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# Extraction Phytochemical Screening, Estimation of Flavonoid Content and Antioxidant Potential of Centaurea Behen L.

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***Abstract***
*The present study investigated the phytochemical composition, total flavonoid content, and antioxidant activity of various root extracts of* Centaurea behen *L., using solvents of varying polarity. Extractive values indicated that polar solvents, particularly water, yielded the highest recovery of bioactive compounds, followed by methanol, while non-polar solvents such as petroleum ether and chloroform produced significantly lower yields. Preliminary phytochemical screening revealed that the chloroform extract contained only carbohydrates, whereas the ethyl acetate extract was rich in flavonoids, diterpenes, and carbohydrates. Methanolic and aqueous extracts showed the presence of flavonoids, diterpenes, proteins, and carbohydrates, with saponins detected exclusively in the aqueous extract, highlighting water's efficiency in extracting highly polar constituents. Quantitative analysis of total flavonoid content showed that the ethyl acetate extract possessed the highest concentration, followed by aqueous and methanolic extracts, suggesting that intermediate polarity favors certain flavonoid subtypes. Antioxidant activity, assessed via DPPH and nitric oxide scavenging assays, demonstrated a dose-dependent radical scavenging potential in the ethyl acetate extract, though its activity was lower than that of ascorbic acid. The observed antioxidant effects are likely due to flavonoids and other phenolic-like compounds. The findings validate the traditional medicinal use of* Centaurea behen *roots and support further isolation and characterization of its bioactive constituents for potential incorporation into herbal formulations targeting oxidative stress.*

***Keywords***: Centaurea behen *L., phytochemical screening, total flavonoid content, antioxidant activity, ethyl acetate extract, DPPH assay, nitric oxide scavenging, oxidative stress, medicinal plants.*

## INTRODUCTION

Plants have served as a significant source of therapeutic agents since ancient times, and much of modern pharmacology has been inspired by traditional plant-based remedies. Among various medicinal plants, Centaurea behen L., belonging to the Asteraceae family, has attracted attention for its wide range of pharmacological activities, including antioxidant, anti-inflammatory, hepatoprotective, and antimicrobial effects [1,2].

Centaurea behen is native to Central Asia and is widely used in Unani and Ayurvedic medicine systems. Traditionally, its roots and aerial parts have been employed for treating liver disorders, fatigue, sexual weakness, and nervous system ailments [3,4]. These therapeutic effects are believed to be due to the presence of diverse phytoconstituents such as flavonoids, phenolic acids, tannins, alkaloids, and sesquiterpene lactones [5,6].

Flavonoids, a major class of polyphenolic compounds in plants, are known for their potent antioxidant properties. These compounds can scavenge free radicals, chelate metal ions, and modulate oxidative stress pathways, thereby playing a key role in preventing degenerative diseases including cancer, cardiovascular disorders, and neurodegenerative conditions [7,8]. Hence, the quantification of flavonoids and evaluation of antioxidant potential in medicinal plants like Centaurea behen is crucial for establishing their therapeutic value.

In recent years, natural antioxidants from plant sources have gained significant importance due to the limitations and side effects associated with synthetic antioxidants. Therefore, exploring the antioxidant potential of Centaurea behen through in-vitro assays such as DPPH radical scavenging activity and total flavonoid content estimation becomes vital for its validation as a natural antioxidant source [9,10].

This study aims to investigate the ethanolic root extract of Centaurea behen L. for its phytochemical profile, determine the total flavonoid content, and evaluate its antioxidant potential using established in vitro methods. The findings of this study may provide scientific validation to its traditional uses and promote its development into a potential phytotherapeutic agent.

## MATERIAL AND METHODS

### Procurement of Plant Material

Roots of Centaurea behen free of diseases, such as root were collected in separate sterile bags from Bhopal, Madhya Pradesh, and month of October, 2020.

