Original Research Article

QUALITATIVE AND QUANTITATIVE DETERMINATION OF SECONDARY METABOLITES AND ANTIOXIDANT POTENTIAL OF *CENTAUREA BEHEN* L. ROOT EXTRACTS

Abstract

Plants have been used by humans as a natural source for treatments and cures since ancient times, and medicinal herbs have gained popularity due to their widespread usage and lack of adverse effects. Plant study has grown in popularity across the world in recent years, and a large body of data has been gathered to demonstrate the enormous potential of medicinal plants employed in diverse traditional systems. Centaurea behen L (C. behen) is a South Asian root that belongs to the Astarcease family and is known as Safed Behman. C. behen is used to treat brain, heart, and liver weakness, as well as palpitation, hepatitis, melancholia, sexual debility, neurasthenia, spermatorrhoea, weariness, and stomach and bowel problems. It is also a heart tonic and is used to treat jaundice. Several sesqueterpene lactones, the guaianolides cyanraopicrin, augerin B, desacylcynaropicrin, grosshemin, and traces of a ketone closely related to solstitialin A were found in aerial sections of C. behen. The goal of this study was to assess in vitro antioxidant activity, as well as qualitative and quantitative phytochemical analyses of C. behen root gathered in the Bhopal region of Madhya Pradesh. The well-known test methodology was used to determine qualitative analysis of various phytochemical ingredients and quantitative analysis of total flavonoids. The antioxidant activity of an ethyl acetate extract of the roots was tested in vitro using conventional techniques against DPPH and nitric oxide (NO) radical scavenging assays. Phenols, flavonoids, tannins, saponins, alkaloids, and other phytochemicals were discovered by phytochemical investigation. Ethyl acetate, methanol, and aqueous root extract of C. behen contained 0.740, 0.381, and 0.465 mg/100mg of total flavonoids, respectively. The anti-DPPH and anti-NO actions of ethyl acetate extracts were concentration dependant. The plant's broad variety of phytochemicals implies that it has medicinal potential, which might be investigated in the pharmaceutical sector as well as in traditional medicine.

Keywords: *Centaurea behen L,* Astarcease, Antioxidant activity, DPPH, NO assay method.

1. INTRODUCTION

Indian medicinal plants are a rich source of pharmacologically active principles and chemicals that are widely employed in home treatments for a variety of diseases [1]. Reactive oxygen species (ROS) are extremely reactive molecules that can serve as both mediators and prooxidants in specific physiological activities. An imbalance between ROS production and

antioxidant capacity causes oxidative stress, which has been linked to the onset and progression of a number of diseases, including cardiovascular dysfunction associated with vascular disease, hyperlipidemia, diabetes, hypertension, and ischemia/reperfusion injury. A set of antioxidant defence mechanisms regulate the potential harm induced by an excess of ROS, with the antioxidant enzymes gluthatione (GSH) peroxidase, superoxide dismutase (SOD), and GSH reductase playing a major protective role [2]. A number of herbal secondary metabolites, including as flavonoid, have been discovered to protect cells from oxidative damage [3]. By scavenging free radicals and decreasing lipid peroxidation, these chemicals have been shown to stabilise RBC membranes [4, 5]. C. behen is a 1.5 m tall annual or perennial herb. It is grown throughout Northern India for its medicinal roots, which are used to treat a variety of ailments. It can be found in India, Pakistan, Israel, Europe, North Africa, and China [6–7]. Tehran, Iraq, and Turkey are also affected [8]. White Behen, Safaid Behmen, Behman abyaz, and White Rhapontic are some of the common names for the plant [7]. The roots of C. behen are used to eliminate lice and give hair a pleasant odour [9]. Roots are nervine and anabolic tonics that enhance the central nervous system and are utilised in renal diseases [6]. The medication Safed behman or Bhamana-i-sufeed is made from the roots of C. behen. These are said to have fattening and aphrodisiac effects. These are also employed in the treatment of calculus diseases. The root is said to be a component of the Unani formulation Davabulmusk, which is used to strengthen cardiac muscles and the central nervous system, as well as Laboobaisagheer, which is utilised in polyurea, nerve tissue building, and renal tissue building [10]. From the roots of *C. behen* a crystalline unsaturated lactone behenin having molecular formula $C_{24}H_{48}O_3$ has been obtained [11].The roots also contain taraxasterol and its acetate, myristate, inulin and a glucoside which on hydrolysis yields centaurea sterol A [7]. The present study was focused to evaluate the phytochemical analysis and antioxidant activity of roots of *C. behen*.

