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ISOLATION OF PHYTO CONSTITUENTS FROM THE ROOTS OF COLEUS FORSKOHLII BY COLUMN AND FLASH CHROMATOGRAPHIC METHOD

Anna Pratima Nikalje^{1,*}, Shradha Baheti¹, Smrutigandha Kamble, Subur Khan², Jayprakash Sangshetti³

¹ Department of Pharmaceutical Chemistry, Y.B. Chavan College of Pharmacy, ²Department of Pharmacognosy,

³Department of Quality Assurance, Dr. Rafiq Zakaria Campus, Rauza Bagh, P.B. No. 33, Aurangabad-431001, Maharashtra, India.

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Abstract:

The herb, Coleus Forskohlii Briq. belongs to family 'labiatae' and is well known for its medicinal use throughout the plains of India. The roots of this plant are therapeutically active due to the presence of the diterpenoid, Forskolin. In the present research work, flash chromatographic method was developed for the isolation of three oleic acids from petroleum ether extract and 1-fluoroforskolin along with four other phyto constituents from methanolic extract of the roots of coleus forskohlii. Three compounds were isolated and identified from the petroleum ether extract of roots of C. forskohlii: 1. n-Hexadecanoic acid, 2. Cis-13-octadecenoic acid, 3. Cis-vaccinic acid and five compounds from methanolic extract of roots: 4. 1-Fluoroforskolin, 5.Stigmasterol, 6. Sclaral, 7. 2-dodecen-yl (-) succinic anhydride, 8. (3R, 4aS, 10aR)-Dodecahydro-3, 4a, 7, 7, 10a-pentamethyl-3-vinyl-1H-benzo[f] chromene. The characterization and confirmation of isolated compounds was done by GC-MS and ¹HNMR spectral studies.

Keywords: Coleus forskohlii, Labiatae, Flash chromatography, Forskolin, oleic acid.

Chemical compounds studied in this article:

n-Hexadecanoic acid (Pub Chem CID: 985); Cis-13-octadecenoic acid (Pub Chem CID: 445639); Cis-vaccinic acid (Pub Chem CID: 5282761); 1-Fluoroforskolin (Pub Chem CID: 540314); Stigma sterol (Pub Chem CID: 5280794); Sclaral (Pub Chem CID 537618); 2-dodecen-yl (-) succinic anhydride (Pub Chem CI*D: 5362708)

Corresponding author:

Anna Pratima Nikalje,

Department of Pharmaceutical Chemistry,

Y.B. Chavan College of Pharmacy,

Harsool, Dr Rafiq Zakaria Marg, Rauza Baug, N 2,

Cidco, Aurangabad, Maharashtra 431003.

Email: annapratimanikalje@gmail.com,

Phone no: +91 9168929111

QR code

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1. INTRODUCTION:

Medicinal plants play an important role in the development of potent therapeutic agents. Plant based drugs provide outstanding contribution to modern therapeutics [1]. The medicinal plant, *Coleus forskohlii* briq which belongs to family Labiatae, is a perennial herb growing throughout the plains of India and in the subtropical Himalayan regions, Nepal, Sri Lanka and Thailand. Coleus species are found as herbs, sub shrubs or shrubs. It grows up to the height of 30 cm to 62 cm.



Fig. 1: Coleus forskohlii plant

Since ancient times it is used for medical treatment in Hindu and Ayurvedic traditional medicine [2]. As shown in Fig.1, they are often succulent with opposite leaves which are usually pubescent, narrowed into petioles. Members of the genus Coleus have square stems, branched, the nodes are often hairy. Inflorescence is terminal or in the upper leaf axils and flowers are arranged in compact cymose clusters. The flowers are very showy bluish to pale lavender colored. The ovary is four parted. The racemes are perfect and the calyx is fined toothed and deflexed in the front. Corolla of the plant is pale blue in color and is bilabiate; the lower lobes are elongated and concave. The tubers or the roots are thick, tuberous, fasciculate, up to 20 cm long and 0.5-2.5 cm thick Conical, fusiform, straight and strongly aromatic. Rootstock is typically golden brown, thick, fibrous, and radials spreading. The plant gets cross pollinated by means of wind or insects. The leaves and tubers have quite different odors, the latter being reminiscent of but quite different from ginger [3]. The entire plant is aromatic (whether fresh or dried).

