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Hplc-Based Quantification Of Phytoconstituents In Ethyl Acetate Root Extract Of Centaurea Behen L

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Abstract
There is at present growing interest, both in the industry and in the scientific research, for aromatic and medicinal plants because of their antimicrobial, antidiabetic, anticancer and antioxidant properties etc. These properties are due to many active phytochemicals including flavanoids, terpenoids, carotenoids, coumarins, curcumines etc. These bioactive principles have also been confirmed using modern analytical techniques. Centaurea behen (C. Behen) is commonly called as White Behen, Safaid Behmen, Behman abyaz and White Rhapontic belongs to the family Asteraceae/Compositae. It occurs in India, Pakistan, Israel, Europe, North Africa and China. The aim of the present study was to examine C. Behen seeds for phytochemical profile and quercetin was detected in ethyl acetate root extract by using RP-HPLC analysis. For future studies, phytochemcials responsible for various activities can be isolated and modified for pharmacological purpose.
Keywords: *C. Behen, Phytochemical profile, Quercetin, RP-HPLC, Flavonoids*

INTRODUCTION

Medicinal plants are very good sources of drugs for traditional systems of medicine. Indian medicinal plants have lot of potential towards curing many diseases. Medicinal plant extracts contain various types of bioactive compounds known as phytochemicals. These phytochemicals can be used in treatment as anticancer, antimicrobial, antioxidant, anti-inflammatory agents etc [1]. Recent studies show that these phytochemicals are safe, broadly effective and have less adverse effects. However in vivo studies of these phytochemicals are necessary to demonstrate their efficacy, safety and to verify their bioavailability [2]. Spices are used all over the world to improve the taste and flavour of food products. In addition, they have medicinal properties, and can be beneficial in the prevention of different human diseases. Epidemiological and *in vitro* studies strongly suggest that phytochemical of spices have potential protective effects against many diseases. Therefore, they could be used as anti-mutagenic, antibacterial, antiviral and anti-inflammatory agents [3]. There is increasing evidence that consumption of phytochemical compounds present in spices may lower the risk of serious health disorders [4, 5].

C. Behen is commonly called as White Behen, Safaid Behmen, Behman abyaz and White Rhapontic belongs to the family Asteraceae/Compositae. It occurs in India, Pakistan [6], Israel, Europe, North Africa and China [7]. It also occurs in Tehran, Iraq and Turkey [8], which is used as remedial agents in various diseases. *C. behen* has been used to treat weakness of brain, heart and liver, palpitation, hepatitis, melancholia, sexual debility, neurasthenia, spermatorrhoea, fatigue and for diseases of the stomach and intestines [9]. It is also used in jaundice and is a heart tonic [10]. The roots of *C. behen* are used for killing the lice and making the hair good smelling [11]. Roots act as nervine and anabolic tonic, strengthen central nervous system and used in affections of kidney [6]. The study was designed to demonstrate the phytochemical constitute. The flavonoid content was determined spectrophotometrically as well as flavonoid characterized by using HPLC.

MATERIAL AND METHOD

Plant Material

Roots of *C. behen* were purchased from local market of Bhopal MP.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade. Quercetin was kindly provided by Scan Research Laboratories, Bhopal (India). Methanol and acetonitrile were of HPLC grade and purchased from Merck Ltd, New Delhi, India. Water used was of HPLC grade from Merck Ltd, New Delhi, India.

Extraction Procedure

Plant material

Roots of *C. behen* free of diseases were collected from local region in separate sterile bags from Bhopal, Madhya Pradesh, and month of October, 2020. Plant material (root part) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time at room temperature. Then the plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was grinded using electronic grinder. Powdered plant material was observed for their colour, odour, taste and texture. Dried plant material was packed in air tight container and stored for phytochemical and biological studies.

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals and solvent used in this study were of analytical grade.

Quantification of flavonoid compounds by HPLC technique

For HPLC investigation of flavonoid compounds the ethyl acetate extracts of *C. behen* root under study were used as a preliminary assessment of various compounds. The HPLC apparatus used for analysis was composed of a waters equipped with a UV dual detector and generated data were analyzed using Waters Ace software. For chromatographic separation Thermo C18 column (250 X 4.6 mm, 5µm) was applied. The chromatographic analysis was performed at ambient temperature on a RP-C18 analytical column with a mobile phase composed of Acetonitrile: Methanol (50:50 v/v) and was isocratically eluted at a flow rate of 1 ml/ min. A small sample volume of 20 µl was used for each sample run, being injected into the HPLC system. The chromatogram was monitored with UV detection at a wavelength of 256 nm. Sample volume (20 ul) and analysis time was 10min for both, standards and samples used for analysis. A quercetin was used as standards. A thermospectronic model of Labindia 3000+UV/VIS Spectrophotometer with 1cm. matched quartz cells were used for determination of λmax. The sample solution was chromatographed and a concentration of quercetin in extract sample was found out using regression equation.

RESULTS AND DISCUSSION

The crude extracts so obtained after the hot continuous percolation extraction process, extracts was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant. Phytochemical analysis of Ethyl acetate extracts of root sample of *C. behen* showed the presence of flavonoid, diterpines and carbohydrate. This study indicates the presence of flavonoids present in sufficiently enough quantity in extract so flavonoid is the phytochemicals that are present in Ethyl acetate extract Table 1.

