

A Review on Forskolin- A Labdane Diterpenoid

Mansi Patel¹, Tanvi Dodiya^{2,*}, Disha Prajapati²

¹Department of Quality Assurance, Parul Institute of Pharmacy & Research, Parul University, Vadodara, Gujarat, India

²Department of Pharmacognosy, Parul Institute of Pharmacy & Research, Parul University, Vadodara, Gujarat, India

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Abstract Forskolin, classified as a labdane-diterpenoid, exhibits the remarkable ability to stimulate vital cellular enzyme adenylyl cyclase, which triggers elevated levels of the critical secondary messenger molecule cyclic AMP across various cell types. This bioactive compound occurs naturally in the roots of *Coleus forskohlii*, a distinguished member of the mint family Lamiaceae. The plant is commonly known as Indian Coleus, makandi, and pashan bhedi. Various chemical and biological synthesis techniques aim to produce forskolin efficiently and sustainably. The extraction of forskolin is achieved through various techniques, including hydrotropic solubilization, ultrasonication, Soxhlet extraction, microwave-assisted solvent extraction, matrix solid-phase dispersion extraction, and heat reflux extraction using various solvents. Analytical methods for the estimation of forskolin encompass UV-visible spectroscopy, liquid chromatography (LC), high-performance liquid chromatography, thin-layer chromatography (TLC), and high-performance thin-layer chromatography. Additionally, forskolin has garnered attention in clinical and experimental studies, revealing diverse pharmacological effects. These include antiplatelet, bronchodilating, vasodilating, antihypertensive, anticancer, antidiabetic, and antiglaucoma properties. Further research on optimizing extraction methods, testing techniques, improving absorption by the body, as well as understanding the mechanisms of how forskolin acts in the body, could expand its medical uses. The versatile nature

of forskolin's pharmacology underscores its potential as a valuable compound in various therapeutic applications.

Keywords Forskolin, Labdane-diterpenoid, *Coleus forskohlii*, Lamiaceae

1. Introduction

Forskolin, a naturally occurring phytoconstituent that stimulates adenylyl cyclase, is used to increase cAMP, and trigger physiological reactions that depend on cAMP [1]. Forskolin (7 β -acetoxy-1 α ,6 β ,9 α -trihydroxy-8,13-epoxy-labd-14-en-11one) is a labdane-diterpenoid obtained from the roots of *Coleus Forskohlii* also called as *Plectranthus barbatus* Andrews [2]. The plant is a member of the Lamiaceae family. The roots of *Coleus forskohlii* are typically fibrous, thick, tuberous, 20cm long, 0.5-2.5cm wide, strongly aromatic, and golden brown in color [3]. *Coleus forskohlii* roots contain an essential oil that emits a spicy fragrance, so it is used as a flavourant in the food sector [4]. Forskolin shows various pharmacological activities such as antiglaucoma, antiobesity, bronchodilating, antithrombotic [3], antihypertensive, platelet aggregation inhibitory [5], and anticancer properties [6]. The structural representation of forskolin is shown in Figure 1.

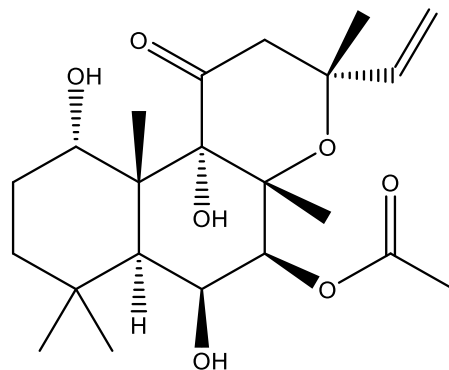


Figure 1. Structure of forskolin

2. Profile of Forskolin

Table 1. Physio-chemical profile of Forskolin [7-9]

Category	labdane-diterpenoid
Synonym	Coleonol, colforsin
Molecular weight	410.5 g/ml
Molecular Formula	C ₂₂ H ₃₄ O ₇
IUPAC name	[(3R,4aR,5S,6S,6aS,10S,10aR,10bS)-3-ethenyl-6,10,10b-trihydroxy-3,4a,7,7,10a-pentamethyl-1-oxo-5,6,6a,8,9,10-hexahydro-2H-benzo[f]chromen-5-yl] acetate
Melting Point	228 °C
Solubility	Soluble in anhydrous DMSO, 100% ethanol, or ethyl acetate.

