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Original Article

Safety Evaluation of *Centaurea behen* Linn.: A Comprehensive Study

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ABSTRACT

Background: Herbal medicines hold significant importance in traditional healthcare practices. As per the World Health Organization (WHO), approximately 70-80% of the world's population relies on traditional remedies to meet their healthcare requirements. Herbs are commonly used for both treatment and prevention of various diseases. However, growing interest in herbal products has raised concerns about their safety. While often perceived as harmless due to their natural origin, instances of toxicity and adverse effects have been reported. As per WHO guidelines, it is essential to conduct safety assessments of herbal medicines and food products to identify potential risks arising from environmental or soil contaminants. **Materials and Methods:** Plants are prone to contamination by harmful substances. This study involved evaluating the safety profile of *Centaurea behen* Linn from the Asteraceae family by analyzing microbial load, heavy metal content through Atomic Mass Spectroscopy (AMS), aflatoxins via Liquid Chromatography-Tandem Mass Spectrometry, and pesticide residues using Gas Chromatography-Tandem Mass Spectrometry. **Results:** Findings revealed that heavy metals such as lead, cadmium, mercury, and arsenic were within acceptable limits as outlined by WHO standards. Furthermore, no Aflatoxins, pesticide residues, or microbial contamination were detected in the crude drug sample. **Conclusion:** The safety evaluation indicates that *Centaurea behen* Linn. is safe for use, with no evidence of toxicity risks.

Keywords: Behman Safed, *Centaurea behen* Linn., Heavy Metals, Pesticidal Residues, Safety Evaluation, WHO Guidelines.

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INTRODUCTION

Centaurea behen Linn. a medicinal herb belongs to the Asteraceae family. The origins of Safed Behman (White Rhapontic) can be traced back to mentions by European authors in Materia Medica and its Arabic equivalent, Bahman Abiaz. Ancient Persian civilizations extensively utilized both Red and White Bahman as medicinal remedies, passing their knowledge to the Arabs, who subsequently introduced it to Western cultures. The term Bahman translates to "the supreme intelligence" and is also the name of a Persian month. According to Burhan-e-Qati, the plant blooms during the month of Behman (January), and the second day of this month is associated with its significance (Yuan *et al.*, 2016; World Health Organization, 2013).

This plant exists in two varieties—white and red. The root of the white variety is noted for its aphrodisiac properties and its ability to reduce fat and alleviate flatulence. Historically, Persians observed the second day of the month Bahman—known as Bahmanjana—as an auspicious day, marked by preparing special meals with meat and grains, adorned with white and red Behman flowers. The day was also regarded as ideal for harvesting medicinal plants, initiating new ventures, wearing fresh clothing, and grooming tasks such as trimming hair or nails (The safety of Herbal Medicine from Prejudice to Evidence).

Unani physicians, particularly Muslim practitioners, have long valued Bahman Safed. It is commonly prescribed for conditions such as calculous diseases and jaundice, being considered both hot and dry (2° each) and a potent aphrodisiac. It is also known to counteract phlegmatic humors. Some sources, such as Ainslie, mistakenly equate its root with that of *Withania somnifera* (Asgandh). The usual dosage is one dirham. Although Red Bahman, or Red Rhapontic, is often associated with its white counterpart in Eastern texts, its origin remains unclear and its root structure differs significantly. According to Hakim Muhammad Husain Khan, author of Makhzan al-Advia, some attribute it to the root of a plant called Kaf-e-Adam.

Classical Unani literature highlights numerous health benefits of Bahman Safed. Modern scientific research has further

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explored its chemical composition, therapeutic applications, and pharmacological properties, offering deeper insights into its medicinal potential.

Herbs frequently contain a substantial amount of molds and bacteria, mainly derived from soil or organic fertilizers. Improper harvesting and production practices, combined with inadequate control of moisture levels during the transportation and storage of herbal medicines, can further contribute to contamination and microbial growth.

Aflatoxins are naturally occurring mycotoxins predominantly produced by *Aspergillus flavus* and *Aspergillus parasiticus*, along with other species such as *A. nomius, A. ochraceoroseus, A. bombycis*, and *A. pseudotamari*. These fungal secondary metabolites are classified into four main types: B1, G1, B2, and G2. Among these, Aflatoxin B1 (AFB1) is the most toxic and is recognized as the most potent natural carcinogen, primarily targeting the liver. The International Agency for Research on Cancer (IARC) has categorized AFB1 as a Group I carcinogen, signifying its high carcinogenic potential.

The consumption of herbal plants and medicines contaminated with aflatoxins or heavy metals is a recognized source of toxicity. Heavy metals enter the environment through natural processes and various anthropogenic activities. Their accumulation in plant tissues depends largely on their soil concentration and bioavailability, but they can also settle on plant surfaces through atmospheric deposition. Due to their persistence and long biological half-life, heavy metals present significant health hazards ("Microbiological Quality Assessment of Some Commercial Herbal Drugs").

