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FORSKOLIN. MORPHOLOGY. EXTRACTION. CHARACTERIZATION

BY

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Abstract. Coleus Forskolii is one of two hundreds species of Coleus from all over the world. These plants grow in tropical and subtropical regions in Asia, Africa and Australia. The main active compound from Coleus F. is a diterpene named Forskolin, found in the roots of the plant. The roots decoction and paste were used in the past in traditional treatment of many diseases. Nowdays Forskolin is used in the treatement of eczemas, psoriazis, cardiovascular disorders, glaucoma, hypertension etc. The isolation of forskolin is realized by the means of organic solvent extraction, followed by purrification through column chromatography, using activated charcoal as an absorbent. The forskolin was analyzed and characterized by TLC, HPTLC, HPLC, FTIR and electrospray ionization MS.

Key words: Coleus Forskolii, Forskolin, extraction and purification methods, physico-chemical characterization.

1. Introduction

Plants are the first medicines used by the mankind and hundreds of plant species are harvested all over the world for their medicinal properties. In spite of modern development of sophisticated pharmaceutical chemicals to treat illnesses, medicinal plants remain an important tool for treating various illnesses. In some regions, traditional medicines made from local plants are the only available and affordable source for treating various ailments. World Health Organization (2003) estimates that 80% of the world’s population depends on traditional medicine for their health needs. In many developed countries, traditional herbal remedies are making a comeback as alternatives to modern medicines.

The existence of traditional medicine depends on plant diversity and the related knowledge of their use as herbal medicine. Thus, among all this areas India is one of the twelve mega diversity hot spot regions of the world and one fifth of all plants found in India are used for medicinal purpose [1], [2]. This area is considered to be the place of origin of *C. forskohlii* [3], [4] which is a plant that grows wild in the subtropical temperate climates of India, Nepal, Burma, Sri Lanka and Thailand. Apparently, it has been distributed to Egypt, Arabia, Ethiopia, tropical East Africa and Brazil [5]. In India, the plant is found mostly on the dry and barren hills [6]. Latitudinal and altitudinal range for the occurrence of the species is between 8º and 31º N and 600 – 800 m, respectively.

Forskolin is extracted from tuber. The tubers are harvested at 75 to 85% moisture level on wet basis and stored at less than 12% moisture after drying. Sun drying required longer period than mechanical drying and recorded the lowest recovery of forskolin. Tubers mechanically dried at 40°C with tuber slice thickness of 0.5 cm and packed in polyethylene lined gunny bag retained the highest amount of forskolin [7].

Forskolin extract has been shown to exhibit several medicinal and cosmetic actions. One of them is to lower elevated blood pressure in different animal species through a vasodilatory effect and had a positive inotropic action on the heart muscle. Forskolin augments myocardial contractility without affecting myocardial oxygen consumption. Forskolin relaxed contracted airways *in vitro* and prevented bronchospasm *in vivo*. In addition, forskolin reduced Schultz-Dale responses of both trachea and parenchyma and protected sensitized guinea pigs during antigen challenge [3]. Forskolin was shown to inhibit immunological histamine release from chopped sensitized lung. It was demonstrated that forskolin inhibited both mediator release and smooth-muscle contraction in the airways. Forskolin increased cyclic AMP levels in neutrophils and macrophages, and was associated with a decrease in the superoxide burst and prostaglandin synthesis. Topical application of forskolin lowered the intraocular pressure (IOP) in rabbits, monkeys and healthy human volunteers. The reduction of IOP was associated with a reduction in aqueous inflow and no change in outflow facility, indicating a potential for forskolin as a therapeutic agent in the treatment of glaucoma [1], [3].

2. Experimental Methods

Forskolin main active ingredients are: diterpenes - Coleonol-D, E, F, coleol, coleonone, barbatusol, plectrin, were isolated from the roots *Coleus forskohlii.* Coleonol and forskolin are stereoisomers. Crocetin dialdehyde, naphthopyrone and 6β-hydroxycarnosol were also isolated from the roots. The molecular structura for forskolin is presented in Fig. 1:

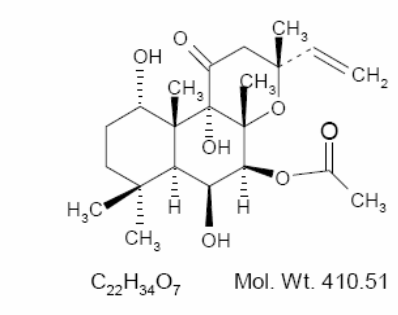


Fig. 1 − Molecular structura for Forskolin.