3. Techniques for the Synthesis of Forskolin

Multiple methods for synthesizing forskolin, encompassing both chemical and biological approaches, have been reported. These techniques span diverse methodologies aiming to produce forskolin efficiently and sustainably.

3.1. Chemical Synthesis Technique

Researchers have achieved a significant breakthrough in forskolin synthesis, a 24-step process yielding hundreds of milligrams of this valuable natural product in a single run. This new method overcame key challenges by incorporating three innovative steps:

1. Strategic Allylic Transposition: This step efficiently rearranges the molecule's carbon skeleton, paving the way for subsequent ring formation.
2. Stepwise Isoxazole Ring Assembly: Building the complex isoxazole ring in a controlled, step-by-step manner ensures accuracy and avoids unwanted side reactions.
3. Citric Acid-Modified Upjohn Dihydroxylation: This clever modification tackles the challenge of

introducing hydroxyl groups onto a particularly stable double bond, enabling further functionalization.

This new synthesis holds exciting potential for developing novel forskolin analogs. Traditional methods often rely on tedious semisynthesis, but this direct approach opens doors to unexplored chemical modifications and potentially even more potent or specialized derivatives [10].

3.2. Biological Synthesis Technique

A research study was conducted to produce forskolin by constructing a *saccharomyces cerevisiae* cell factory. Initially, a strain was engineered to accumulate 13R-manoyl oxide (13R-MO), a critical precursor of forskolin, at a level of 145.8 mg/L. Subsequently, specific genes, including CfCYP76AH15, CfCYP76AH11, CfCYP76AH16, ATR1, and CfACT1-8, were integrated into this chassis strain, resulting in forskolin production reaching 76.25 µg/L. Identification of cytochrome P450 enzymes (P450s) as rate-limiting factors led to optimizations that increased the forskolin titer to 759.42 µg/L. Further metabolic engineering strategies, such as regulation of gene copy numbers, amplification of the endoplasmic reticulum area, and enhancement of cofactor metabolism, were implemented to boost forskolin

production. This resulted in forskolin yields of 21.47 mg/L in shake flasks and 79.33 mg/L in a 5 L bioreactor. These findings provided insights into the synthesis of natural terpenoids in *S. cerevisiae*, especially those with complex synthetic pathways involving multiple P450s [11].

4. Extraction Techniques of Forskolin

Various methods have been discovered for extracting forskolin from *Coleus forskohlii*, as shown in Table 2.

5. Analytical Methods for Determination of Forskolin

Thin-layer chromatography (TLC) was widely utilized as the predominant and versatile method for herbal analysis, before the advent of instrumental chromatography techniques such as gas chromatography and HPLC. TLC involves the distribution of a solute between two phases: a stationary phase, which operates through adsorption, and a liquid mobile phase. The adsorbent is a thin and even layer of finely powdered drug material applied to a sheet or plate made of glass, plastic, or metal. The separation is based on the partitioning alone or a combination of partitioning and

adsorption, depending on the specific support material and the use of different solvents [19]. Methods for estimation of forskolin by TLC are illustrated in Table 3.

High-performance liquid chromatography stands as a powerful and widely employed analytical technique for both qualitative and quantitative assessment of phytoconstituents in herbal extracts. This sophisticated method involves the separation of complex mixtures into individual components with high precision and efficiency. HPLC offers numerous advantages, making it a preferred method in analytical applications. These include rapidity, specificity, accuracy, precision, and ease of automation [20]. Various methods for estimation of forskolin by HPLC are mentioned in Table 4.

High-performance thin-layer chromatography represents an advanced and automated version of the traditional thin-layer chromatography technique. HPTLC is employed for identifying constituents, assessing impurities, and Quantifying bioactive elements. Its advantages over TLC include enhanced accuracy, reproducibility, and ease of result documentation [19]. Forskolin was estimated by high-performance thin-layer chromatography (HPTLC) by using Precoated Silica gel 60F₂₅₄ as the stationary phase. Various Mobile phases, detection wavelength, and R_f value are mentioned in Table 5.