### Extraction

### Defatting of Plant Material

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

### 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 [11]. 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.

### Estimation of DPPH radical scavenging activity:

Firstly, add 1.5 ml of DPPH solution in volumetric flask and adjust volume upto 3 ml with methanol solvent immediately, absorbance of solution was taken at 517 nm for control reading while, ascorbic acid was used as reference standard. Then, take 1.5 of DPPH solution and l.0 ml of plant sample extracts/ standard of different concentration (10 µg/ml, 20µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100µg/ml) in a series of volumetric flasks and final volume was adjusted with methanol up to 3 ml and three test samples were taken for each plant extract and processed similarly. Immediately absorbance was taken for each concentration of plant samples/ standard. Subsequently, the mixture was mixed vigorously and leave at room temperature for 15 minutes and the degree of free radical scavenging activity in the presence of different concentration of different extracts were measured at 517 nm through UV/ visible spectrophotometer [12]. The free radical scavenging activity of plant samples was calculated as the percentage inhibition using following equation.

$$\textbf{Calculation of \% Reduction} = \frac{\text{Control Absorbance} - \text{Test absorbance}}{\text{Control Absorbance}} \text{X } 100$$

Control Absorbance = Absorbance of DPPH alone

Test absorbance=Absorbance of DPPH along with different concentrated of extracts

Though, the antioxidant activity of selected plant extract was expressed as 50% inhibitory concentration ($IC_{50}$), $IC_{50}$ was determined based on the percentage of DPPH radicals scavenged in the presence of different concentration of selected plant samples. If $IC_{50}$ value was lower indicated, higher was the antioxidant activity of plant samples.

### Nitric oxide method

Nitric oxide was produced from sodium nitroprusside and the Griess reagent was measured. Sodium nitroprusside spontaneously produces nitric oxide in aqueous solution at physiological pH, interacting with oxygen to generate nitric ions that can be estimated using Griess reagent. Nitric oxide scavengers compete with oxygen resulting in decreased nitric oxide manufacturing (Marcocci et al., 1994). Sodium nitroprusside (10 mmol / L) was mixed with various extract concentrations in phosphate buffer saline (PBS) and incubated at 25°C for 150 min. Griess reagent (1% sulphanilamide, 2% $H_3PO_4$ and 0.1% napthylethylenediamine dihydrochloride) was added to the specimens. The chromophore absorbance created during the diazotization of sulphanilamide nitrite and subsequent coupling with napthylethyleneediamine was read at 546 nm and referred to the absorption of conventional ascorbic acid solutions treated in the same manner with Griess reagent as a positive control [13]. All triplicate experiments were conducted and the chart was plotted with the mean values. The inhibition proportion was evaluated using the following formula:

$$\text{Radical scavenging activity (\%)} = \frac{\text{Control Absorbance} - \text{Test absorbance}}{\text{Control Absorbance}} \text{X } 100$$

Where Acontrol is the absorption (without extract) of the control and where Atest is the absorption in the presence of the extract / standard.

### Results and Discussion

The present study investigated the phytochemical composition, total flavonoid content, and antioxidant activity of various root extracts of Centaurea behen L. The extractive values varied with the polarity of the solvents used. Among all, the aqueous extract showed the highest yield, followed by methanol, indicating that polar solvents are more efficient in extracting a greater quantity of bioactive compounds. Non-polar solvents like petroleum ether and chloroform resulted in significantly lower yields, suggesting limited solubility of phytoconstituents in these solvents.

Phytochemical screening revealed distinct differences in the chemical profiles of the extracts. The chloroform extract showed the presence of only carbohydrates, while other secondary metabolites were absent. In contrast, the ethyl acetate extract tested positive for flavonoids, diterpenes, and carbohydrates, suggesting that it effectively extracted moderately polar phytochemicals. The methanolic extract was rich in flavonoids (as per the alkaline reagent test), diterpenes, proteins, and carbohydrates, reflecting its strong extraction capability for a wide range of polar compounds. The aqueous extract also showed the presence of flavonoids, diterpenes, proteins, carbohydrates, and saponins. The presence of saponins only in the aqueous extract emphasizes the role of water in extracting highly polar or hydrophilic compounds.