(7β-Acetoxy-8,13,-epoxy-1α,6β,9α-trihydroxy labd-14-en-11-one)

Forskolin, the major diterpenoid isolated from the Indian herb, *Coleus forskohlii* is a promising drug for the treatment of glaucoma, congestive cardiomyopathy and asthma because of its unique adenylate cyclase stimulant activity. It has been shown to be a hypotensive agent with spasmolytic, cardiotonic & platelet aggregation inhibitory activity [8].

For the physico-chemical characterisation of coleus, the organic extract from the roots of the plant has been used, obtained by extraction with a solvent selected from the group consisting of ethyl acetate, methanol, ethanol and dichloromethane.

Generally organic solvents such as aromatic hydrocarbons, aliphatic or aromatic halogenated ethers of dialkyl ketones, alkanols, carboxylic acids and esters or other solvents such as dimethyl formamide, dioxan, tetrahydrofuran and diethylsulfoxyde can be used. Among the above solvents, preferred is toluene, xylene, methylene chloride, chloroform, ethyl acetate, methanol or ethanol.

The report of the plant material to the extracting agent is not critical (generally being between 1:5 and 1:20 parts per weight), preferably 1:10. The extraction is realised at temperatures between room temperature and the boiling point of the solvent used for extraction. An advantageous technique of extraction is the Soxhlet extraction.

It may be advantageous in some cases when is necessary to evaporate the solvent, for example by freezing it and resume the crude extract for purification. In the context of the present aims, the alcoholic extraction is particularly interesting, especially at the end of procedure for obtaining extract due to the usually low toxicity of alcohol. Another particularly advantageous solvent is ethyl acetate [9].

Specific variants of the process are described in literature. In general, the concentration of extracts used for the preparation of a cosmetic or pharmaceutical composition, expressed in dry weight is between 0.001% and 2%, preferably from 0.01% and 0.5% by weight, based on the total weight of the composition [1], [8].

The forskolin was then purified by the means of column chromatography. Thus Forskohlii roots extract is passed through a column using activated charcoal as adsorbent. The elution was carried out under reduced pressure to speed up the process. Activated charcoal acted as a reversed phase adsorbent and allowed elution of forskolin without much impurities. The residue, obtained from elute was purified and crystallized using different solvent mixtures to obtain pure forskolin. The forskolin isolated was analyzed

and characterized by UV, IR, RP-HPLC, electrospray ionization MS, (1)H NMR and (13)C NMR. The yield was 0.097% w/w (RSD 5.6%). The purity was 96.9% w/w (RSD 0.3%) as determined by RP-HPLC. The present method enables researchers to produce high-purity forskolin in their labs by using common chemicals [2], [3].

2.1. Analytical Specifications for the Coleus Forskohlii – Extract [2]

Item : *Coleus forskohlii* extract (≥ 20% Forskolin)
Description : Brown to light brown powder, with characteristic odour and taste.
Identification :
1) Comparison with the standard TLC profile.
2) Positive for forskolin
TESTS LIMITS PROTOCOL
Physico-chemical analysis
Loss on drying (Moisture) < 5% w/w As per I.P / B.P
Acid insoluble ash < 1% w/w As per I.P / B.P
Heavy metal analysis
Lead < 10 ppm By A.A.S.
Cadmium < 2 ppm By A.A.S.
Arsenic < 2 ppm As per U.S.P
Microbiological tests
Total Viable Aerobic Count < 10^4 cfu g-1 As per I.P / B.P
Total Fungal count < 10^2 cfu g-1 As per I.P / B.P
Total Enterobacteriaceae < 10^2 cfu g-1 As per B.P.
E. Coli Absent As per I.P / B.P
Salmonella typhi Absent As per I.P / B.P
S. aureus Absent As per I.P / B.P
Solvent residues
Organic solvents < 200 ppm By GC
Mycotoxin analysis
Aflatoxins (Total B1,B2,G1,G2) < 5 ppb As per A.O.A.C
Phytochemical analysis
Forskolin ≥ 20.0% w/w HPLC(High Performance Liquid Chromatography)/
HPTLC (High Performance Thin Layer Chromatography)

2.2. Identification of Crude Drug by TLC [3]

Sample detail: *Coleus forskohlii* crude drug
Adsorbent: Precoated silicagel (Al - Sheet)
Mobile Phase: Benzene: Ethyl Acetate 85:15
Sample preparation: Known amount of *Coleus forskohlii* root was extracted in acetonitrile. The chloroform layer was concentrated and applied on the TLC plate.
Solvent front run upto: 9 cm^2
Detection: Anisaldihyde Sulphuric acid reagent (Fig. 2), Vanillin sulphuric acid (Fig. 3).

