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Method of Extraction and Determination of Forskolin from *Coleus forskolii* of Nepal

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ABSTRACT
Coleus forskolii variety of Nepal has got very less concentration of forskolin <0.13±0.05 %. A new and highly specific method has developed by Phytolabs in order to extract a well purified forskolin from the *C. forskolii* variety of Nepal. A rapid and validated method was developed for the evaluation of forskolin in *Coleus forskohlii.* Forskolin was quantitated by reverse-phase High performance liquid chromatography (HPLC) with a photodiode array detector at 210 nm. The temperature was held constant at 30 °C, and the retention time of forskolin was approximately 6.9 min with purity of 82 % when compared with 70 % standard provided by GSN pharma. The samples were extracted with Acetonitrile by sonication. TLC has confirmed the forskolin with R_f value 0.25±0.02 evaluated through validated method.

Keywords: *C. forskolii*, forskolin, HPLC, TLC.

1. Introduction

Coleus forskolii grows wild in arid and semi-arid regions of India, Nepal and Thailand; the roots have long been used in Ayurvedic medicine [1]. A member of the mint family, it has been traditionally used to treat heart and lung disease, intestinal spasms, insomnia, and convulsions [1]. Forskolin. (Fig1).

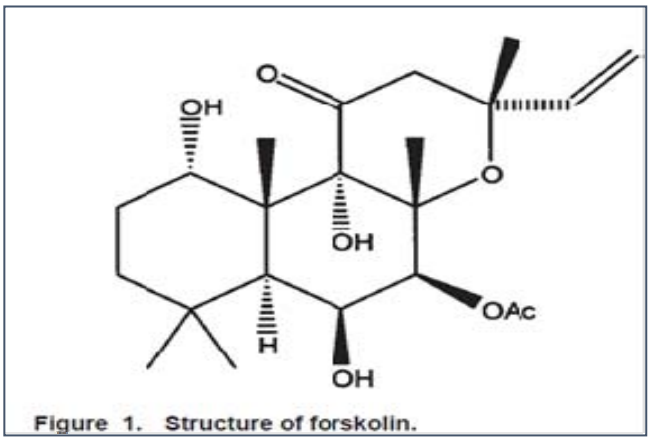

Figure 1. Structure of forskolin.

A labdane diterpenoid, is considered the active secondary metabolite because of its ability to activate the enzyme adenylate cyclase [2]. Recent research has shown that forskolin has positive effects against a wide range of conditions such as asthma, glaucoma, hypertension, hair loss, cancer, and obesity [3-9].

In 1984, Inamdar *et al* [10]. reported a comparison of thin-layer chromatography (TLC), gas–liquid chromatography (GLC), and high performance liquid chromatography (HPLC) for the quantitative estimation of forskolin [10]. A Validated Stability-Indicating TLC Method for Determination of Forskolin in Crude Drug and Pharmaceutical Dosage Form was first reported by Sayeed Ahmad *et al* [11] Of the methods studied, normal- phase HPLC was determined to be the best procedure for the analysis of the extract of *C. forskohlii*. The present study improved the extraction procedure and HPLC method.

2. Methods

2.1 Extraction of Forskolin

A new and rapid method has been developed to extract forskolin from *C. forskohlii* variety collected from Nepal. *C. forskolii* tubers are collected washed and dried. After drying the tubers are pulverized into granules. By using routine methods whole forskolii extracted in crude form using methanol as solvent. Methanol extract is concentrated and chloroform is added to concentrate and equal volume of water is added to the separating funnel and shaked well. Allow to settle and separate the chloroform layer. Repeat the water treatment two to three times and collect chloroform layer. Concentrate the chloroform layer. Precipitate the Forskolin using ice cold n-hexane. A reddish brown to brown colour powder of Forskolin is obtained.

2.2 TLC Method

UV6000LP detector, using Shimadzu an RP18 column, 150 × 4.6 mm, 5 µm particle size (Waters) at ambient temperature. The mobile phase consisted of water (A) and Acetonitrile (B). At a flow rate of 0.5 mL/min, the gradient elution was 50A/50B to 43A/57B in 10 min, hold at 43A/57B for 10 min. Each run was followed by a 5 min wash with methanol and an equilibration period of 10 min. The detection wavelength was 210 nm, and the injection volume was 5 µL.