2. MATERIAL AND METHOD

2.1 Plant material

Roots of C. behen that were disease-free were gathered in separate sterile bags from the local area in Bhopal, Madhya Pradesh, in the month of October 2020. The plant material (root section) chosen for the study was properly cleaned under running tap water and then rinsed in distilled water before being allowed to dry at room temperature for a period of time. Without being contaminated, the plant material was then shade dried for 3 to 4 weeks. The dried plant material was ground using an electric grinder. Powdered plant material was tested for colour,

odour, taste, and texture. Dried plant material was stored in an airtight container for phytochemical and biological research.

2.2 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). The chemicals and solvents utilised in this experiment were all of analytical grade.

2.3 Defatting of plant material

136 gram shade dried powder of root of *C. behen* was extraction with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place.

2.4 Successive extraction with different solvents by maceration method

Water, methanol, ethyl acetate, and chloroform were used to extract plant material in four distinct polarity solvents. The maceration process was used to remove powdered plant components. To get the dry concentrated extract, the resulting content was filtered using Whatman filter paper no. 1 and stored for solvent evaporation. The extractive yield was calculated by weighing the dried crude concentrated extract, which was then transferred to glass vials (6 2 cm) and kept in a refrigerator (4°C) until utilised for analysis [12].

2.5 Phytochemical screening

The presence of bioactive chemicals was detected using standard phytochemical screening procedures [13, 14]. Visual assessment of colour change or precipitate development following the addition of specific chemicals to the solution indicated the tests.

2.6 Flavonoid content total

Olufunmiso et al., 2011 [15], a technique for determining total flavonoid content, was used. The absorbance of the reaction mixture was measured at 420 nm using a UV/visible spectrophotometer after 1 ml of 2 percent AlCl3 methanolic solution was added to 3 ml of extract or standard and allowed to stand for 60 minutes at room temperature. The flavonoid content was determined using a standard graph of quercetin, and the findings were represented in mg/g of quercetin equivalent.

2.7 Antioxidant activity

2.7.1 DPPH radical scavenging assay

The DPPH scavenging activity was determined using a modified Olufunmiso et al., 2011 [15] technique. The DPPH scavenging activity was measured using a spectrophotometer. The stock solution (6 mg in 100 mL methanol) was produced to give an initial absorbance of 1.5

mL in 1.5 mL methanol. After 15 minutes, there was a decrease in absorbance in the presence of sample extract at various concentrations (10-100 g/ml). 1.5 mL of DPPH solution was added to 3 mL of methanol, and the absorbance was measured at 517 nm for the control reading. 1.5 ml of DPPH and 1.5 ml of varying concentrations of the test sample were placed in a succession of volumetric flasks, and the final volume was adjusted to 3 ml using methanol. Three test samples were collected and processed in the same way. The average was then determined. The absorbance at zero time was measured for each concentration. The absorbance of DPPH with various doses exhibited a final decline after 15 minutes at 517 nm. The following equation was used to compute the % inhibition of the free radical DPPH: [(absorbance of control - absorbance of sample)/absorbance of control] 100 percent inhibition IC_{50} was computed based on the proportion of DPPH radicals scavenged, even though the activity is represented as a 50 percent inhibitory concentration (IC_{50}). The stronger the antioxidant activity, the lower the IC_{50} value.

2.7.2 ***Nitric oxide (NO^o) radical scavenging assay***

The capacity of the extracts to scavenge NOo radicals is determined by the Griess reagent's suppression of NOo radicals produced from sodium nitroprusside in phosphate buffer saline solution (1 percent sulfanilamide, 2 percent orthophosphoric acid and 0.1 percent naphthyl ethylenediamine dihydrochloride). Nitric oxide scavengers compete with oxygen, resulting in reduced nitrite ion formation, which may be measured at 546 nm [16]. Sodium nitroprusside (0.6 ml, 5 mM) solution was combined with and without altering the concentration of the extracts or Ascorbic acid (2 ml, 10-200g/ml) and incubated for 5 hours at 25 2°C. The absorbance of the purple coloured azo dye chromophore was measured at max 546 nm using a UV-Vis spectrophotometer after the incubated solution (2 ml) was combined with an equivalent amount of Griess reagent. The NO^o radical scavenging ability was calculated using following formula: $$\text{Scavenging activity (\%)} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} x100$$