The genus Coleus consists of 150 species. Some of the species are *C. forskohlii*, *C. Spicatus*, *C. amboinicus*, *C. Malabaricus*, *C.barbatus*, *C.aromaticus*, *C. blumei* [4]. The root portion of the plant has been traditionally used for medicinal purpose and contains the active constituent, forskolin which is the primary constituent of clinical interest in *C. forskohlii* [5]. Forskolin was named after the Finnish botanist, Forskal. Historically, it has been used to treat hypertension, congestive heart failure, eczema, colic, respiratory disorders, painful urination, insomnia and convulsions. Clinical studies of the plant and the forskolin constituent support these

traditional uses and also indicate that it may have therapeutic benefit in asthma, angina, psoriasis and prevention of cancer metastases. Other minor di terpenoids such as deacetyl forskolin, 9-deoxyforskolin, 1, 9-deoxyforskolin, 1, 9-dideoxy-7-deacetylforskolin and four other di terpenoids, have been reported to be present in the roots of *C. forskohlii* [6].

From the literature survey, it was observed that, in this traditional Ayurvedic systems of medicine, many activity studies were carried out for the various parts and different extracts for Coleus forskohlii and it was proved that the plant is useful in the treatment of heart diseases and hypertension, abdominal respiratory disorder, insomnia, convulsions, asthma, bronchitis, intestinal disorders, burning sensation, constipation, epilepsy and angina. The roots are also used in treatment of worms and to alleviate burning in festering boils. When mixed with mustard oil, the root extract is applied to treat eczema and skin infections. The plant is also used for veterinary purposes. Essential oil has potential use in food flavoring industry and can be used as an antimicrobial agent. Recently, this plant has tremendous use in fat loss and weight management.

However, the phyto constituents are not well characterized and are not well established for their activities, therefore, there is a need for more elaborate and detail study on the characterization of the phyto constituents of Coleus forskohlii. The objective of the present research work is to develop a flash chromatographic method for isolation of new phyto constituents from roots of C. forskohlii. The extracts were subjected to column chromatography and flash chromatography for isolation of phyto constituents. These isolated fractions were characterised by qualitative GC-MS and H1NMR analysis. Thus, we report herein, the isolation and identification of three compounds from the petroleum ether extract of roots of C. forskohlii: 1. n-Hexadecanoic acid, 2. Cis-13-Octadecenoic acid, 3. Cis- vaccinic acid and five compounds from methanolic extract of roots: 4. 1-Fluoroforskolin, 5.Stigmasterol, 6. Sclaral, 7. 2dodecen-yl (-) succinic anhydride, 8. (3R, 4aS, 10aR)-Dodecahydro-3, 4a, 7, 7, 10a-pentamethyl-3-vinyl-1H-benzo[f] chromene. This is the first report on the isolation of these compounds from the roots of C. forskohlii.

2. MATERIALS AND METHODS:

The Silica Gel for column and flash chromatography (230-240 #) was obtained from Spectro Chem Pvt. Ltd. Mumbai. The Silica Gel 60 F254 pre-coated plates were purchased from Merck Specialties Pvt. Ltd. Mumbai. Petroleum ether (EP), methanol (EP), toluene (EP) was purchased from Dipa Chemical Industries, Aurangabad. Chloroform (EP) and glacial acetic acid (EP) was purchased from Fisher Scientific Chemicals, Ahmadabad. Aldehydes such as anisaldehyde and vanillin were obtained from Fine Chem. Industries, Mumbai.