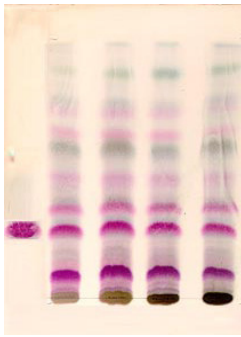


Fig. 2 − TLC for standard Forskolin.

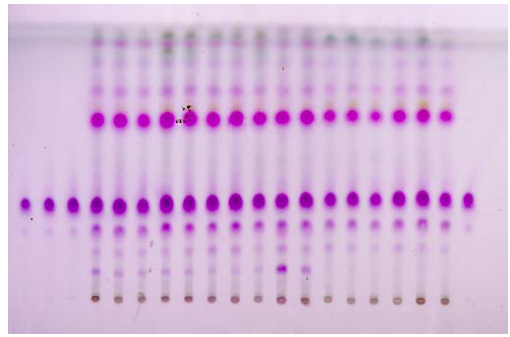


Fig. 3 − TLC for various samples of Coleus forskolii root extract.

2.3. Estimation of Forskolin in Coleus Corskohlii Extract [3]

There are two methods for the estimation of Forskolin content in the extract. Method 1 - HPLC & Method 2 - HPTLC. Both methods are accurate and reproducible.

a) Method I (by HPLC):

Summary: Forskolin in *Coleus forskohlii extract* is separated from its related compounds and other impurities by High Performance Liquid Chromatography (HPLC). The separated compounds are identified with the retention time in comparison with the pure compound and quantified with the corresponding peak area. The results were found to be accurate and reproducible.

ANALYSIS:

Chromatographic system**:** High Performance Liquid Chromatographic system equipped with LC8A pump, SPD-M 10A*vp* Photo Array Detector in combination with Class LC 10A software.

Chromatographic conditions**:**

Mobile phase**:** Acetonitrile : Water 50:50

Column**:** ODS (Octadecyl silane) C18, 5 µ size, 250 x 4.6 mm (Supelco)

Detector**:** SPD-M 10A*vp* Photo Array Detector

Wave length: 220 nm

Flow rate: 1.6 mL/min

Inject volume: 20 µL

Standard preparation: Weigh accurately 10 mg of Forskolin in a 25 mL volumetric flask. Dissolve in 15 mL of Acetonitrile and make up to 25 mL with Acetonitrile.

Sample preparation: Weigh accurately 250 mg of sample (equivalent to 20 mg of Forskolin). Dissolve in 25 mL of Acetonitrile with the aid of heat, filter and make up to 100 mL with Acetonitrile in a volumetric flask.

Procedure: Set the instrument as per the chromatographic condition as prescribed above. By means of suitable syringe inject 10 µL of Standard preparation and record the chromatogram. Inject another 4 times and calculate the mean area and the RSD. The RSD should not be more than 2%. Inject 10 µL of sample preparation and record the chromatogram. Calculate the percentage of forskolin content from the peak areas.

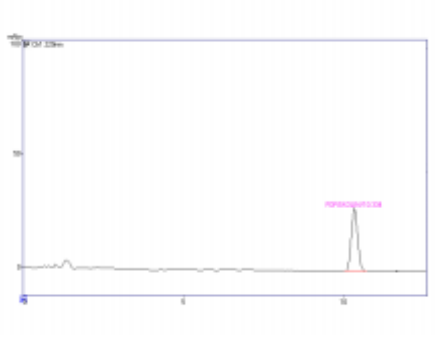


Fig. 4 − HPLC Spectra for standard Forskolin.

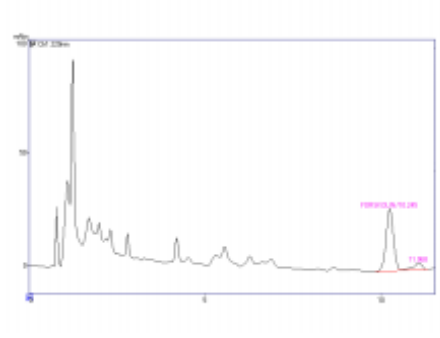


Fig. 5 − HPLC Spectra for samples extracts.

b) Method II (by HPTLC):

Summary: Forskolin in *Coleus forskohlii* extract is separated from its related compounds by Thin Layer Chromatography (TLC). The separated compounds are identified with the RF values in comparison with the pure compound and quantified with the corresponding peak area. The results were found to be accurate and reproducible.

