmar, Srilanka, Thailand and Africa. In India, it is reported to be distributed in dry hills of Westeren Uttarpradesh, Gujarat, parts of Orissa, Western ghats and in kitchen gardens in Northern Karnataka (Belgaum and Dharward districts) for its carrot like tubers, which are used as condiments in the preparation of pickles.

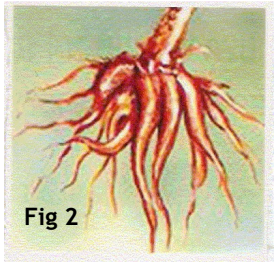


Fig 2

Forskolin has a unique property of activating almost all hormone sensitive adenylate cyclase enzymes in a biological system (2). Forskolin is reported to be useful in the treatment of congestive heart failure, glaucoma, asthma and certain type of cancers (3). It is an important plant used against various disorders in indigenous systems of medicine such as anti-aging, antioxidant, as a remedy for heart, abdominal and respiratory disorders (4). In addition, it has been shown to have anti-inflammatory property (5). The total synthesis of forskolin has been reported (6), it is uneconomic because of structural complexity of the molecule and the synthetic forskolin is not as effective as procured from natural source. *C. forskohlii* is the only source for this compound. Indiscriminate collection of *C. forskohlii* has led to rapid depletion of wild populations resulting in its listing as a plant vulnerable to extinction in India (7).

Chemistry

Forskolin (C_{22} $H_{34}O_7$) is a 7β-acetoxy 8,13-epoxy -1α, 6β,9α-trihydroxy labd-14-en-11-one.The structures of forskolin and its analogs are shown in Fig. 3. Between 1974 and 1986 researchers at the CDRI, Lucknow isolated a group of diterpenoid possessing the basic skeleton of 11-oxo-manoyl oxide from the medicinal plant *C. forskohlii*. The most active diterpene was characterized and named Coleonol (11). Thereafter De Souza *et al.*, (1) Hoechst India Limited, Mumbai, named the same compound as forskolin (1) with pharmaceutical activities similar to coleonol. The only difference between the structures of coleonol (11) and forskolin was the configuration of the 7-acetoxy group of forskolin. Hoechst group assigned the β-configuration to the 7-acetoxy group of forskolin, where as the CDRI group assigned α-sterochemistry to the group at that position. Later on both compounds were assigned the same structure and stereochemistry, the 7-acetoxy group having the β-configuration as in forskolin. Recently a series of compounds have been reported (1-9) with β-configuration of the 7-oxygenated group, i.e. deacetyl forskolin (2), 9-deoxy forskolin (3), 1,9-dideoxy forskolin (4), 1,9-dideoxy-7-deacetylforskolin (5), colenol (6), 7β-acetoxyl-6β, 9α-dihydroxy-8,13-epoxy-labd-14-en-11-one (7), 6β, 7β, 9α- trihydroxy- 8,13-epoxy- labd-14-en-11-one (8), 6β,dihydroxy-8,13-epoxy-labd-14-en-11-one (9) and 8,13-epoxy-epoxy-labd-14-en-11-one (10). α-configuration of the 7-oxygenated group were coleonol B and C (15,16), deoxycoleonol (13) coleonol E (14), coleonol D (17) and coleonol F (12) compounds in which C-11 is not oxygenated have also been reported such as coleonone (8) .

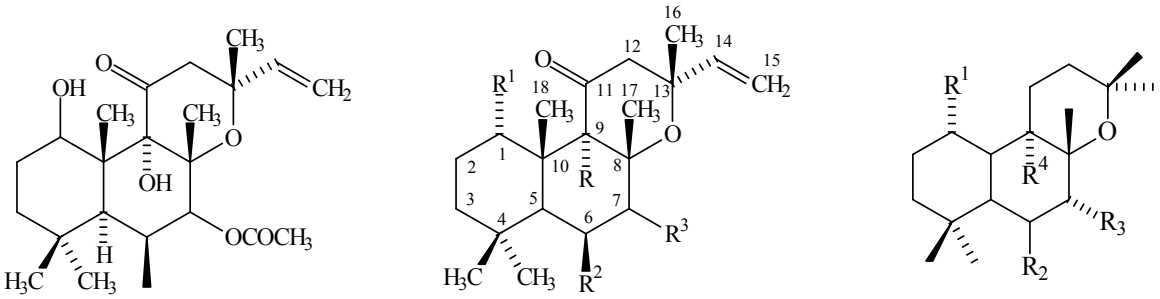
Biogenesis

The forskolin is biosynthesized from acetate-mevalonate pathway. In the postulated biosynthetic pathway 8,13-epoxy-labd-14-en-11-one is the first mono oxygenated labdane type diterpene to be formed on biosynthetic pathway leading from the labdane diterpene skeleton, subsequent addition of oxygen gives 1,9-dideoxy forskolin, 9-deoxyforskolin and forskolin with other terpenes. Forskolin is the last compound to be formed in the biogenetic sequence (8).