Table I: Observation table for Extractive Value

Sr. No.	Extracts	Nature of Extract	Colour of Extract	Weight (g)	% Yield w/w
1	Pet-ether (60-80°C)	Solid sticky	Dark yellow	50	25
2	Methanol	Semisolid viscous	Brownish black	45	22.5

Table II: Observation table of preliminary tests

Test	Saponins	Tannins	Alkaloids	Flavanoids	Terpenes	Glycoside
Petroleum Ether Extract	-	+	+	-	+	-
Methanol Extract	+	-	+	+	+	-

(+ indicated presence and - indicated the absence of the respective natural product)

2.1 Collection and drying of plant material

The roots of *C. forskohlii* were purchased from local market of Veraval, dist. Junagadh, Gujarat, in the month of May. Authenticity of the plant materials was confirmed by the Professor from Botany department

2.2 Preparation of Extracts

200gms of the air-dried and coarsely powdered roots of coleus forskohlii were subjected to successive hot continuous extraction (soxhlet) with petroleum ether and methanol for 24 hours. Each time before extracting with next solvent the powdered material was dried at room temperature. After the effective extraction, solvents were concentrated using rotary flash evaporator and the extract obtained with each solvent was weighed. Their percentage yields obtained are shown in the Table I. The petroleum ether extract was filtered and evaporated under reduced pressure using rotary evaporator. The extracted plant material was then air-dried, repacked in the soxhlet apparatus and exhaustively extracted with chloroform for 24 hours. The extracted plant material was then air-dried, repacked in the soxhlet apparatus and exhaustively extracted with methanol (98.8%) for 15 hours. The methanolic extract was filtered and evaporated under reduced pressure using rotary evaporator. All the isolated plant extracts were characterized for the presence of various chemical constituents by preliminary qualitative tests which revealed the presence of saponins, tannins, alkaloids, flavanoids and diterpenoids.

2.3 Preliminary Qualitative Tests

Characterization of all plant extract has been done for various chemical constituents by subjecting the root extract to various preliminary qualitative tests and the results are as indicated in Table II. of Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. The roots were dried under shade and protected from sunlight for 20 days. Further drying in hot air oven at was carried out at 40°C for 7 days. Dried material was powdered using mixer grinder.

Extraction and isolation of the chemical constituents from the roots of *C. forskohlii*

2.4 Thin Layer Chromatography

The objective of the thin layer chromatographic study was to characterize the extract with respect to the number of separable phyto constituents. Activation of TLC plate was done by heating in oven for 30 min at 105 °C.

Petroleum Ether Extract: The petroleum ether extract was unsaponified and the extract was applied on TLC plates. The sample was applied by dipping the capillary into the extract sample solution and applying the spot on to the thin layer plate at a point about 2 cm from the bottom. Spot was air dried [7]. The glass chamber for TLC was saturated with mobile phase of petroleum ether: ethyl acetate (9:1) for 30 min. TLC plates were kept in chamber and the mobile phase was allowed to run up to 12-15 cm. Plates were removed, dried and upon spraying with visualizing agent vanillin- sulphuric acid reagent the TLC plate displayed 6 different spots.

Methanolic extract: The sample was prepared by mixing the extract in methanol. The procedure followed was same as described above for petroleum ether extract, except that the mobile phase used is chloroform alone. The TLC plates were dried and upon spraying with visualizing agent vanillinsulphuric acid reagent the TLC plate displayed 06 different spots.

2.5 Column Chromatography

Petroleum extract: The separation of the extract constituents was done by column chromatography. The 75 cm long, clean and dried glass column was used. The silica gel for column chromatography (230-240 #) was activated at 110°C. The silica was mixed with mobile phase and poured into the column preventing any air bubble to trap in. The silica gel was then allowed to stabilize in the column. The sample was prepared by vigorously mixing the petroleum ether extract with silica gel solvent, the solvent was evaporated to form a free flowing material. This sample was charged into the column. The sample was eluted in gradient manner [8]. Initially petroleum ether was used. Then polarity of mobile phase was increased by adding ethyl acetate in increasing order such as petroleum ether: ethyl acetate composition was first taken as 9:1 then 8:2 and so on. Total 30 fractions, each containing 10 ml, were collected. The sample was recovered by distillation. Fractions giving identical spots on TLC were mixed as one fraction. However, fractions showing inseparable mixture of several compounds were rejected. Purity of dried separated samples was again checked by TLC method by UV (365nm) light and by using visualizing agent vanillin- sulphuric acid solution.