ANALYSIS:

Chromatographic system: High Performance Thin Layer Chromatographic system equipped with Densitometer (Shimadzu flying spot densitometer CS9301), Applicator (CAMAG-Linomat IV) Developing chamber (CAMAG Twin trough 10 x 10 cm) were used.

Mobile phase: Benzene: Ethyl Acetate 85:15

Adsorbent: Silica gel 60 F254 (Merck Al. Sheets - 1.05554)

Solvent front run up to: 9 cm^2

Detection: 550 nm after spraying with Vanillin sulphuric acid

Standard preparation: Weigh accurately 12.5 mg of Forskolin Ref. Standard to a 25 mL volumetric flask. Dissolve with 50 mL of chloroform and make up to 100 mL with chloroform, from this pipette 5 mL into a 100 mL volumetric flask and make up the volume with chloroform.

Sample preparation: Weigh the extract accurately equivalent to 50 mg of Forskolin into a 100 mL volumetric flask. Dissolve with the aid of chloroform and make up to 100 mL with chloroform, from this pipette 5 mL into a 100 mL volumetric flask and make up the volume with chloroform.

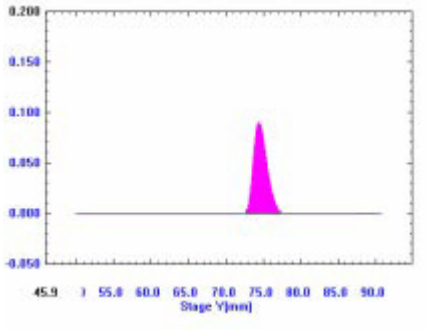


Fig. 6

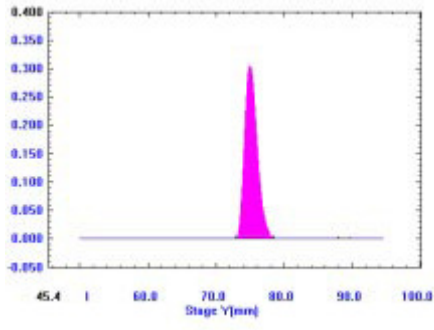


Fig. 7

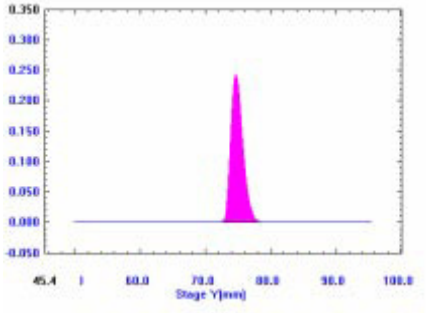


Fig. 8

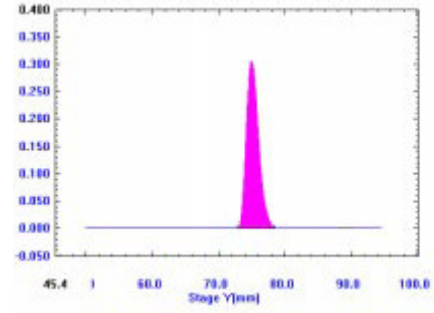


Fig. 9

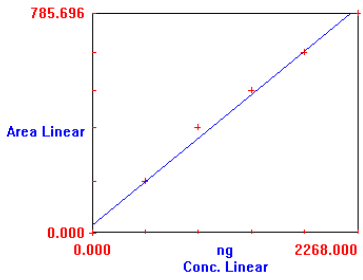


Fig. 10 − Etalon Curve for sample extracts.

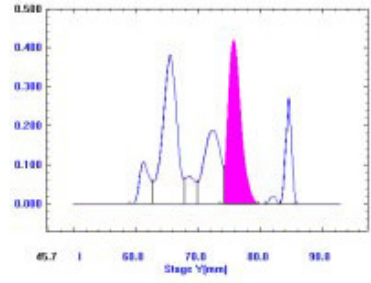


Fig. 11 − HPTLC spectra for sample extracts.