Tissue culture

Forskolin and its analogs are obtained from *C. forskohlii*. Forskolin is usually produced by extraction from roots of the plant. Forskolin content of the roots obtained from natural habitats ranges from 0.04 - 0.44% of dry cell weight, 0.1% being most common (9). Attempts have been made to provide additional sources to provide the pharmaceutical demand for forskolin through plant biotechnology technique. Mersinger *et al.,* (10) established suspension cultures of *C. forskohlii* for production of forskolin in a 20-l air bioreactor to a maximum of 730mg/kg after 19 days of fermentation. Kries and Reinhard (11) reported the production of forskolin (325mg) in 300L bioreactor at the end of 17 days of culture. Krombholz (12) initiated root cultures

Fig. 3



Forskolin

S.No.	R^1	R^2	R^3	R^4
1	OH	OH	OAc	OH
2	OH	OH	OH	OH
3	OH	OH	OAc	H
4	H	OH	OAc	H
5	H	OH	OH	H
6	H	H	H	H
7	H	OH	OAc	OH
8	H	OH	OH	OH
9	H	OH	H	H
10	H	H	H	H
11	OH	OH	OAc	OH
12	H	OAc	OH	OH
13	OH	OH	OAc	H
14	H	OH	OAc	H
15	OH	OAc (C-16 methyl is β-oriented)	OH	OH
16	OH	OAc	OH	OH
17	H	OH	OAc	OH

from primary callus or Indole butyric acid (IBA) treated suspension cultures and maintained on Gamborg (B_5) (13) medium containing IBA (1mg/l) and reported on the growth behavior of transformed and untransformed roots in batch culture in shake flasks and 20-l glass jar. Sen *et al.,* (14) reported forskolin production in different tissue culture systems viz shoot tip culture, rhizogenic callus culture and root organ suspension respectively on Murashige and Skoog (MS) media (15) and Whites (16) basal media with or with out growth regulators. Tripathi *et al.,* (17) reported the production of forskolin by root callus cultures. Maximum growth rate was obtained in medium containing sucrose (7% w/v). Biomass production was highest with 1-napthalene acetic acid (NAA) (4 mg/l) maximum production of forskolin (0.075% of dry weight cells) was observed in medium containing indole-3-acetic acid (IAA) (0.5 mg/l), indole-3-butyric acid (IBA) (0.5 mg/l), glycine (5 mg/l), casein hydrolysate (200 mg/l) and sucrose (7% w/v). Mamatha *et al.,* (18) reported the enhanced bioproduction of

forskolin by 150% by treating the cell cultures of *C. forskohlii* with ancymidol (50μM).

Sharma *et al.,* (19) reported the *in-vitro* clonal multiplication of *C. forskohlii* which was been achieved on MS medium supplement with kinetin (2.0mg/l) and indole acetic acid (IAA) (1.0mg/l) using nodal segments 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 (1.0mg/l). The micropropagated plants were successfully established under field conditions. Forskolin content in tubers of plants obtained by micropropagation was found to be 0.1% (dry wieght), and which were comparable to the wild plants. Tefera and Narayanaswamy (20) initiated callus cultures and regeneration from shoot tip explants of aseptically grown. A rapid initiation and proliferation of callus was obtained on MS basal medium containing IAA (1.0 mg/l) and BAP (1.5mg/l). Adventious shoots were obtained from compact greenish callus on passage to MS basal medium containing various concentrations and combinations of IAA and kinetin, but the best response was in the medium containing IAA (1.0mg/l) and kinetin (2.0mg/l), on further subculturing of individual shoots onto hormone free MS medium developed into normal plantlets. Bhattacharya and Bhattacharya (21) developed the protocol for clonal multiplication of *C. forskohlii* in one step with in 35-40 days by culturing stem tip explants in MS medium containing IAA (0.1mg/l) and kinetin (0.1mg/l) through direct multiplication at the rate of 12.5 shoots per explants. This enables easy, large scale (1224 plants by two succesive subcultures) multiplication of the endangered medicinal plant *C. forskohlii* with the high survival rate of the *in vitro* raised plants in *ex vitro* conditions and this seems to be a major advantage compared to the published protocol for commercial purposes. Bhattacharya *et al.,* (22) developed the most-suitable way of conservation and recovery of true-to-type elite *C. forskohlii* plant through the use of *in vitro* culture and encapsulation technique. Reddy *et al.,* (23) developed a protocol for a high frequency shoot organogenesis and mass propagation of *C. forskohlii* from leaf derived callus. Optimal callus was developed from mature leaves on MS medium supplemented with kinetin (0.5mg/l) alone. Shoots were regenerated from the callus on MS medium supplemented with kinetin (1mg/l) and NAA (0.1mg/l). The highest rate of shoot multiplication was achieved at the sixth subculture and more than 150 shoots were produced for the callus clump. Regenerated shootlets were rooted spontaneously on half strength MS medium devoid of growth regulators. The amount of forskolin in *in vitro* raised plants and wild plants were estimated and found that they produce comparable quantity of forskolin. Krombholz et al., (12) reported that transformed root cultures were established by infecting surface sterilized leaves with *Agrobacterium rhizogenes* strain 15834. The transformation was confirmed by mannopine detection and yielded 4.5 mg/l forskolin. Mukherjee *et al.,* (24) reported *Agrobacterium tumefecians* mediated tumour tissue and shooty tetratomas for potential forskolin detection in tumours callus (0.002%w/w dry weight), rhizogenic callus (0.011%w/w dry weight) and root cultures (0.014% w/w dry weight) but not in shooty tetratomas. Zhouli *et al.,* (25) reported the hairy root cultures of *C. forskohlii* from roots, stem and leaves by infecting with *A. rhizogenes* strain pRi 15834. Sasaki *et al.,* (26) reported high forskolin yielding hairy root clone by infection with the *A. rhizogenes* MAFF03-1724 strain. The highest forskolin yield (ca 1.6 mg/100 ml flask) was obtained at week 5.