Methanolic extract: Similar procedure was followed for methanolic extract by using gradient manner. Initially chloroform was used. Then polarity of mobile phase was increased by adding methanol in increasing order like chloroform: methanol in the ratio 9.5: 0.5 then 9:1 and so on.

2.6 Flash Chromatography

The fractions obtained from column chromatography were subjected to TLC and the fractions giving good separation were subjected to flash chromatography (YMC, Japan). The liquid fractions selected were allowed to dry by evaporating the solvent till semisolid extract was obtained. The cartridge used was Sepa flash-silica gel, of capacity 4gm and max operating press-200 psi. The silica gel for flash chromatography (230-240 #) was activated at 110°C. The sample was prepared by mixing the semisolid extract with silica powder in mortar and pestle, solvent was evaporated till free flowing powder was obtained. The sample was loaded in the cartridge. Cartridge was attached to flash column [9]. By using gradient mode two mobile phases were used with increasing polarity i.e. petroleum ether (non-polar) and ethyl acetate (polar) for petroleum ether extract and similarly, two mobile phases i.e. chloroform (nonpolar) and methanol (polar) were used for methanolic extract. The entries were made with different mobile phase ratios into the software to obtain a linear line. Linear line indicates the increasing composition of mobile phase from non-polar to polar mobile phase. Then column was allowed to equilibrate with mobile phase. After completion of equilibrium, run mode was selected. The duration of extraction process was 40 min at a flow rate of 5 ml/min. The collecting wavelength (collecting wavelength was selected by absorbance of the extract on

spectrophotometer) was 284 nm and monitor wavelength was 295 nm. The flash chromatographic extracts were collected in test tubes. The correlation of peaks of absorbance was made with extracts of test tubes and then they were tested for purity by TLC.

3. RESULTS AND DISCUSSION:

In the present study, shade dried roots of *Coleus forskohlii* briq having bioactiveqal phyto constituents, were isolated and studied for the presence of new phyto constituents by spectroscopic analysis. Literature survey reveals various pharmacological activities of the plant as in hypertension, respiratory disorder, insomnia, convulsions, asthma, bronchitis, intestinal disorders.

The dried root powder was studied for preliminary phytochemical analysis and subjected to soxhlet extraction (continuous hot extraction) using solvents of increasing polarity i.e. petroleum ether and methanol. Petroleum ether extract was further subjected to TLC, column chromatography and flash chromatographic separation and the fractions were characterised by GC-MS analysis. The mass spectra studies and their fragmentation pattern shows three different compounds respectively, as follows:

Compound 1: n-Hexadecanoic acid Compound 2: Cis-13-octadecenoic acid

Compound 3: Cis-vaccinic acid

These are the common types of oleic acid. Oleic acid is a fatty acid that occurs naturally in various animal and vegetable fats and oils. It is odourless, colourless oil; although commercial samples may be yellowish. Cis-9-octadecenoic acid is obtained from olive oil and from quite all seed oils.

Oleic acid is a common monounsaturated fat in human diet. Monounsaturated fat consumption has been associated with decreased low-density lipoprotein (LDL) cholesterol, and possibly increased high-density lipoprotein (HDL) cholesterol. Oleic acid may hinder the progression of adreno leuko dystrophy (ALD), a fatal disease that affects the brain and adrenal glands. Oleic acid may be responsible for the hypotensive (blood pressure reducing) effects of olive oil [10].