Table 2. Extraction of Forskolin

Sr.no.	Extraction method	Solvents	% yield	References
1.	Three-phase partitioning	t-butanol	0.197 ± 0.003% w/w	[12]
2.	Ultrasound-assisted three-phase partitioning	t-butanol	0.51 ± 0.01 w/w	[12]
3.	Enzyme assisted three phase partitioning	t-butanol	0.53 ± 0.01 w/w	[12]
4.	Ionic liquid-based ultrasonic-assisted extraction	tetramethyl guanidium lactate (TMGL)	0.508 ± 0.006% w/w	[13]
5.	Microwave-Assisted Extraction	methanol	1.0% w/w	[14]
6.	Supercritical fluid extraction	methanol	0.608 ± 0.11% w/w	[15]
7.	charcoal column chromatography	toluene, n-hexane	0.097% w/w	[16]
8.	Hydrotropic Extraction	sodium cumene sulfonate	1.5% w/w	[17]
9.	Matrix Solid-phase Dispersion	ethyl acetate, methanol	0.057 ± 0.002 % w/w	[18]
10.	Soxhlet extraction	methanol	0.056 ± 0.001 % w/w	[18]
11.	Ultrasonic-assisted extraction	methanol	0.054 ± 0.002 % w/w	[18]
12.	Heat reflux extraction	methanol	0.051 ± 0.002 % w/w	[18]

Table 3. TLC of forskolin

Sr No.	Stationary phase	Mobile phase	Detection	Retardation factor (R _f)	References
1.	Pre-coated silica gel plates 60F ₂₅₄	benzene: ethyl acetate (85:15, v/v)	562nm		[21]
2.		benzene: methanol (9:1, v/v)		0.25 ± 0.02	[22]
3.		benzene: ethyl acetate (85:15, v/v)		0.21	[23]

Table 4. HPLC of forskolin

Sr No.	Stationary phase	Mobile phase	Detection	Retention time	References
1.	3.9 mm x 30cm prepacked with silica	hexane, ethyl acetate and dichloromethane (70:20:10, v/v/v)		15.38 min	[21]
2.	C18 column (250mm x 4.6mm, 5 µm)	water (A): acetonitrile (B) linear gradient	210nm	3.5min	[24]
3.	C18 column (250mm x 4.6mm, 5 µm)	acetonitrile: water (45:55, v/v)	220nm	12.376 min	[25]
4.	C18 column (250mm x 4.6mm, 5 µm)	acetonitrile: water (50:50, v/v)	218nm	15.70 min	[26]
5.	RP-18 column (150 × 4.6 mm, 5 µm)	acetonitrile: water (50:50, v/v)	210nm	6.9min	[22]
6.	HiQ Sil C18-HS column	methanol:acetonitrile:water (60:30:10, v/v/v)	220nm	4.199min	[27]
7.	C18 (150 mm × 10 mm, 5µm)	acetonitrile:water (68:32, v/v)	210nm	6.2min	[28]
8.	C18 (250mm x 4.6mm, 5 µm)	acetonitrile: Water (50:50, v/v)	220nm		[29]
9.	RP-18 column (250 X 46 mm, + precolumn 40 x 46 mm; 5 µm)	acetonitrile: water, adjusted to a pH of 2.5 by the addition of orthophosphoric acid.	202nm	11.4min	[30]
10.	C18 (4.6 x 150mm)	water: acetonitrile (60:40, v/v)	202nm	7.61min	[23]
11.	C18 column (4.6 mm x 250 mm)	acetonitrile: water (50:50, v/v)	220nm		[31]
12.	C18 (250 mm × 4 mm, 5 µm)	water: acetonitrile (50:50, v/v)	210nm	10.3min	[16]
13.	RP-18 column (150 x 4.6 mm, 5 m)	water: acetonitrile (50:50, v/v)	210nm	6.8min	[32]
14.	RP-18 column (150 x 4.6 mm, 5 m)	acetonitrile: water (80: 20, v/v)	210nm		[33]
15.	RP-18 (30 x 3.5 mm)	methanol: water (60:40, v/v)	210nm	7.83min	[34]
16.	C18 (250mm x 4.6mm, 5 µm)	acetonitrile: water (50:50, v/v)	210nm	7.0±0.2min	[35]

