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Exploring the safety profile of *Doronicum hookeri*: Insights and findings

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Abstract
Background: Herbal medicines play a crucial role in traditional healthcare systems. According to the World Health Organization (WHO), an estimated 70-80% of the global population depends on traditional remedies for their health needs. These herbs are widely utilized for the treatment and prevention of various health conditions. However, the increasing popularity of herbal products has raised concerns about their safety. Despite being considered safe due to their natural origins, there have been cases of toxicity and adverse effects. The WHO emphasizes the importance of conducting safety evaluations for herbal medicines and food products to identify potential risks associated with environmental or soil contaminants.
Methods: Plants are susceptible to contamination by harmful substances. In this study, the safety profile of *Doronicum hookeri* (*Darunaj Aqrabi)* from the Asteraceae family was assessed by evaluating microbial load, heavy metal content using Inductively coupled plasma mass spectrometry (ICPMS), aflatoxins through Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), and pesticide residues via Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS).
Results: The analysis showed that the levels of heavy metals, including lead, cadmium, mercury, and arsenic, were within the acceptable limits set by WHO standards. Additionally, no aflatoxins, pesticide residues, or microbial contamination were found in the crude drug sample.
Conclusion: The safety evaluation demonstrates that *Doronicum hookeri* (*Darunaj Aqrabi)* is safe for use, with no indication of toxicity or associated risks.

Keywords: *Doronicum hookeri*, Darunaj Aqrabi, WHO guidelines, safety evaluation, pesticidal residues, heavy metals, aflatoxins, microbial load, Unani medicine

Introduction

Doronicum hookeri C.B.Clarke ex Hook.f (*Darunaj Aqrabi*) is a significant ancient medicinal plant. The name *Darunaj* is believed to originate from the Persian word "Daruna," meaning heart [1]. This traditional herb is thought to have originally come from Greece and Syria, with some species of this genus also found in the Himalayan region of India [2]. The plant has an aromatic root [3] and its shape resembles the tail of a scorpion, leading to the name *Darunaj Aqrabi* (with *Aqrabi* meaning scorpion). Its roots are commonly used in folk medicine and are known as "Darunaj Aqrabi" in Unani medicine and "Leopard's Bane" in English. It is mentioned in nearly all major Unani texts, including *Advia Qalbia* by Ibn Sina, for its cardiac tonic and invigorating properties [4]. Due to its tonic and antidote characteristics, as well as its protective qualities, the root is frequently used in compound formulations with tonic effects, especially for vital organs like the heart.

Classical Unani literature highlights numerous health benefits of Darunaj Aqrabi. Modern scientific research has further explored its chemical composition, therapeutic applications, and pharmacological properties, offering deeper insights into its medicinal potential.

Herbs frequently contain a substantial number 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 primarily 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 major types: B1, G1, B2, and G2. Among them, Aflatoxin B1 (AFB1) is the most toxic and is recognized as the strongest natural carcinogen, with a particular affinity for the liver.

The International Agency for Research on Cancer (IARC) has classified AFB1 as a Group I carcinogen, indicating its significant carcinogenic potential.

The consumption of herbal plants and medicines contaminated with aflatoxins or heavy metals is a well-established source of toxicity. Heavy metals enter the environment through both natural processes and various human activities. Their accumulation in plant tissues is primarily influenced by soil concentration and bioavailability, though they can also settle on plant surfaces due to atmospheric deposition. Due to their persistence and long biological half-life, heavy metals pose serious health risks [5].

Key heavy metals, such as cadmium, arsenic, mercury, and lead, are of particular concern. The harvesting and cultivation of medicinal plants 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 heightened risk of contamination can lead to serious health issues when these plants are consumed [6].

Materials and Methods

Collection and Authentication

The test drug, Darunaj Aqrabi (*Doronicum hookeri*), 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.

An herbarium specimen of the test drug was prepared and deposited in the Ibn Baytar Advia Museum previously known as Mawalid-e-Salasa 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 *Doronicum hookeri* 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.

(A) Heavy Metal Analysis

The analysis of heavy metals was performed to measure the levels of metal impurities in the test drug. Contamination with heavy metals such as arsenic, cadmium, mercury, and lead can arise 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 specified in the ASU Guidelines (Table-1), using Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

(B) Aflatoxin Estimation

Sample Preparation

A 2 g sample was mixed with 20 ml of a 60% acetonitrile-water solution and blended at high speed for two minutes. The mixture was then centrifuged at 1600 rpm for ten minutes, and the supernatant was carefully separated. To reduce the solvent concentration to 10% or lower, 2 ml of the supernatant was combined with 48 ml of phosphate-buffered saline (PBS) at pH 7.4. The prepared solution was 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 the same flow rate and then quickly dried. 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, and 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).

(C) 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.

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.

(D) 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 Inductively Coupled Plasma Mass Spectrometry (ICP-MS), aflatoxins via LC-MS/MS, and pesticide residues through GC-MS/MS, as presented in Tables 1-4.

Table 1: Heavy Metal Analysis of *Doronicum hookeri*.

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

LOQ = Limit of Quantification

Table 2: Test for Aflatoxins in *Doronicum hookeri*.

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

Table 3: Microbiological test of *Doronicum hookeri*

S. No.	Test Parameters	Test Result mg/kg	LOQ (mg/kg)	Max Permissible Limits as per API
1	Total Bacterial Count (cfu/gm)	42000	-	100000
2	Total Yeast and Mould (cfu/gm)	250	-	1000

Table 4: Any Specific Pathogens

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

Table 5: Pesticidal Residue in *Doronicum hookeri*.

S. No.	Pesticide Residue (mg/kg)	Results	Limit of Quantification	Max Permissible Limits as per API (mg/kg)
1	Alachlor	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
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 Quitozenepentachloroanilline)	Not Detected	0.1	1.0

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 [7,8].

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 *Doronicum hookeri* was analyzed for microbial load, including the total bacterial count and total yeast and mould count [9]. 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 [10]. 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 [11, 12]. Screening of the test drug for aflatoxins, was 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 [13]. 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 [14]. 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.

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.

Conflict of Interest

No conflict of interest as declared by the authors.

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