Similarly, the methanolic extract was further processed for purification by TLC, column chromatography and flash chromatography. These purified fractions were characterized by GC-MS and ¹HNMR spectroscopic analysis and from the mass spectral studies and their fragmentation pattern along with ¹HNMR spectral interpretation, these compounds were confirmed as:

Compound 4: 1-Fluoroforskolin

Compound 5: Stigma sterol

Compound 6: Sclaral (3a, 6, 6, 9a-Tetramethyldodecahydronaphtho [2, 1-b] furan-2-ol)

Compound 7: 2-dodecen-yl (-) succinic anhydride

Compound 8: (3R, 4aS, 10aR)-dodecahydro-3, 4a, 7, 7, 10a-pentamethyl-3-vinyl-1H benzo[f] chromene

1-Fluoroforskolin is the halogen derivative of Forskolin which is the main chemical constituent of Coleus forskohlii and it is responsible for all the

therapeutic activities of the plant. It lowers blood pressure and intra-ocular pressure, inhibits platelet aggregation, promotes vasodilatation, thyroid hormone secretion and stimulates lipolysis in fat cells. It also has a positive inotropic action on cardiac tissue via increased cAMP levels.

Stigmasterol (also known as Wulzen anti-stiffness factor) is one of a group of plant sterols, or phyto sterols, that are chemically similar to animal cholesterol. Phyto sterols are insoluble in water but soluble in most organic solvents and contain one alcohol functional group. It is used as a precursor in the manufacture of semi synthetic progesterone, a valuable human hormone that plays an important physiological role in the regulatory and tissue rebuilding mechanisms related to estrogens effects, as well as acting as an intermediate in the biosynthesis of androgens, estrogens and corticoids. It is also used as the precursor of vitamin D₃ [11].

Dodecenyl succinic anhydride obtained from methanolic extract of roots of *Coleus forskohlii* have structural resemblance with phenytoin which is used as an anticonvulsant agent, therefore anticonvulsant activity of roots of *Coleus forskohlii*; may be due the dodecenyl succinic anhydride. It is used as fixative in electron microscopy and a curing agent for epoxy resins.

Thus in the present research work, it can be concluded have developed that we the new flash method chromatographic isolation for and characterisation of phyto constituents of roots of Coleus forskohlii and lead to the identification of the eight new phyto constituents; 3 from petroleum ether extract and 5 from methanolic extract; which were not reported earlier in the roots of Coleus forskohlii.

The petroleum ether extract led to the isolation of three compounds (1, 2, and 3) as shown in Fig.2. EI-

MS analysis as in figure 4, shows that **compound 1** has a molecular weight of 256 [M+1] with molecular formula $C_{16}H_{32}O_2$. It shows base peak at m/z 60 and mass fragments at m/z 57, 60, 73, 83, 97, 115, 129, 143, 157, 171, 185, 199, 213, 227, 239, 256. Fragment 185 corresponds to $C_{11}H_{21}O_2^+$ and 71 corresponds to $C_5H_{11}^+$.

Compound 2 has a molecular weight of 282 [M+1] with molecular formula $C_{18}H_{34}O_2$. It shows base peak at m/z 55 and mass fragments at m/z 60, 69, 73, 83, 97, 111, 125, 137, 151, 165, 180, 193, 207, 222, 235, 246, 264, 282 in Figure 5. Fragment 223 corresponds to $C_6H_{31}^+$ and 99 correspond to $C_7H_{15}^+$.

EI-MS revealed molecular weight of **Compound 3** as 282 [M+1] with molecular formula $C_{18}H_{34}O_{2}$. It shows base peak at m/z 55 and mass fragments at m/z 55, 60, 69, 73, 83, 97, 111, 125, 137, 151, 165, 180, 193, 207, 222, 235, 246, 264, 282 in Figure 6. It was found to be the structural isomer of compound 2.