3. RESULTS AND DISCUSSION

The roots of C. behen were harvested in Bhopal, Madhya Pradesh, India. Air-dried and extracted using the maceration method. To get the actual yield of extraction, the crude extracts produced after each of the maceration extraction processes were concentrated on a water bath by fully evaporating the solvents. Table 1 shows the yield of extracts produced from plant roots using petroleum ether, chloroform, ethyl acetate, methanol, and water as solvents. Table 2 shows the findings of a qualitative phytochemical examination of C. behen's crude powder roots. Flavonoids, diterpines, carbohydrate, and proteins were found in

methanolic and ethyl acetate extracts of C. behen roots, however all phytoconstituents were lacking in chloroform extracts, and carbohydrate was detected in C. behen extract. The extracts' total flavonoids content was calculated as a percentage of quercetin equivalent per 100 mg dry weight of sample. Total flavonoids concentration of methanolic, aqueous, and ethyl acetate extracts of C. behen roots were 0.381, 0.465, and 0.740, respectively, according to Table3 and Figure1. The hydrogen donating nature of extracts was evaluated using the DPPH radical scavenging assay [17]. The inhibitory concentration 50 percent (IC50) of C. behen ethyl acetate extract was determined to be 69.72g/ml when compared to ascorbic acid (17.68g/ml) in DPPH radical scavenging activity. Table 4 and Figure 2 show dose-dependent action in relation to concentration. NO scavenging effects were seen in extracts, which competed with oxygen for the ability to directly react with NO, inhibiting the production of nitrite ions [18]. In comparison to ascorbic acid (IC50 17.68g/ml), C. behen ethyl acetate extract demonstrated nitric oxide (NOo) radical scavenging activity with an IC50 value of 65.43g/ml. C. behen extract had a lot of action. Figure 3 and Table 5

Table 1: Results of percentage yield of roots extracts

S. No.	Solvents	% Yield (W/W)
1.	Pet. ether	0.274
2.	Chloroform	0.366
3.	Ethyl acetate	0.557
4.	Methanol	5.444
5.	Aqueous	6.987

Table 2: Phytochemical evaluation of *C. behen* roots extracts

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
1.	**Alkaloids**				
	Hager's Test:	-ve	-ve	-ve	-ve
2.	**Glycosides**				
	Legal's Test:	- ve	- ve	- ve	- ve
3.	**Flavonoids**				
	Alkaline Reagent Test:	- ve	+ve	+ve	+ ve
	Lead acetate Test:	- ve	+ve	- ve	+ ve

4.	**Diterpenes** Copper acetate Test:	- ve	+ ve	+ve	+ ve
5.	**Phenol** Ferric Chloride Test:	- ve	- ve	- ve	- ve
6.	**Proteins** Xanthoproteic Test:	- ve	- ve	+ve	+ ve
7.	**Carbohydrate** Fehling's Test:	+ ve	+ ve	+ ve	+ ve
8.	**Saponins** Froth Test:	-ve	-ve	-ve	+ ve

Table 3: Results of flavonoids content of root extracts of *C. behen*

S. No	Extracts	Total flavonoids content (mg/ 100 mg of dried extract)
1	Ethyl acetate	0.740
2	Methanol	0.381
3	Aqueous	0.465

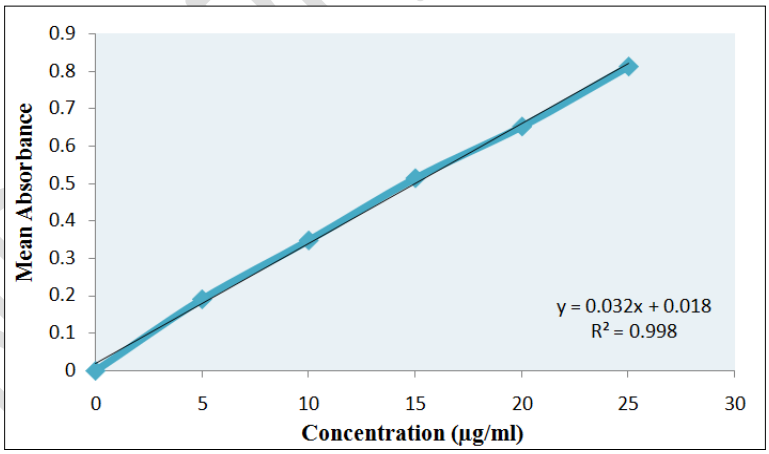

Fig. 1: Graph of estimation of total flavonoids content

Table 4: % Inhibition of ascorbic acid and ethyl acetate extract of *C. behen* using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethyl acetate extract
1	10	44.65	21.85