Key heavy metals include cadmium, arsenic, mercury, and lead. The harvesting and cultivation of plants having medicinal property near industrial areas or locations where these metals are improperly disposed of should be avoided; as such environments increase the likelihood of heavy metal accumulation in plants. This elevated risk of contamination can lead to significant health concerns when these plants are consumed (National Center for Complementary and Integrated Health, National Institute of Health (NIH).

MATERIALS AND METHODS

Collection and Authentication

The test drug, Bahman Safed (*Centaurea behen* Linn.), was sourced from Dawakhana Tibbiya College, Aligarh Muslim University (AMU), Aligarh, Uttar Pradesh, India. Its identity was verified based on Unani classical literature and botanical literature in the lab of pharmacognosy, Department of Ilmul Advia, A.K.T.C. and further authenticated in the Department of Botany AMU, Aligarh.

A herbarium specimen of the test drug prepared and deposited in the Mawaleed-e-Slasah museum of Ilmul Advia Department, AMU, for the purpose of future reference. The collected drug was subsequently powdered and stored in an airtight container for use in experimental studies.

The powdered fruits of *Centaurea behen* Linn. were analyzed to assess the concentration of Aflatoxins, heavy metals, pesticide residues, and microbial load. These evaluations were conducted at AGSS Analytical and Research Lab Pvt. Ltd., Delhi, India (ULR No. TC121152400009268F; Report No. AGSS/AP/24071300009). The sample was submitted on July 13, 2024, and the results were reported on July 18, 2024.

Heavy Metal Analysis

The analysis of heavy metals was conducted for measuring levels of impurities of metals for the test drug. Impurity with heavy metals like arsenic, cadmium, mercury, and lead may result from various sources, including environmental pollution. The heavy metal content was assessed at AGSS Analytical and Research Lab Pvt. Ltd., Delhi, following the testing protocol outlined in the ASU Guidelines (Table 1) using Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Aflatoxin Estimation

Sample Preparation

A 2 g sample was combined with 20 mL of a 60% acetonitrile-water solution and blended at high speed for 2 min. The mixture was then subjected to centrifugation at 1600 g for 10 min, and the supernatant was carefully separated. To reduce the solvent concentration to 10% or less, 2 mL of the supernatant was mixed with 48 mL of Phosphate-Buffered Saline (PBS) at pH 7.4. The prepared solution was then passed through an immunoaffinity column at a flow rate of 5 mL/min.

The column was rinsed by passing 20 mL of distilled water through it at a similar flow rate and then dried quickly. Subsequently, 1.5 mL of distilled water was added to the sample eluate. A 500 µL portion of the prepared sample was injected into the LC-MS/MS system for analysis (LC-Perkin, MS Applied Biosystems, Model No. 2000).

LC-MS/MS Parameters

- Mobile Phase: A - 100% Water, B - 100% Acetonitrile.
- Column: ZORBAX Rx C18, narrow base (2.1 x 150 mm, 5 µm).
- Column Oven Temperature: 30°C.
- Flow Rate: 0.750 ml/min.

The concentration of aflatoxins was determined by comparing the peak heights or areas of the sample to those of a known Aflatoxin standard (Table 2).

Microbial Load Analysis

The World Health Organization (WHO) mandates the determination of microbial load in all herbal drugs to ensure safety for human use. The microbial load assessment was conducted following WHO guidelines (Table 3).

Method: Total Bacterial Count

Sample Preparation and Pre-treatment

The preparation process was tailored to the nature of the test sample. The antimicrobial properties of the sample were neutralized or diluted appropriately. The test sample was diluted using Buffered Sodium Chloride-Peptone Solution (pH 7.0, MM1275-500G, Himedia Labs, Mumbai, India).

For Water-Soluble Materials

- A 10 g portion of the test sample was dissolved in lactose broth (M1003-500G, Himedia Labs, Mumbai, India), which was verified to be free of bacterial activity under test conditions unless stated otherwise.
- The solution was diluted to 100 mL using the same medium, and the pH was adjusted to approximately 7.

For Non-Fatty, Water-Insoluble Materials

- A 10 g portion of the test sample was dissolved in lactose broth with no antimicrobial activity under test conditions.
- A suitable surfactant solution containing Polysorbate 20R (M1307-500G, Himedia Labs, Mumbai, India) and 1 mg/mL of Potassium Tellurite (FD052, Himedia Labs, Mumbai, India) was added to aid dilution.