The methanolic extract led to the isolation of five compounds (4, 5, 6, 7 and 8) as shown in Fig.3. EI-MS analysis reveals molecular weight of compound 4 as 392 [M+1] with molecular formula C₂₂H₃₃FO₇. It shows base peak at m/z 193 and mass fragments at m/z 55, 67, 95, 107, 121, 147, 175, 193, 203, 221, 243, 271, 289, 316, 332, 357, 375, 392 as shown in Fig.7. Fragment 316 corresponds to C₁₆H₂₅FO₅⁺, 96 corresponds to C₆H₈O ⁺. ¹HNMR revealed doublet at δ value at 0.99 ppm and 1.04 ppm which showed presence of angular -CH3. It also showed singlet at 1.33 ppm and 2.21 ppm representing -CH₃ of cyclohexane and -CH₃ of ester respectively. Multiplet at 2.46-2.71 ppm revealed -CH₂ of cyclohexane. Singlet at δ value 3.5 and 3.65 ppm identified -OH Group and singlet at 5.16 ppm showed -CH₂ of ethylene.

Fig. 2: Phyto constituents of petroleum ether extract of roots of *C. forskohlii*; Compound (1) n-Hexadecanoic acid, (2) cis-13-Octadecenoic acid, (3) cis-vaccinic acid.

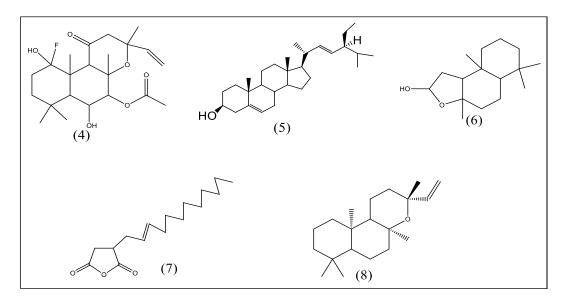


Fig.3: Phyto constituents of methanolic extract of roots of *C. forskohlii*; Compound (4) 1-Fluoroforskolin, (5) Stigmasterol, (6) Sclaral, (7) 2-dodecen-yl (-) succinic anhydride), (8) (3R, 4aS, 10aR)-dodecahydro-3, 4a, 7, 7, 10a-pentamethyl-3-vinyl-1H-benzo[f] chromene.

EI-MS analysis shows that **Compound 5** has a molecular weight of 412 [M+1] with molecular formula $C_{29}H_{48}O$. It shows base peak at m/z 83 and mass fragments at m/z 55, 69, 83, 91, 105, 119, 133, 152, 173, 213, 229, 255, 271, 285, 300, 314, 351, 369, 397, 412 as shown in Fig.8. Fragment at 273 corresponds to $C_{19}H_{29}O_2^+$ and 151 correspond to $C_{10}H_{15}$ O^+ . ¹HNMR revealed doublet at δ value 0.91 ppm for angular -CH₃, triplet at 1.48 ppm represents hetero -CH₂ group, multiplet at 1.86-2.23 ppm showed -CH₂ of cyclohexane, singlet at 3.65 ppm represents -OH Group, singlet at 5.38 ppm showed -CH₂ of ethylene.

Compound 6 in EI-MS analysis revealed molecular weight as 252 [M+1] with molecular formula $C_{16}H_{28}O_2$. It shows base peak at m/z 69 and mass fragments at m/z 55, 62, 81, 95, 109, 125, 137, 149, 163, 177, 192, 219, 237, 252 as in Fig.9. Fragment at 192 corresponds to $C_{14}H_{24}^+$ and 60 correspond to $C_{2}H_{4}O2^+$. ¹H NMR represents doublet for δ value 0.99 ppm showing angular - CH₃, multiplet at 1.39-1.65 ppm revealed -CH₂ of cyclohexane, singlet at 3.45 ppm and 5.38 ppm represents presence of -OH Group and -CH₂ of ether linkage, respectively.

Compound 7 revealed molecular weight as 266 [M+1] in EI-MS analysis with molecular formula $C_{16}H_{26}O_2$. It shows base peak at m/z 41 and mass fragments at m/z 41, 55, 69, 83, 97, 109, 123, 137, 151, 166, 181, 195, 209, 223, 237, 251, 266 as

shown in Fig.10. Fragment at 167 corresponds to $C_{12}H_{23}^+$ and 60 correspond to $C_2H_4O2^+$. ¹H NMR represents triplet at δ value 0.92 ppm for presence of -CH₃ of alkyl side chain, singlet at 1.34 ppm revealed -CH₂ of alkyl side chain, quartet at 2.50 ppm and singlet at 5.50 ppm showed presence of -CH₂ of hetro ring and -CH₂ of ethylene group, respectively.