2.3 TLC Method

The method was developed on TLC aluminium plates precoated with silica gel 60F-254 using solvent system benzene: methanol (9:1, v/v)

3. Result and Discussion

3.1 HPLC

Forskolin was quantified using HPLC as 82% purity when compared with 70% standard provided by GSN pharma see fig -2, fig-3 and fig-4.,

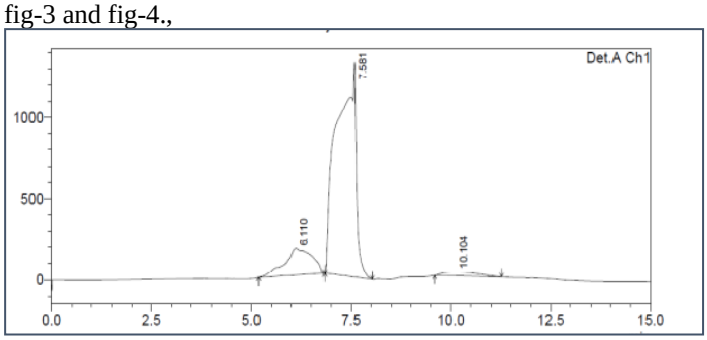

Fig 2: Forskolin extracted from *C. forskolii* collected from Nepal detected at 210 nm with RT 6.9 min

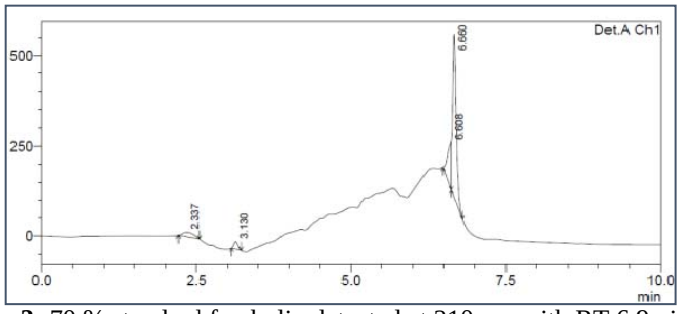

Fig 3: 70 % standard forskolin detected at 210 nm with RT 6.9min.

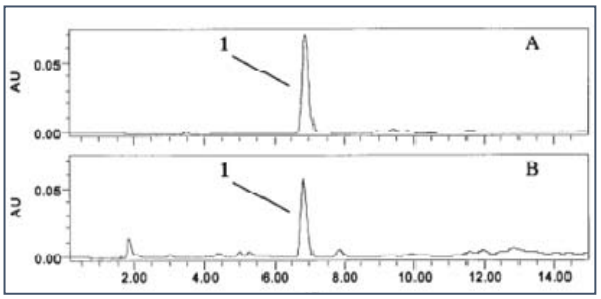

Fig 4: Method adopted by Schaneberg and Khan *et al*, forskolin determined at RT 6.8

3.2 TLC

Thin layer chromatography has determined the forskolin as a compact spot at R_f 0.25±0.02 see fig-5, 6.

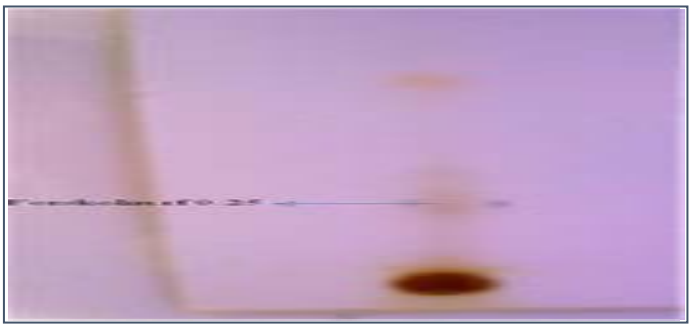

Fig 5: Forskolin TLC with R_f 0.25

Fig 6: forskolin TLC with R_f 0.25 under long U.V. (Left, sample and right, standard)

4. Conclusion

The method adopted in this paper for extraction of forskolin has shown good results and validated by different validated testing methods like HPLC and TLC. High performance liquid chromatographic method has shown more accurate and validated method for determination and quantification of forskolin followed by TLC R_f value confirmation. In the present study we have determined and quantified the forskolin purity by both HPLC and TLC methods.

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