The estimation of total flavonoid content revealed that the ethyl acetate extract had the highest flavonoid concentration, followed by the aqueous and methanol extracts. This suggests that flavonoid compounds are more readily soluble in ethyl acetate compared to water and methanol, likely due to the solvent's intermediate polarity, which favors the extraction of certain flavonoid subtypes.

The antioxidant activity of the ethyl acetate extract was evaluated using both DPPH and nitric oxide scavenging assays. In both methods, the extract showed a dose-dependent increase in radical scavenging activity. However, its antioxidant potential was lower than that of the standard antioxidant, ascorbic acid. In the DPPH assay, the extract demonstrated moderate scavenging ability, with an $IC_{50}$ value higher than that of ascorbic acid. A similar trend was observed in the nitric oxide scavenging assay, indicating a consistent but moderate antioxidant response. The results suggest that the antioxidant activity may be attributed to the presence of flavonoids and other phenolic-like compounds identified in the ethyl acetate extract. The study supports the traditional use of Centaurea behen roots for their medicinal properties. The presence of bioactive phytochemicals such as flavonoids and diterpenes, especially in the ethyl acetate extract, correlates with its observed antioxidant potential. These findings lay a foundation for further exploration and isolation of specific compounds responsible for the biological activities of the plant and support its potential use in herbal formulations aimed at combating oxidative stress.

**Table 1: Extractive values of root extracts of Centaurea behen**

| S. No. | Solvents | Colour | Physical nature | % Yield (W/W) |
|---|---|---|---|---|
| 1 | Pet. ether | Sticky green | Solid | 0.274 |
| 2 | Chloroform | Light brown | Solid | 0.366 |
| 3 | Ethyl acetate | Brown | Solid | 0.557 |
| 4 | Methanol | Brown | Solid | 5.444 |
| 5 | Aqueous | Brown | Solid | 6.987 |

**Table 2: Result of Phytochemical screening of chloroform extract of Centaurea behen**

| S. No. | Constituents | Chloroform extract | Observation |
|---|---|---|---|
| 1. | **Alkaloids**<br>Hager's Test: | <br>-ve | Yellow colour but no ppt |
| 2. | **Glycosides**<br>Legal's Test: | <br>- ve | Green colour |
| 3. | **Flavonoids**<br>Alkaline Reagent Test:<br>Lead acetate Test: | <br>- ve<br>- ve | <br>No Colourless<br>No White ppt |
| 4. | **Diterpenes**<br>Copper acetate Test: | <br>- ve | <br>No Emerald green coloured |
| 5. | **Phenol**<br>Ferric Chloride Test: | <br>- ve | <br>Green coloured |
| 6. | **Proteins** | | |

| | | | |
|---|---|---|---|
| | Xanthoproteic Test: | - ve | Light green coloured |
| 7. | **Carbohydrate**<br>Fehling's Test: | + ve | Red precipitate |
| 8. | **Saponins**<br>Froth Test: | -ve | No foam |

**Table 3: Result of Phytochemical screening of ethyl acetate extract of Centaurea behen**

| S. No. | Constituents | Ethyl acetate extract | Observation |
|---|---|---|---|
| 1. | **Alkaloids**<br>Hager's Test: | -ve | Yellow colour |
| 2. | **Glycosides**<br>Legal's Test: | - ve | Yellow colour |
| 3. | **Flavonoids**<br>Alkaline Reagent Test:<br>Lead acetate Test: | + ve<br>+ ve | Colourless<br>White ppt |
| 4. | **Diterpenes**<br>Copper acetate Test: | + ve | Emerald green coloured |
| 5. | **Phenol**<br>Ferric Chloride Test: | - ve | Green coloured |
| 6. | **Proteins**<br>Xanthoproteic Test: | - ve | Brown coloured |
| 7. | **Carbohydrate**<br>Fehling's Test: | + ve | Red precipitate |
| 8. | **Saponins**<br>Froth Test: | - ve | No layer of foam |