EI-MS analysis showed that **Compound 8** has a molecular weight of 290 [M+1] with molecular formula $C_{29}H_{48}O$. It shows base peak at m/z 257 and mass fragments at m/z 43, 55, 67, 81, 95, 109, 123, 137, 149, 163, 177, 192, 205, 220, 230, 245, 257, 275, 290 as in figure 11. Fragment at 192 corresponds to $C_{14}H_{24}^+$ and 98 correspond to C_6H_{10} O⁺. ¹H NMR revealed singlet at δ value 1.0 ppm for angular -CH₃, multiplet at 1.3-1.72 ppm and doublet at 5.10, 5.30 ppm revealed -CH₂ of cyclohexane and -CH₂ of ethylene respectively. Triplet at δ value 5.73 ppm represents -CH of ethylene group.

The structure and the probable mass fragmentation pattern of compound 1 to compound 8 are as shown in Fig. 4-Fig.10. Spectrum 1-8 provides mass spectra of compounds 1-8.

Fig. 4: Structure and fragmentation of compound 1 from petroleum ether extract.

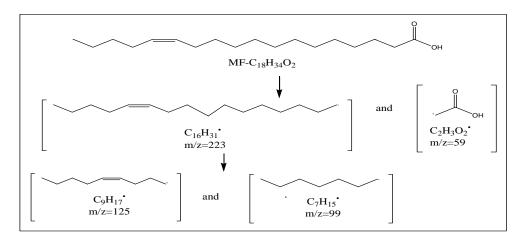


Fig. 5: Structure and fragmentation of compound 2 from petroleum ether extract.

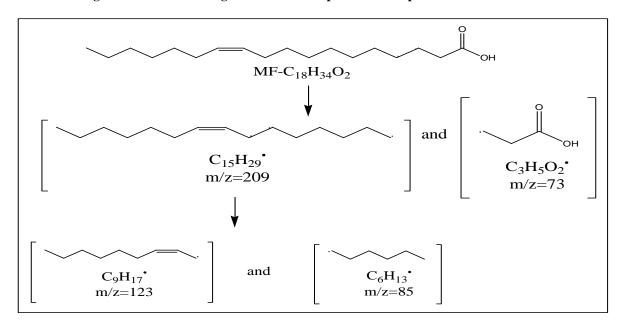
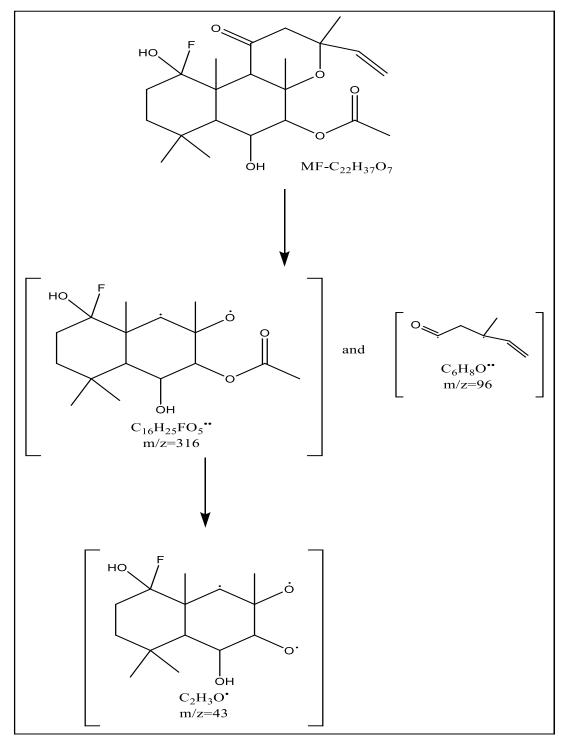


Fig. 6: Structure and fragmentation of compound 3 from petroleum ether extract.