Table 5. HPTLC of forskolin

Sr No.	Mobile phase	Spraying Reagent	Detection	Retardation factor (R _f)	References
1.	ethylacetate:hexane: formic acid (7:2.9:0.1 v/v)	anisaldehyde-sulphuric acid	555nm	0.46±0.012	[36]
2.	benzene: ethyl acetate (85:15, v/v)	vanillin sulphuric acid	550 nm		[29]
3.	benzene: ethyl acetate (85:15, v/v)	anisaldehyde- sulphuric acid	315nm	0.33 ± 0.01	[37]
4.	toluene: ethyl acetate: methanol (90:30:0.5, v/v/v)	anisaldehyde- sulphuric acid	545nm	0.64 ± 0.02	[38]
5.	benzene: ethyl acetate (7.5:2.5, v/v)		200nm		[39]
6.	benzene and ethyl acetate (75:25, v/v)		200nm	0.49±0.01	[40]
7.	benzene:methanol (9:1, v/v)	anisaldehyde- sulphuric acid	545nm	0.25 ± 0.02	[41]
8.	toluene:methanol (18:1.5, v/v)	anisaldehyde- sulphuric acid	545nm	0.21 ± 0.02	[42]
9.	Toluene:ethyl acetate:methanol (9:3:0.05, v/v/v)	anisaldehyde- sulphuric acid	545nm	0.48	[43]
10.	toluene: methanol (18:1.5, v/v)	anisaldehyde- sulphuric acid	545nm	0.27±0.02	[44]

6. Pharmacology of Forskolin

Table 6. Pharmacological activities of forskolin

Pharmacological activity	Mechanism	Evidence	Inference of study	Reference
Antiglaucoma	Reduces aqueous humor inflow; may also increase outflow (needs further research)	- Forskolin nanocrystals show promise in pH and thermoreversible polymeric in situ gel-forming nanosuspensions. - Increases aqueous humor outflow in rabbit eyes	- Forskolin could be a potential treatment for glaucoma by lowering intraocular pressure. - More research is needed to confirm efficacy and understand the mechanism of action in humans	[45,46]
Bronchodilating	Relaxes airway smooth muscle, inhibits histamine and leukotriene release	- Prevents or reverses contractions of airway smooth muscles in vitro and in vivo. - Suppresses histamine and leukotriene production in vitro.	- Forskolin could be a prototype for new asthma drugs due to its strong bronchodilating activity	[47,48]
Antiplatelet	Inhibits platelet aggregation	- Total inhibition of aggregation in platelet-rich plasma at 10-20 μ M forskolin. - Hinders binding of platelet-activating factor (PAF) to its receptor	- Forskolin has the potential to reduce the risk of blood clots and cardiovascular events.	[49,50]
Antihypertensive	Relaxes vascular smooth muscle	- Lowers blood pressure in spontaneously hypertensive rats.	- Forskolin shows promise as an antihypertensive compound due to its effectiveness at small doses and minimal impact on the central nervous system.	[51]
Antiobesity	May increase lean body mass and reduce fat mass	- Changes in hip and waist circumference observed in obese patients treated with forskolin, suggesting fat mass reduction and bone mass increase. - Reductions in fat mass and body fat percentage, and increases in lean body mass and testosterone in overweight/obese men treated with forskolin.	- Forskolin may be beneficial for weight management and body composition improvement.	[52,53]
Antidiabetic	Lowers blood glucose levels, may also enhance testosterone and antioxidant function	- Reduces blood glucose levels in rats. - Enhances testosterone, antioxidant function, and sperm concentration in diabetic rats.	- Forskolin has potential as an antidiabetic drug, but more research is needed to understand its effects on humans.	[54,55]
Anticancer	Decreases tumor colonization; may inhibit proliferation and survival of cancer cells	- Decreases tumor colonization in the lungs of mice. - Low-dose combination of forskolin and rolipram inhibits proliferation and survival of colon cancer cells.	- Forskolin may have potential for cancer prevention and treatment, but further research is needed.	[56,57]

7. Conclusions

Forskolin is a labdane-diterpenoid that is obtained from the roots of *Coleus forskohlii*. It holds the potential to treat various diseases due to its versatile pharmacological properties. This review discusses several extraction methods of forskolin. Methanol is the most commonly used solvent in forskolin extraction. This review preliminary focuses on the chemical and biological synthesis techniques of forskolin and various pharmacological activities shown by forskolin such as antiglaucoma, antiobesity, bronchodilating, antithrombotic, antihypertensive, platelet aggregation inhibitory, and anticancer properties and various analytical methods including TLC, HPTLC, and HPLC. This review reports 10 HPTLC methods, 16 HPLC methods, and 3 TLC methods for the estimation of forskolin. As the outcome, it can be assumed that recent trends and existing analytical methods show that the data is useful for developing and validating analytical methods.

Conflict of Interest

The authors have no conflicts of interest regarding this investigation.

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