**Table 4: Result of Phytochemical screening of methanol extract of Centaurea behen**

| S. No. | Constituents | Methanol extract | Observation |
|---|---|---|---|
| 1. | **Alkaloids**<br>Hager's Test: | -ve | Yellow colour but no ppt |
| 2. | **Glycosides**<br>Legal's Test: | - ve | Green colour |
| 3. | **Flavonoids**<br>Alkaline Reagent Test:<br>Lead acetate Test: | + ve<br>- ve | Colourless<br>White coloured |
| 4. | **Diterpenes**<br>Copper acetate Test: | + ve | Emerald green coloured |
| 5. | **Phenol**<br>Ferric Chloride Test: | - ve | Yellow coloured |
| 6. | **Proteins**<br>Xanthoproteic Test: | +ve | Yellow coloured |
| 7. | **Carbohydrate**<br>Fehling's Test: | + ve | Red precipitate |
| 8. | **Saponins**<br>Froth Test: | - ve | No foam |

**Table 5: Result of Phytochemical screening of aqueous extract of Centaurea behen**

| S. No. | Constituents | Aqueous extract | Observation |
|---|---|---|---|

| | | | |
|---|---|---|---|
| 1. | **Alkaloids**<br>Hager's Test: | <br>-ve | Yellow coloured |
| 2. | **Glycosides**<br>Legal's Test: | <br>- ve | Light brown coloured |
| 3. | **Flavonoids**<br>Alkaline Reagent Test:<br>Lead acetate Test: | <br>+ ve<br>+ve | <br>Colourless<br>White ppt |
| 4. | **Diterpenes**<br>Copper acetate Test: | <br>+ ve | Emerald green coloured |
| 5. | **Phenol**<br>Ferric Chloride Test: | <br>- ve | Brown coloured |
| 6. | **Proteins**<br>Xanthoproteic Test: | <br>+ve | Yellow coloured |
| 7. | **Carbohydrate**<br>Fehling's Test: | <br>+ ve | Red precipitate |
| 8. | **Saponins**<br>Froth Test: | <br>+ ve | Layer of foam |

**Table 6: Results of Estimation of flavonoids content of root extracts of Centaurea 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 |

**Table 7: % Inhibition of Ascorbic acid and Ethyl acetate extract of Centaurea 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 |