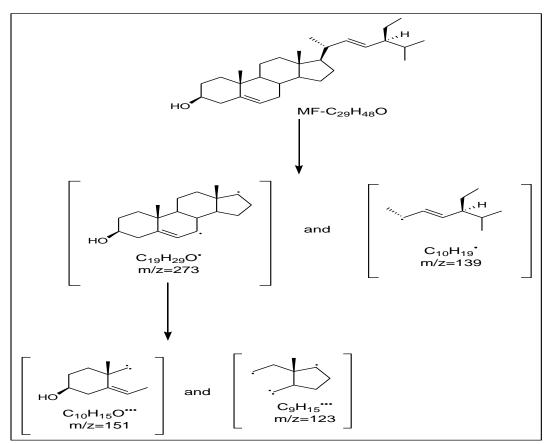


Fig. 8: Structure and fragmentation of compound 5 from methanolic extract.

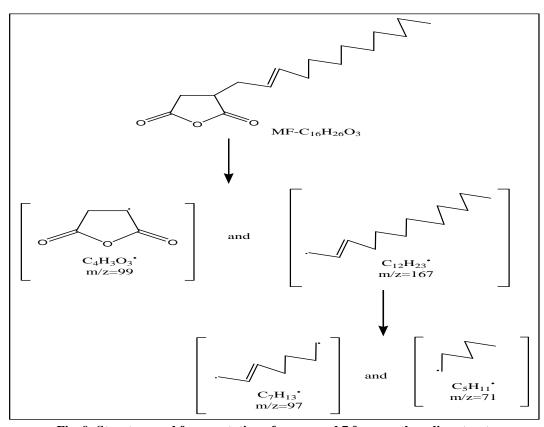


Fig. 9: Structure and fragmentation of compound 7 from methanolic extract.

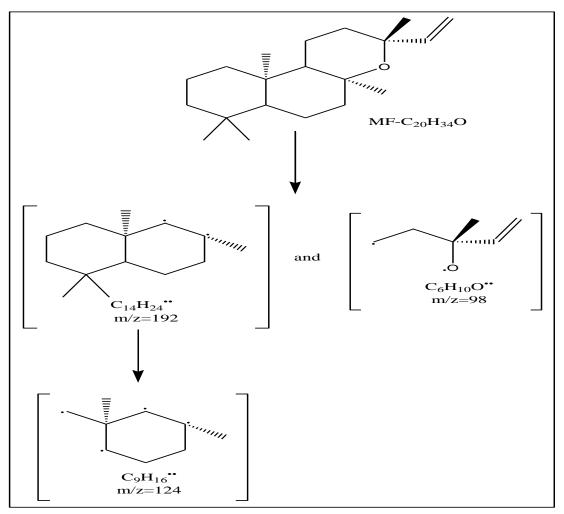


Fig. 10: Structure and fragmentation of compound 8 from methanolic extract

4. CONCLUSION:

In the present research work, a new flash chromatographic method is developed for isolation and characterization of phyto constituents of roots of C. forskohlii and eight new therapeutically active phyto constituents were identified. Compound 1-3 were isolated from petroleum ether extract and compound 4-8 were isolated from methanolic extract. Thus, we have isolated and identified three compounds from the petroleum ether extract of roots of C. forskohlii: 1. n-Hexadecanoic acid, 2. Cis-13-octadecenoic acid, 3. Cis-vaccinic acid and five compounds from methanolic extract of roots: 4. 1-Fluoroforskolin, 5.Stigmasterol, 6. Sclaral, 7. 2-dodecen-yl (-) succinic anhydride, 8. (3R, 4aS, 10aR)-Dodecahydro-3, 4a, 7, 7, 10a-pentamethyl-3-vinyl-1H-benzo[f] chromene.

These compounds have not been reported earlier in the roots of *C. forskohlii*. The structures of these phyto constituents were confirmed on the basis of GC-MS and ¹HNMR spectral data.

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The authors declare no conflict of interest.

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