Analysis of forskolin in Homoeopathic tinctures by validated HPLC method

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Abstract: A simple, short and easiest procedure for quantitative determination of forskolin in market and in house samples (homoeopathic mother tinctures) was performed in which forskolin can be regarded as one of the active constituent. This method comprises of extraction of forskolin from Coleus forskolii plant and subsequently preparation of mother tinctures (in house and market samples) thereafter analysis by UV and HPLC methods where forskolin was used as internal standard. This method was successfully applied to the analysis of mother tinctures obtain from C. forskolii and further comparison of them with standard of forkolin. Thus the determination of forskolin can be used as a convenient method of standardization of selected homoeopathic mother tinctures.

Keywords: Forskolin; HPLC; U.V.; quantification; homoeopathic mother tincture.

Introduction

Medicinal plants are resources of new drugs. The plant part used for the preparation of homoeopathic mother tincture may be the seeds, berries, leaves, barks, roots, fruits, or other plant’s parts. The medicinal effects of plants are due to metabolites (Primary and secondary) especially bioactive compounds produced by plants species include terpenoids, nitrogen metabolite (including, non-protein amino acids, amines, cyanogenic glycosides, glucosinolates and alkaloids) and phenolics.

The terpenoids sometimes called isoprenoids are a large and diverse class of naturally occurring organic compounds similar to terpenes, derived from five carbon isoprene units assembled and modified in thousands ways. Isoprenoids occur in higher plants are known for many biological activities (Sparg et al. 2004). Diterpenes are C-20 compounds formed by combination of four isoprene (C5) units, it may be acyclic, mono, bi-tri or tetra cyclic compound and posses various functional group and unsaturation at different positions. The carbon skeleton of labdanes, derived from 2E, 6E, 10E-geranyl pyrophosphate. Attachment of two tertiary methyls at C-4 one angular methyl group at €-10, an olefinic linkage involving Ĉ-8 or an oxygen functional group on this carbon atom or one of its immediate neighbours and six carbon atom chain at C-9 of a decalin nucleus constitute the basic frame work of bicyclic labdane diterpenes. Labdanes were so named because the first members of the class were originally obtained from labdanum, a resin derived from rockrose plants (Cocker et al. 1956).

C. forskohlii contains diterpenes whose basic skeleton is 11-oxo-manoyl oxide (8, 13-epoxy-labd-14-en-11-one). Forskolin was discovered in the year 1974 and was initially referred to as coleonol. After the identification of other coleonols and diterpenoids the name was later changed to forskolin.

C. forskohlii. belonging to family Lamiaceae is a perennial branched aromatic herb found in subtropical western Himalayas, Nilgiri hills, Gujarat , Bihar, and also cultivated in Maharaashtra growing up to 45-60cm tall (Valdes et al. 1987). The plant is found mostly on the dry and barren hills (Anon 1950). Latitudinal and altitudinal range for the occurrence of the spe-
cies is between 80 and 310 N and 600 – 800 m, respectively.

It is indigenous to India and is recorded in Ayurvedic Materia Medica under the Sanskrit name ‘Makandi’ and ‘Mayani. It has been distributed to Egypt, Arabia, Ethiopia, tropical East Africa and Brazil (Willemse 1985). Forskolin occurred exclusively in \( C. forskohlii \) and could not be detected in six other Coleus species \( \text{viz.} \), \( C. amboinicus \), \( C. blumei \), \( C. caninus \), \( C. malabaricus \), \( C. parviflorus \) and \( C. spicatus \).

A variety of biological activities have been determined for labdane diterpenes including antibacterial, antifungal, antiprotozoal, and anti-inflammatory activities (Atta-Ur-Rahman 2008).

In view of the therapeutic importance of forskolin, analysis of forskolin content is required for which different chromatographic methods are employed for quantification of forskolin. Gas-liquid chromatography (GLC) method is the first developed method (Inamdar et al. 1980). Later on, thin layer and high performance liquid chromatographic (HPLC) methods are employed. HPLC method is found to be more rapid and less sensitive than GLC and used to monitor variation in forskolin content in different germplasm (Inamdar et al. 1984). A monoclonal antibody specific for forskolin has been developed for affinity isolation of forskolin and it has been used for extremely sensitive quantification of forskolin in plant tissues at different stages of development (Yanagihara et al. 1996). Nuclear Magnetic Resonance (NMR) and a Gas Chromatography-Mass Spectroscopy (GC-MS) method are also used for forskolin quantification (Demetzos et al. 2002). Reversed-phase liquid chromatography with a photodiode array (PDA) detector at 210 nm is successful in the qualitative and quantitative evaluation of forskolin in plant material and in market products claiming to contain forskolin (Schanebera and Khan 2003).