Table 1: **Heavy Metal Analysis of *Centaurea behen* Linn.**

Sl. No.	Test Parameters	Test Result mg/kg	LOQ (mg/kg)	Permissible Limits as API
1	Lead as Pb (mg/kg)	0.05	2.50	10.0 max
2	Mercury as Hg (mg/kg)	0.05	0.50	1.0 max
3	Arsenic as As (mg/kg)	0.05	1.25	3.0 max
4	Cadmium as Cd (mg/kg)	0.05	0.25	.30 max

LOQ = Limit of Quantification.

Table 2: **Test for Aflatoxins in *Centaurea behen* Linn.**

Sl. No.	Aflatoxins	Results	Limit of Quantification	Permissible Limit as per API
1	Aflatoxin B1	1.0 u/gm	0.001	2.0 max
2	Aflatoxin B2	1.0 u/gm	0.001	5.0 max
3	Aflatoxin G1	1.0 u/gm	0.001	5.0 max
4	Aflatoxin G2	1.0 u/gm	0.001	5.0 max

Table 3: **Microbiological test of *Centaurea behen* Linn.**

Sl. No.	Test Parameters	Test Result mg/kg	LOQ (mg/kg)	Permissible Limits as API
1	Total Bacterial Count (cfu/gm)	55000	-	100000 max
2	Total Yeast and Mould (cfu/gm)	300	-	1000 max

Any Specific Pathogens

Sl. No.	Test Parameters	Test Result mg/kg	LOQ (mg/kg)	Permissible Limits as API
3	E. coli/gm	Absent	-	Absent
4	Salmonella/gm	Absent	-	Absent
5	S. aureus/gm	Absent	-	Absent
6	P. aeruginosa/gm	Absent	-	Absent

- The final volume was adjusted to 100 mL with the same medium, and the pH was set to about 7.

Testing Procedures

Plate Count for Bacteria

- A 1 mL aliquot of the pre-treated sample was mixed with approximately 15 mL of liquefied casein-soybean digest agar (M290-500G, Himedia Labs, Mumbai, India) in a 90 mm Petri dish, maintained at a temperature not exceeding 45°C. Alternatively, the sample was spread on the surface of the solidified medium.
- Two Petri dishes were prepared for each dilution, inverted, and incubated at 30–35°C for 48–72 hours or until a reliable count was obtained.
- The number of colonies formed was counted, with results calculated from plates containing up to 300 colonies.

Plate Count for Fungi

- A 1 mL aliquot of the pre-treated sample was mixed with approximately 15 mL of liquefied Sabouraud glucose agar with antibiotics (MI472-500G, Himedia Labs, Mumbai, India) in a 90 mm Petri dish, maintained at a temperature not exceeding 45°C. Alternatively, the sample was spread on the solidified medium.
- Two Petri dishes were prepared for each dilution, inverted, and incubated at 20–25°C for five days or until reliable counts were obtained.
- The colonies were counted, with results calculated from plates containing no more than 100 colonies.
- This method ensures an accurate determination of bacterial and fungal loads in herbal drugs.

Table 4: Pesticidal Residue in *Centaurea behen* Linn.

Sl. No.	Pesticide Residue (mg/kg)	Results	Limit of Quantification	Permissible Limit (mg/kg)
1	Alachor	Not Detected	0.02	0.02
2	Aldrin and Dieldrin (Sum of)	Not Detected	0.04	0.05
3	Azinophos-methyl	Not Detected	0.04	1.0
4	Bromopropylate	Not Detected	0.08	3.0
5	Chlordane (Sum of cis, trans and oxychlordane)	Not Detected	0.04	0.05
6	Chlorfenvinphos	Not Detected	0.04	0.5
7	Chlorpyrifos	Not Detected	0.04	0.2
8	Chlorpyrifos-methyl	Not Detected	0.04	0.1
9	Cypermethrin (and isomers)	Not Detected	0.1	1.0
10	DDT (Sum of p,p-DDT,,p,p-DDE and p,p-TDE)	Not Detected	0.04	1.0
11	Deltamethrin	Not Detected	0.1	0.5
12	Diazinon	Not Detected	0.04	0.5
13	Dichlorvos	Not Detected	0.04	1.0
14	Dithiocarbametes (as CS2)	Not Detected	0.01	2.0
15	Endosulfan (Sum of isomer and Endosulfansulphate)	Not Detected	0.04	3.0
16	Endrin	Not Detected	0.04	0.05
17	Ethion	Not Detected	0.04	2.0
18	Fenitrothion	Not Detected	0.04	0.5
19	Fenvalerate	Not Detected	0.1	1.5
20	Fonofos	Not Detected	0.04	0.05
21	Heptachlor (Sum of Heptachlor and Heptachlor epoxide)	Not Detected	0.04	0.05