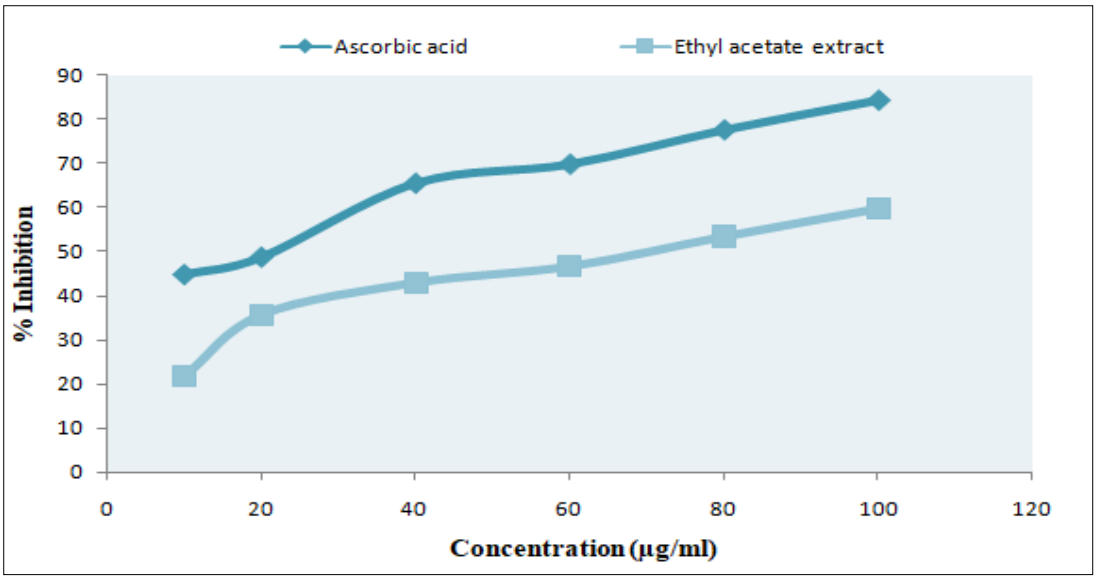


**Figure 1: % Inhibition of ascorbic acid and Ethyl acetate extract of Centaurea behen using DPPH method**

**Table 8: % Inhibition of ascorbic acid and Ethyl acetate extract of Centaurea 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 |

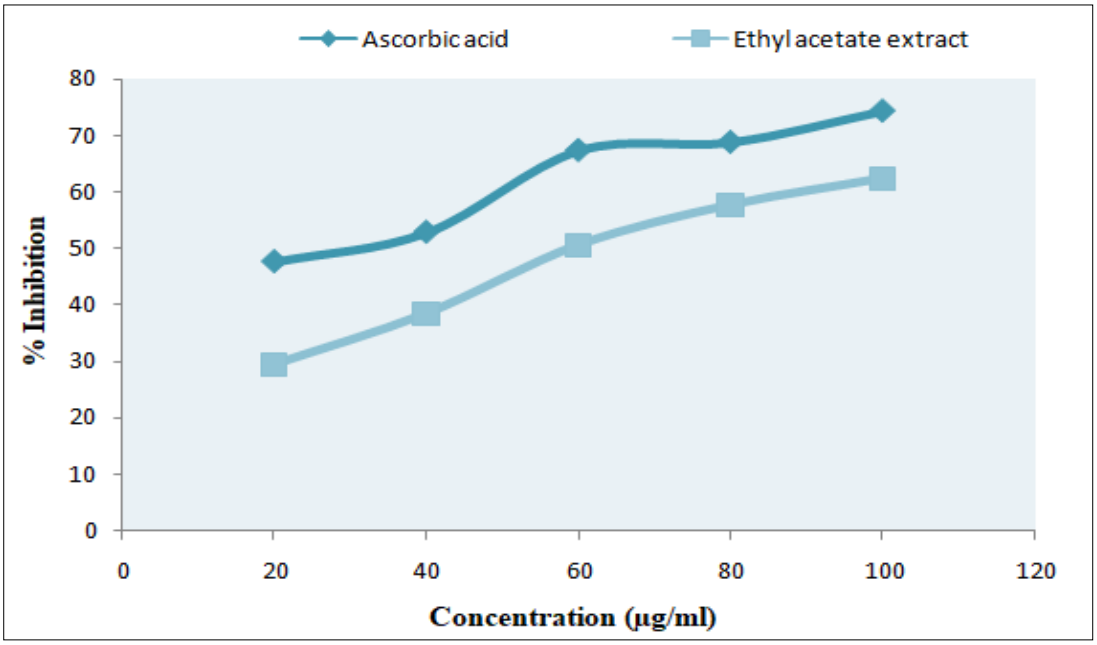


**Figure 2: % Inhibition of ascorbic acid and Ethyl acetate extract of Centaurea behen using NO method**

## CONCLUSION

The present study highlights the significant phytochemical diversity and moderate antioxidant potential of Centaurea behen L. root extracts. The variation in extractive yields with different solvents underscores the importance of solvent polarity in phytochemical extraction. Among the tested extracts, ethyl acetate demonstrated the highest total flavonoid content and notable antioxidant activity, though it remained less potent than ascorbic acid. The presence of bioactive compounds such as flavonoids, diterpenes, proteins, carbohydrates, and saponins supports the plant's traditional medicinal uses in Unani and Ayurvedic systems. These findings validate Centaurea behen as a promising source of natural antioxidants and provide a scientific basis for its potential inclusion in herbal formulations aimed at managing oxidative stress-related disorders. Further studies involving isolation, structural characterization, and in-vivo evaluation of its active constituents are warranted to fully establish its therapeutic potential.

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