Procedure: Set the applicator as per the condition prescribed by the manufacturer. By means of suitable syringe apply 5, 10, 15, 20 µL of standard preparation in 4 different tracks at 1cm height and 4 mm-band width with 7 mm distance between the tracks. Apply 5 – 10 µL of sample preparation in another two tracks. Allow the plate to develop in the above mentioned solvent system up to 9 cm and remove. Dry it in a current of air and spray 10% Vanillin sulphuric acid. Heat the plate at 105ºC for 10 min. Record the chromatograms

of standard and sample. Make a calibration curve with the concentration on the x-axis and area on y-axis and calculate the content of forskolin in the extract by extrapolation.

2.4. Analitical Aspects of Forskolin Tests Results [3]

Description White crystalline powder

Solubility: Soluble in Chloroform, benzene, methanol, dichloromethane, sparingly soluble in Pet ether.

Identification (by Spectroscopy & Chromatography)

FTIR Identical with reference standard

MASS SPECTRUM Characteristic of Forskolin

TLC & HPTLC Gives single spot

HPLC Retention time matches with the reference standard

Loss on drying at 105°C < 1%w/w

Melting range2 230 - 232ºC

Purity (Forskolin content) > 97%w/w.

The analysis with Mass Spectroscopy has been made by using an JEOL - JMX-DX303 instrument attached with a JMA-DA5000 Data system.

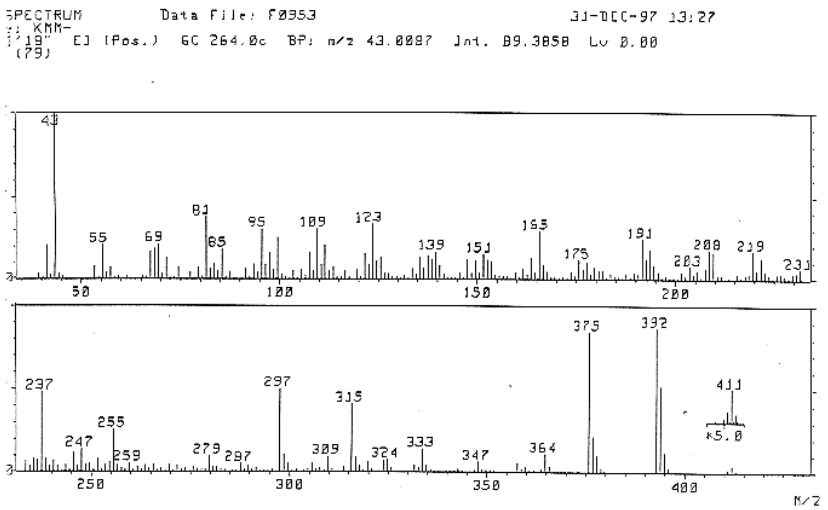


Fig. 12 − Mass spectrum for forskolin.

High resolution MS, showed M+ ion at 411 (nearest whole number of Forskolin molecular weight 410.5) corresponding to the molecular formula C22H34O7. Major fragment peaks at m/e 392, 375, 364 etc.

The FTIR spectra have been recorded in KBr pellet, using a: Fourier Transmission Infrared Spectra Make: SHIMADZU.

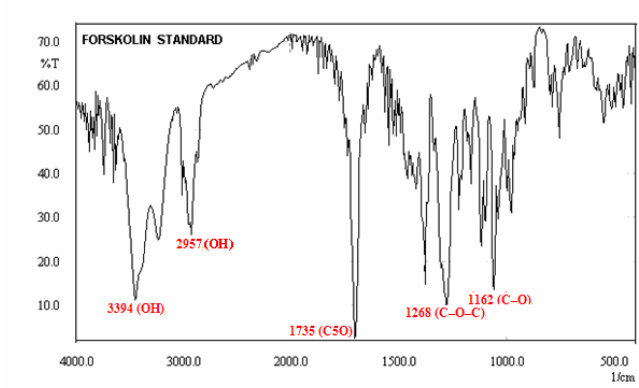
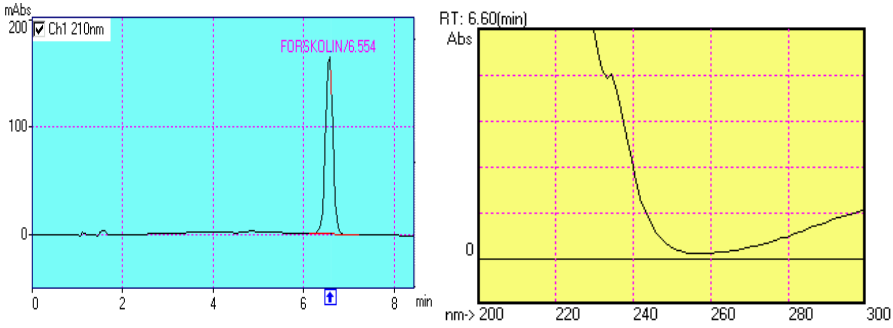


Fig. 13 − FTIR Spectra for Forskolin.