Quercetin, a member of flavanol family is predominantly present in several plants. The method described here can be used for quantitation of quercetin and other flavanols from the same family in various herbal extracts and their formulations, and can be remarkably important for QC testing of raw material extracts and herbal formulations containing quercetin. It has been successfully applied in our laboratory for quantitation of quercetin in the extracts of *C. behen* for their chemical standardization. The results of HPLC were shown in table 2.

Table 1: Phytochemical Screening of *C. behen* Extract

S. No.	Constituents	Ethyl acetate extract
1.	**Alkaloids** Hager's Test:	 -ve
2.	**Glycosides** Legal's Test:	 - ve
3.	**Flavonoids** Alkaline Reagent Test: Lead acetate Test:	 +ve +ve
4.	**Diterpenes** Copper acetate Test:	 + ve
5.	**Phenol** Ferric Chloride Test:	 - ve
6.	**Proteins** Xanthoproteic Test:	 - ve
7.	**Carbohydrate** Fehling's Test:	 + ve
8.	**Saponins** Froth Test:	 -ve

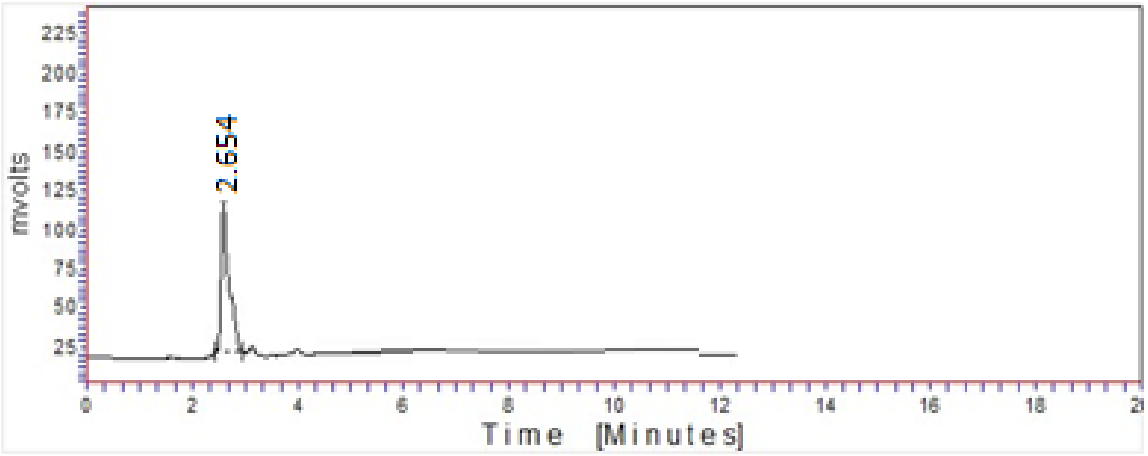
(+ve)= Present, (-ve) = absent

Table 2 Characteristics of the analytical method derived from the standard calibration curve

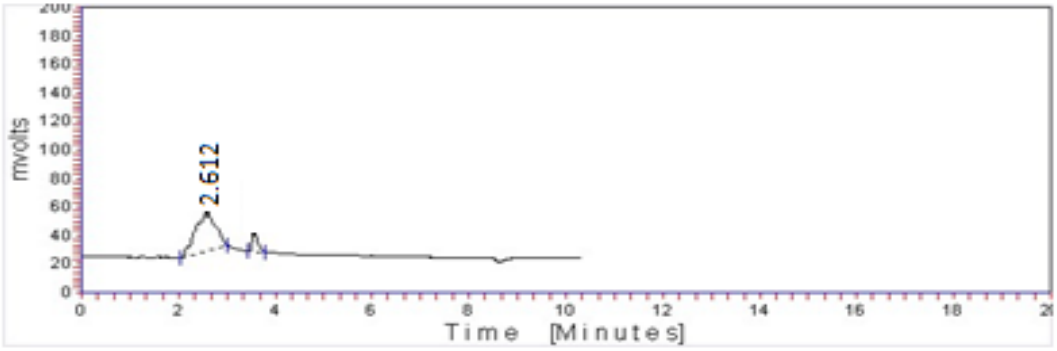
Compound	Linearity range µg/ml	Correlation co-efficient	Slope	Intercept
Quercetin	5-25	0.999	28.64	-1.134

Table 3 Quantitative estimation of quercetin in Ethyl acetate extract

S. No.	Extract	RT	Area	% Assay
1.	*C. behen*	2.604	245.658	0.8671



(A)



(B)

Figure 1 Chromatogram of (A) Standard Quercetin (B) Ethyl acetate extract of *C. behen*

CONCLUSION

The present study concluded that this medicinal plant viz. *C. behen* is a promising source of various activities and may be efficient as preventive agents in the pathogenesis of some diseases. However, the strength of the existing data is not enough to suggest a reasonable mode of action. Further phytochemical studies are also required to isolate and characterize active ingredients that are responsible for its antioxidant activity and to explore the existence of synergism if any, among the compounds.

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