Sl. No.	Pesticide Residue (mg/kg)	Results	Limit of Quantification	Permissible Limit (mg/kg)
22	Hexachlorobenzene	Not Detected	0.04	0.1
23	Hexachlorocyclohexane isomer (other than γ)	Not Detected	0.04	0.3
24	Lindane (γHexachlorocyclohexane)	Not Detected	0.04	0.6
25	Malathion	Not Detected	0.04	1.0
26	Methidathion	Not Detected	0.04	0.2
27	Parathion	Not Detected	0.04	0.5
28	Parathion Methyl	Not Detected	0.04	0.2
29	Permethrin	Not Detected	0.04	1.0
30	Phosalone	Not Detected	0.04	0.1
31	Piperonylbutoxide	Not Detected	0.04	3.0
32	Primiphos Methyl	Not Detected	0.04	4.0
33	Pyrethrins (Sum of isomer)	Not Detected	0.1	3.0
34	Quintozen (Sum of Quitozenepentachloroanillineand methyl pentachlorophenylsulphide)	Not Detected	0.1	1.0

Pesticide Residue Analysis

A 2 g sample of the test drug was extracted using 5 mL of ethyl acetate. The mixture was subjected to extraction for two minutes and then centrifuged at 10,000 rpm for another two minutes. The supernatant layer was collected, and a 1 mL aliquot was injected into a GC-MS/MS system to analyze and quantify the pesticide residues.

RESULTS

The safety profile of the test drug was assessed through the evaluation of microbial load, heavy metals using Atomic Absorption Spectroscopy (AAS), aflatoxins via LC-MS/MS, and pesticide residues through GC-MS/MS, as presented in Tables 1-4.

DISCUSSION

Herbal drugs are often perceived as inherently safe due to their natural origin, but this assumption is not always accurate. This highlights the critical need for safety evaluations of herbal medicines and food products in compliance with WHO guidelines. These evaluations typically include the assessment of heavy metals, microbial load, aflatoxins, and pesticide residues (BMC Complementary Medicine and Therapies).

Microbial contamination in herbal drugs not only compromises their efficacy but can also lead to toxicity, rendering the drugs unsuitable for human consumption. Contaminated herbal products may result in unintended health issues rather than addressing the intended ailments. Consequently, investigating microbial contamination in herbal drugs is of paramount importance. In this study, the Unani herbal drug *Centaurea behen* Linn. was analyzed for microbial load, including the total bacterial count and total yeast and mold count. As per WHO norms, the serial dilution method revealed that the total bacterial count in the drug sample was within permissible limits. Pathogenic bacteria, such as *Enterobacteriaceae, E. coli, Salmonella* sp., *Pseudomonas aeruginosa*, and *Shigella*, were not detected in the sample (Table 1).

Medicinal plants often contain varying levels of heavy metals, which may arise from environmental contamination or absorption from the atmosphere. While some heavy metals are essential in trace amounts, excessive levels can pose severe health risks. Ensuring quality control and standardized screening of herbal products is vital to protect consumers from toxicity. Heavy metal contamination in the test drug was assessed using Atomic Absorption Spectrometry (AAS). Lead was found within permissible limits, while mercury, arsenic, and cadmium were absent (Table 2).

Aflatoxins, a group of mycotoxins produced primarily by *Aspergillus* species, are toxic secondary metabolites with a strong link to fungal development. These carcinogenic compounds, particularly Aflatoxin B1, target the liver and pose significant health risks. The International Agency for Research on Cancer (IARC) classifies Aflatoxin B1 as carcinogenic, while Aflatoxins G1, B2, and G2 are considered possible carcinogens. Screening of the test drug for aflatoxins, conducted using Liquid Chromatography-Mass Spectrometry (LC-MS/MS), confirmed the absence of aflatoxins in the sample (Table 3).

Herbal drugs may also contain pesticide residues from agricultural practices, such as spraying, soil treatment during cultivation, or fumigant use during storage. Comprehensive testing for pesticide residues in herbal drugs is essential to ensure safety. The test drug sample was processed using a standard extraction procedure, with impurities removed by partition or absorption, and individual pesticides quantified using GC-MS. The pesticide residues in the test drug were found to be within acceptable limits (Table 4).

CONCLUSION

The World Health Organization (WHO) has mandated the evaluation of the safety profile for all finished products. This study assessed the safety parameters of the test drug, including microbial load, contamination with heavy metals such as lead, cadmium, mercury, and arsenic, as well as the presence of aflatoxins and pesticide residues. The findings revealed that the test drug posed no harm, with all measured values falling within permissible limits. Therefore, it can be concluded that the test drug is safe for use and carries no risk of toxicity (Rajkumar Dattatraya B, Sriram P. Evaluation of Microbial Load of Herbal Raw Materials: a Necessary Quality Control Measure to Ensure Safety of Finished Herbal Preparations. Adv Biotech & Micro).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUTURE SCOPE

The safety profile plays a crucial role in assessing the safety and potential toxicity of herbal drugs by identifying contaminants such as heavy metals, microbial pathogens, aflatoxins, and pesticide residues.

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