Extraction

Srivastava *et al.,* (4) reported the extraction of powdered drug (1gm) by refluxing for 5 min on water bath with 5ml benzene, then filtered and filtrate was used for analysis, Mersinger (10) reported the extraction of cell material by harvesting, freeze-drying and extracting twice with dichloromethane for 30 min under reflux. The solvent was removed under reduced pressure and the residue was dissolved in methanol and subjected to analysis. Inamdar (27) reported the extraction of dried and finely powdered roots (1g) of *C. forskohlii* with benzene (3 x 50ml) at 70^0C for 2 hrs. The benzene extracts were filtered and concentrated in vacuum; TLC and HPLC assayed the residue for its content of forskolin.

Thin layer chromatography (TLC)

The standard solution was prepared by dissolving accurately weighed forskolin(1mg) in HPLC grade methanol(1ml).The extracts derived from calli were applied on precoated silica gel G plate (Merck) with the help of capillary tube along with the authentic sample. The chromatogram was run with solvent systems benzene: ethyl acetate (85:15) and toluene: ethyl acetate (85:15). The diterpenoids were detected by immersing the TLC plate into anisaldehyde-sulfuric acid (0.5ml of anisaldehyde was mixed with 10 ml of 98%v/v glacial acetic acid, followed by 85 ml of methanol and 5ml of concentrated sulphuric acid) reagent and heating for 3-4 min at 140^0 C (27).

High performance liquid chromatography (HPLC)

Mukheerjee *et al.,* (25) performed the HPLC analysis of the extracts and standard solutions, by injecting 10μl of the each standard solutions and extracted with Hamilton syringe, using C-18 column (Tracer Analitica, Nucleosil-100, 25cmX0.4cm) with Photodiode Array (SPD-M10A VP model) detector. The mobile phase (acetonitrile:water 65:35, pH 2.5 by adding orthophosphoric acid) which was pumped isocratically at a flow rate of 1ml/min, and forskolin was detected at 202nm. Sasaki *et al.,* (26) reported the HPLC method with minor changes. In this method the mobile phase was (acetonitrile:water 60:30, pH 2.5 by adding orthophosphoric acid) and forskolin was detected at 218 nm.

Conclusion

The literature indicates there is a need to undertake systematic study on cell suspension cultures and transformed root cultures to enhance the production of therapeutically important forskolin by employing a variety of elicitors, precursors and metabolic engineering of biosynthetic pathway of forskolin for higher productivity through cell and transformed root cultures before the commercialization of the techniques.

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PHCOG MAG: NEWS

Sri Sarthak Bhattacharya- Sr.Lecturer of Faculty of Pharmacy, Shri Ram Institute of Technology, Jabalpur (M.P.) was invited by Bangladesh Aushad Shilpa Samity (RAPI) to present a paper in Asian Pharma Expo 2005 held at DCFCC Center, Dhaka, Bangladesh between 15th to 18th February. 2005. Hon'ble Prime Minister. Govt. Of Bangladesh "Khlaleda Zia" inaugurated this biggest event - Gateway to South East Asia Pharma market in which more than 18 countries participated. Sri Bhattacharya presented his paper on Herbal Drug Technology entitled "RECENT ADVANCEMETNS OF HERBAL DRUG INTERMEDIATES AND THEIR IMPACT ON PHARMACEUTICAL CONCERNS" and was appreciated by various dignitaries. Sri Bhattacharya is a member of International society for ethnopharmacology, IPA, ICS and Phcog.net. He has published several research papers, review articles in national and international journals.

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