CROMATOGRAM PDA SPECTRUM

Fig. 14 − HPLC chromatogram for Forkolin.

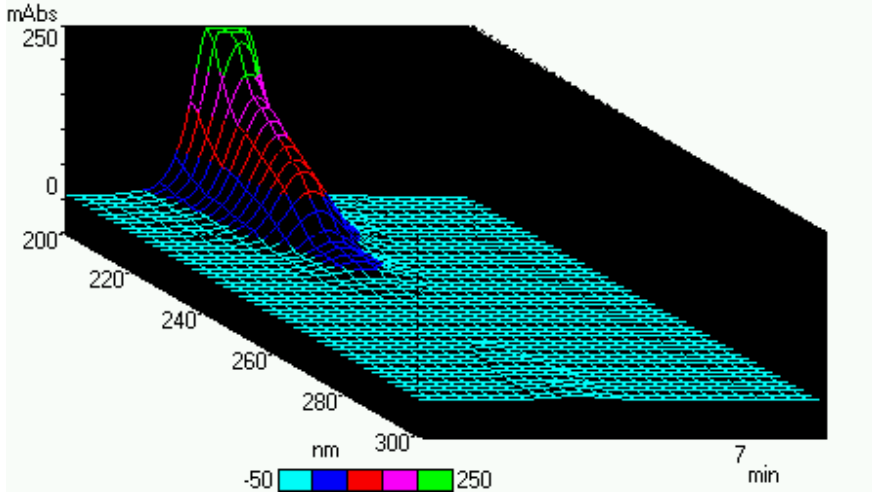


Fig. 15 − Forskolin HPLC 3D- VIEW.

** Peak Report ** FORSSTD1. K07 98/01/08 20:01:16

PKNO	ChNO	TIME	AREA	HEIGHT	CONC	MK	PLATE #	TAILING FACTOR	NAME
1	1	6.554	1882933	166853	181.2565	*	7365	1.03	FORSKOLIN
TOTAL			1882933	166853	181.2565				

3. Conclusions

It is well known the therapeutic effect that various plant exhibit, thus that even after thousand of years of using them the modern society still consider them al alternative medicine, sometime even more effective than synthetised drugs.

Among all those plant, the newest trend is forskolin extract, obtained from Coleus Forskolii tubers. For extract various organic solvents could be used, the most recomended being ethanol and ethy acetate. Also for medical purposes the forskolii oil could be used, obtained by direct pressingof tubers.

The obtained extracts could then be purified by the means of column chromatography, using active charcoal as sorbent.

The obtained forskolin have been characterized by using physicol-chemical methods, such as: TLC, HPTLC, HPLC and MS.

Our next aim is to find out if the specias of Coleus from our country contain forskolin and if they have, than we will try to extract it and to characteriyed it in order to compaire it with the comercial available products.

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FORSKOLINA. MORFOLOGIE. EXTRACȚIE. CARACTERIZARE

(Rezumat)

Coleus Forskolii este una din cele 200 de specii de Coleus din lume. Această plantă poate fi găsită în zonele tropicale şi subtropicale din Asia, Africa, şi Australia. Principalul component activ din Coleus Forskolii este diterpena numită Forskolina, gasită în rădăcina plantei. Decoctul sau pasta din rădăcina de Coleus era folosită în trecut în tratamentul tradițional al diferitelor afecțiuni. În present Forskolina este utilizată în tratamentul eczemelor, psoriazisului, afecțiunilor cardiovasculare, hipertensiune, glaucom etc. Extracția Forskolinei din rădăcina de Coleus se face cu ajutorul solvenților organici, urmată de o purificare prin cromatografie pe coloană, utilizând cărbunele activ drept sorbent. Forskolina a fost analizată şi caracterizată cu ajutorul următoarelor metode fizico-chimice: TLC, HPTLC, HPLC, FTIR şi electrospray ionization MS.