2	20	48.62	35.75
3	40	65.34	42.96
4	60	69.65	46.62
5	80	77.41	53.36
6	100	84.13	59.65
IC_{50}		17.68	69.72

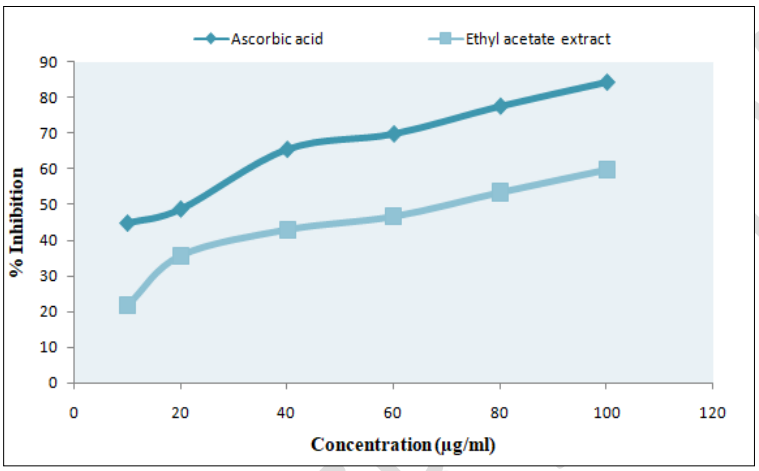

Fig. 2: Inhibition of ascorbic acid and ethyl acetate extract of *C. behen* using DPPH method

Table 5: % Inhibition of ascorbic acid and ethyl acetate extract of *C. behen* using NO method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethyl acetate extract
1	20	47.70	29.54
2	40	52.92	38.55
3	60	67.43	50.63
4	80	68.89	57.68
5	100	74.42	62.36
IC 50		24.63	65.43

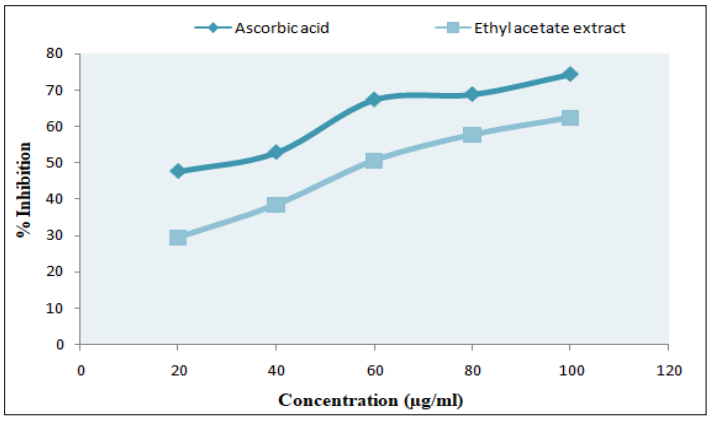

Fig. 3: % Inhibition of ascorbic acid and ethyl acetate extract of *C. behen* using NO method

4. CONCLUSION

It can be concluded that the phytochemical investigation provided valuable information about the various phytoconstituents present in the plant, which will aid future investigators in selecting the appropriate extract for further investigation of isolating the active principle, as well as an understanding of the various phytochemicals that have been discovered to have a wide range of activities. In vitro antioxidant investigations support the total flavonoid content of ethyl acetate, methanol, and aqueous roots extract. Under in vitro circumstances, the extract may efficiently scavenge different reactive oxygen species/free radicals. This might be because it can produce a large number of stable oxidised products after oxidation or radical scavenging. The extracts' wide spectrum of action shows that antioxidant activity is mediated by many pathways. The extract's various antioxidant activities exhibited in this study clearly reveals the plant's potential application value. Further research into the antioxidant role of the plants mentioned above in diverse systems might lead to the discovery of natural antioxidants.

NOTE:

The study highlights the efficacy of " traditional medicine " which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

.COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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