U.V. spectral studies of diterpene are helpful in detecting the presence of a particular type of chromophore (molecule absorbs certain wavelengths of visible light and transmits or reflects others. The existence of \( \alpha, \beta \)-unsaturated carboxyls (near 250 nm), \( \gamma, \delta \)-unsaturated ketone (210-239, 214-244 and 300 nm) and conjugated diene system is established by studying their respective bands (Mohd.Ali 2001).

Materials and Methods

Standard sample of forskolin was purchased from Sigma Aldrich. Mother tinctures (in-House and market samples) were prepared in our in-House Pharmacy. Crude drug (authentic) samples were collected by one of the author from Survey of Medicinal Plants & Collection Unit (SMPCU) Ooty Tamil Nadu and identified by Dr. S. Baburaj (Scientist) for comparative study. Solid phase extraction (SPE) column Agilent eclipse (XDB) was obtained from Agilent. Acetonitrile, methanol and water were of HPLC grade used and purchased from Fisher Scientific USA. Solvents were filtered through a Millipore filter (0.45µm) before use.

Chromatographic Condition

Agilent 1200 Series HPLC auto sampler system, consisting of pumps, degasser and PDA detector with chemstation software.

Separations was carried out with Agilent Eclipse XDB RP C18 column (5 \( \mu \), 250x4.6 mm i.d.) protected by a guard column (5 \( \mu \), 20x3.0 mm i.d.) containing the same packing. The mobile phase was isocratic i.e. 45 volumes of acetonitrile and 55 volumes of water which remains constant throughout whole procedure. The flow rate was 1 ml per minute, the analysis was monitored at 220 nm and adsorption spectra of compounds were recorded between 200-300 nm. The column temperature was 25 °C and the sample injection volume was 20 \( \mu \)l and run time 30 Min. The forskolin content was identified by comparing their retention time and U.V. spectra with the standard.

Sample preparation

Powdered sample were put in in 50 % (v/v) ethanol by using percolation method prescribe in Homoeopathic Pharmacopoeia of India (H.P.I) and left at room temperature for at least three weeks before use as a mother tincture for analysis.
1ml of mother tinctures of both *C. forskolii* in house and market samples were taken, filtered through a membrane filter (0.45 µm) and evaporated to dryness. Dissolve the dried material in 1ml of methanol.

**Standard preparation**

51.68 mg forskolin standard was taken in volumetric flask. Add about 50 ml methanol and shake well to dissolve completely. Make up the volume upto 100 ml with methanol and mix properly. Before HPLC analysis standard solution was filtered through a polytetrafluoroethane (PTFE) membranes filter (0.45 µm) prior to injection.

**U.V. spectrophotometric studies**

Spectrophotometer set at 220 nm, samples and standard were put in cuvette. Before analysis cuvettes were washed with ethanol and analysis were performed on Agilent 8453 spectrophotometer and Chemstation UV-VIS software was used for the UV. analysis.

**Sample preparation**

Samples (in house and market) used for U.V analysis were prepared by mixing one part of Mother tincture and ninety nine parts of absolute alcohol (1:99) and filtered through membrane filter prior to U.V. analysis.

51.68 mg forskolin standard was taken in volumetric flask. Add about 50 ml methanol and shake well to dissolve completely. Make up the volume up to 100 ml with methanol and mix properly.

**Results**

It can be concluded on the basis of above results that the sample collected from SMPCU OOTY, for preparation of mother tincture having higher content of forskolin in comparison from the sample collected from market.

UV analysis also performed on the above two samples with reference solution. Maximum absorbance was found at 220 nm for reference, 210 nm for in house and 190 nm for market sample of mother tincture which also in the favour of above said result (Figure 1 to 4).

**Figure 1**: Chromatogram of forskolin Standard
Analysis of forskolin in Homoeopathic tinctures

Figure 2: Chromatogram of Mother Tincture (market sample) of *C. forskolii*

Figure 3: Chromatogram of Mother Tincture (in house sample) of *C. forskolii*

Figure 4: U.V. Spectra of Spectra of Standard (1), in house sample (2) and market samples (3)
Discussion

Raw material used in the preparation of mother tincture (In house sample) was cultivated in Survey of Medicinal Plants Unit, Ooty, Tamil Nadu and freshly collected without any adulterations so it contains excess amount of bioactive compounds than that of market samples. More study required to validate the result of other mother tinctures in respect of authentic as well as market sample.

Calculations:

Content of forskolin =
Area of sample X Dilution of Std.
----------------------------- X %purity of std
Area of standard X Dilute of sample

For market sample of Coleus forskolii
60.8 51.68 97.31
----- X ------ X -------- = 0.29 gm/l
1044 100 100

For in house sample of Coleus forskolii
3370.2 51.68 97.31
----- X ------ X -------- = 1.62 gm/l
10440 100 100

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References:


