Original Research Article

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

Abstract

Plants have served human beings as a natural source for treatments and therapies from ancient times, amongst them medicinal herbs have gain attention because of its wide use and less side effects. In the recent years plant research has increased throughout the world and a huge amount of evidences have been collected to show immense potential of medicinal plants used in various traditional systems. *Centaurea behen L* (*C. behen*) is a root belongs to the family Astarcease, native to South Asia and is commonly known as Safed Behman. *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. It is also used in jaundice and is a heart tonic. Aerial parts of *C. behen* afforded several sesqueterpene lactones, the guaianolides cyanraopicrin, augerin B, desacylcynaropicrin, grosshemin and traces of a ketone which is closely related to solstitialin A. The aim of the present study was to evaluate *in vitro* antioxidant activities, qualitative and quantitative phytochemical analysis of root of *C. behen* collected from Bhopal region of Madhya Pradesh. Qualitative analysis of various phytochemical constituents and quantitative analysis of total flavonoids were determined by the well-known test protocol available in the literature. The *in vitro* antioxidant activity of ethyl acetate extract of the roots was assessed against DPPH and nitric oxide (NO) radical scavenging assay methods using standard protocols. Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids etc. The total flavonoids content of ethyl acetate, methanol, and aqueous root extract of *C. behen* was found to be 0.740, 0.381 and 0.465 mg/100mg respectively. The activities of ethyl acetate extracts against DPPH and NO assay method were concentration dependent. The diverse array of phytochemicals present in the plant thus suggests its therapeutic potentials which may be explored in drug manufacturing industry as well as in traditional medicine.

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

1. INTRODUCTION

Indian medicinal plants are considered a vast source of several pharmacologically active principles and compounds, which are commonly used in home remedies against multiple ailments [1]. Reactive oxygen species (ROS) are highly reactive molecules which may be both important mediators of some physiological functions and also potential prooxidants. Imbalance between ROS generation and antioxidant capacity induces a condition known as oxidative stress which may play a major role in the initiation and progression of numerous pathologies including cardiovascular dysfunction associated with vascular disease, hyperlipidemia, diabetes mellitus, hypertension and ischemia/reperfusion injury. The potential damage caused by an excess of ROS is controlled by a series of antioxidant defence mechanisms and among them, a key protective role is played by the antioxidant enzymes gluthatione (GSH) peroxidase, superoxide dismutase (SOD) and GSH reductase [2]. Several herbal secondary metabolites such as flavonoid have been found to protect cells from oxidative damage [3]. These compounds have been evidenced to stabilize RBC membrane by scavenging free radicals and reducing lipid peroxidation [4, 5]. *C. behen* is an annual or perennial herb upto 1.5 m tall. It is cultivated throughout Northern India for the sake of its highly medicinal roots, which are used as remedial agents in various diseases. . It occurs in India, Pakistan [6], Israel, Europe, North Africa and China [7]. It also occurs in Tehran, Iraq and Turkey [8]. The plant is commonly known as White Behen, Safaid Behmen, Behman abyaz and White Rhapontic [7]. The roots of *C. behen* are used for killing the lice and making the hair good smelling [9]. Roots act as nervine and anabolic tonic, strengthen central nervous system and used in affections of kidney [6]. Roots of *C. behen* constitute the drug Safed behman or Bhamana-i-sufeed. These are credited with fattening and aphrodisiacal properties. These are also used in calculus affections. The root is reported to be an ingredient of the the Unani formulation Davabulmusk, used to strengthen cardiac muscles and central nervous system; Laboobaisagheer, used in polyurea, tissue building of nerves and kidney, and Mahajan-e-til, used in dyspepsia, congested liver and anorexia [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* 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.

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).All the chemicals and solvent used in this study were 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

Plant material were extracted in four solvents of different polarity viz water, methanol, ethyl acetate and chloroform. Powdered plant materials were extracted by maceration method. The resultant content was filtered with whatman filter paper no.1 and kept for evaporation of solvent to get the dry concentrated extract. The dried crude concentrated extract was weighed to calculate the extractive yield then transferred to glass vials (6 ×2 cm) and stored in a refrigerator (4°C), till used for analysis [12].

2.5 Phytochemical screening

Phytochemical screening to detect the presence of bioactive agents was performed by standard procedures [13, 14]. After the addition of specific reagents to the solution, the tests were detected by visual observation of color change or by precipitate formation.

2.6 Total flavonoid contents

The total flavonoid content was determined using the method of Olufunmiso *et al.*, 2011 [15]. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 60 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer The content of flavonoids was calculated

using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/g).

2.7 Antioxidant activity

2.7.1 DPPH radical scavenging assay

DPPH scavenging activity was measured by modified method of Olufunmiso *et al.*, 2011 [15]. DPPH scavenging activity was measured by the spectrophotometer. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10-100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm. The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%. Though the activity is expressed as 50% inhibitory concentration (IC50), IC50 was calculated based on the percentage of DPPH radicals scavenged. The lower the IC50 value, the higher is the antioxidant activity.

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

The determination of NO^o radical scavenging ability of the extracts is based on the inhibition of NO^o radical generated from sodium nitroprusside in phosphate buffer saline solution by Griess reagent (1% sulfanilamide, 2% orthophosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride). Scavengers of nitric oxide act against oxygen, prompting to lessened production of nitrite ions which can be monitored at 546 nm [16]. Briefly, sodium nitroprusside (0.6 ml, 5 mM) solution was mixed with and without varying the concentration of the extracts or Ascorbic acid (2 ml, 10-200µg/ml) and incubated at 25 ± 2°C for 5 h. Incubated solution (2 ml) was mixed with equal volume of Griess reagent and absorbance of the purple colored azo dye chromophore was measured at λmax 546 nm using UV-Vis spectrophotometer. 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 collected from the local area of Bhopal, MP, India. Air-dried and extracted by maceration extraction process. The crude extracts so obtained after each of the maceration extraction process were concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The yield of extracts obtained from the roots of the plants using petroleum ether, chloroform, ethyl acetate, methanol and water as solvents are depicted in the Table 1. The results of qualitative phytochemical analysis of the crude powder roots of *C. behen* are shown in Table 2. methanolic and ethyl acetate extracts of roots sample of *C. behen* showed the presence of flavonoids, diterpines, carbohydrate and proteins but in chloroform extracts all phytoconstituents was absents and carbohydrate was present in *C. behen* extract. The total flavonoids content of the extracts was expressed as percentage of quercetin equivalent per 100 mg dry weight of sample. The total flavonoids estimation of methanolic, aqueous and ethyl acetate extracts of roots of *C. behen* showed the content values of 0.381, 0.465 and 0.740 respectively Table3 & Figure1. DPPH radical scavenging assay measured hydrogen donating nature of extracts [17]. Under DPPH radical scavenging activity the inhibitory concentration 50% (IC_{50}) value of *C. behen* ethyl acetate extract was found to be 69.72µg/ml as compared to that of ascorbic acid (17.68µg/ml). A dose dependent activity with respect to concentration was observed Table 4 & Figure 2. Extracts showed NO scavenging effects by competing with oxygen to react with NO directly hence inhibited the nitrite ion formation [18]. *C. behen* ethyl acetate extract showed nitric oxide (NO^o) radical scavenging activity with IC_{50} value of 65.43µg/ml, as compared to that of ascorbic acid (IC_{50} 17.68µg/ml). *C. behen* extract showed significant activity Table 5 and Figure 3.

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: Lead acetate Test:	- ve - ve	+ve +ve	+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

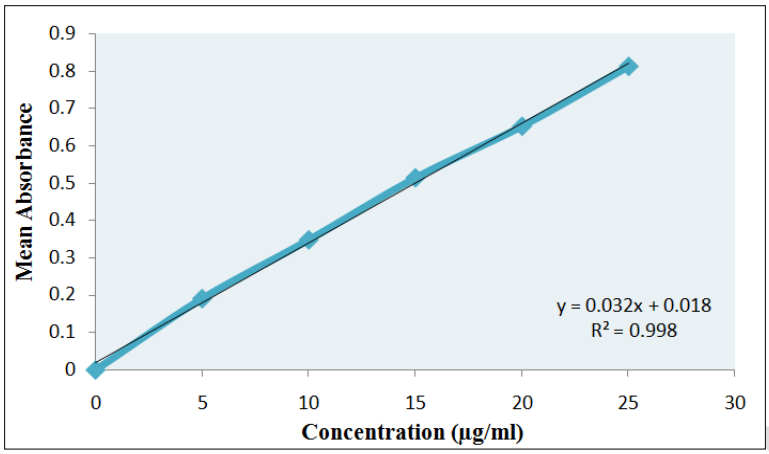

Fig. 1: Graph of estimation of total flavonoids content

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

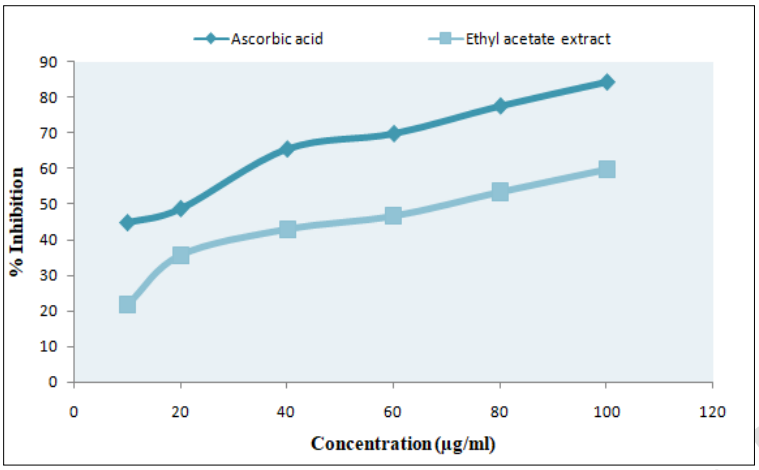

Fig. 2: Inhibition of ascorbic acid and ethyl acetate extract of *C. behen* using DPPH method

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

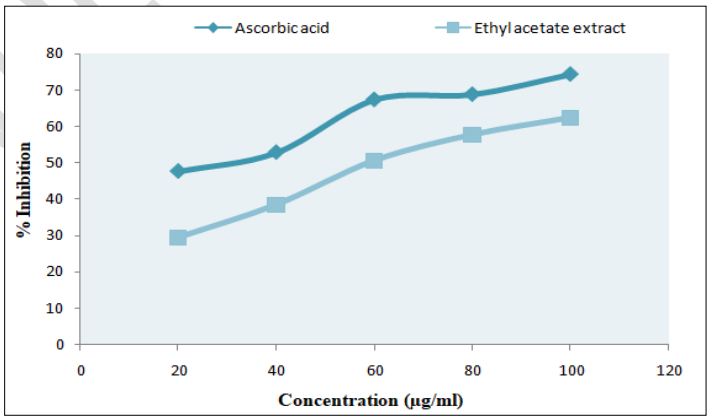

Fig. 3: % Inhibition of ascorbic acid and ethyl acetate extract of *C. behen* using NO method

4. CONCLUSION

It can be concluded that from present investigation the phytochemical investigation gave valuable information about the different phytoconstituents present in the plant, which helps the future investigators concerning the selection of the particular extract for further investigation of isolating the active principle and also gave idea about different phytochemical have been found to possess a wide range of activities. The total flavonoid content in ethyl acetate, methanol and aqueous roots extract is further proved by in vitro antioxidant studies. The extract, which can effectively scavenge various reactive oxygen species/free radicals under in vitro conditions. This may be due to the number of stable oxidized products that it can form after oxidation or radical scavenging. The broad range of activity of the extracts suggests that multiple mechanisms are responsible for the antioxidant activity. The multiple antioxidant activity of extract demonstrated in this study clearly indicates the potential application value of the plant. Further studies, on the use of above plant for their antioxidant role in various systems may provide potential 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.

REFERENCES

1. Chatopadhyay I, Biswas K, Bandhopadhyay U, Banerjee RK. *Curr Sci*., 2004; **87:**44.
2. Zawadzka-Bartczak E, Kopka L, Gancarz A. *Aviat Space Environ Med*., 2003; **74:**654-658
3. Kumar S, Pandey A. *SCI World J*., 2013; **23:**1-16.

4. Yu L. *J Agric Food Chem*., 2001; **49:**3452-3546.
5. Ebrahimzadeh M, Nabavi S, Nabavi S, Eslami B. *Cent Eur J Biol*., 2010; **5:**338-345.
6. Khare CP. Indian medicinal plants. 2007; pp 36. Springer-Verlag Berlin/Heidelberg.
7. Khare CP. Indian Herbal Remedies: Rational Western Therapy, Ayurvedic and Other Traditional Usage, Botany, 2004; pp 137-138. Springer.
8. Mirza M, Najafpour NM, Dini M. *Iranian Journal of Aromatic and Medicinal Plants,* 2003; **19:**227-32.
9. Abivardi C. Iranian entomology: An Introduction. 2001; pp 520. Springer, Berlin.
10. Pullaiah T. Encyclopedia of world medicinal plants. 2006; pp 500. Regency publications, New Delhi, India.
11. Bhargava PN, Dutt S. *Proceedings of the Indian Academy of Sciences*, 1944; **19:**163-166.
12. Mukherjee PK. Quality control of herbal drugs. 2nd Ed. Business Horizons; 2007.
13. Khandelwal KR. Practical pharmacognosy technique and experiments. 23rd Ed. Nirali Prakashan; 2005.
14. Kokate CK. Practical pharmacognosy. 4th Ed. Vallabh Prakashan; 1994.
15. Olufunmiso OO, Afolayan AJ. *BMC Complement Alter Med.,* 2011; **11:**130.
16. Shirwaikarkar A, Somashekar AP. *India J Pharm Sci.*, 2003; **65:**67-9.
17. Hudson BJ. Food antioxidants. In: Gordon MH, editor. The Mechanism of Antioxidant Action *in Vitro*. London: Elsevier Applied Science; 1990.
18. Sunil C, Ignacimuthu S. *Food Chem Toxicol*., 2011; **49:**1604-9.