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Evaluation of the safety profile of "Talmakhana" the seed drug of *Hygrophila auriculata* (Schumach.) Heine used in Unani medicine

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Abstract
Background: Talmakhana (*Hygrophila auriculata* (Schumach.) Heine), a widely used Unani seed drug, is valued for its aphrodisiac, diuretic, tonic, and hepatoprotective properties. Despite its therapeutic relevance, scientific evaluation of its safety parameters remains limited.
Objective: To assess the safety profile of Talmakhana seeds through analysis of microbial load, heavy metals, aflatoxins and pesticide residues in accordance with WHO and ASU (Ayurveda, Siddha, Unani) guidelines.
Methods: Standardized analytical procedures were employed, including microbial limit testing, Atomic Absorption Spectrophotometry (AAS) for heavy metals, LC-MS/MS for aflatoxins, and GC-MS/MS for pesticide residues determination.
Results: Microbial load values (44,000 cfu/g bacteria; 180 cfu/g fungi) were within acceptable limits, and no pathogenic organisms were detected. Heavy metals (Pb, As, Cd, Hg), aflatoxins (B1 and total), and all 34 screened pesticides were below the limit of quantification.
Conclusion: Talmakhana seeds exhibit a favourable safety profile and comply with international quality standards, supporting their continued safe use in Unani medicine.

Keywords: Talmakhana, *Hygrophila auriculata* (Schumach.) Heine, Unani medicine, Safety evaluation, Heavy metals, Microbial load, Aflatoxins, Pesticide residues

1. Introduction
Herbal medicines have remained an integral part of human healthcare since ancient times, forming the backbone of several traditional medical systems. Among these, the Unani system of medicine is recognized as one of the most well-developed, scientifically organized, and historically enriched healing traditions [1]. Emerging from the Greco-Arab medical heritage, Unani evolved through the foundational contributions of early Greek and Roman physicians such as Buqrat (Hippocrates, 460-370 BC) and Jalinoos (Galen, 129-210 AD), and was later advanced by eminent Arab and Persian scholars including Al-Razi (Rhazes, 850-925 AD) and Ibn Sina (Avicenna, 980-1037 AD). Their extensive writings transformed Unani into a systematic medical discipline. In India, the system was further strengthened by scholars like Hakim Ajmal Khan and remains widely practiced today under the AYUSH framework [2]. Unani medicine is based on a holistic approach to health, focusing on the balance of temperament (Mizaj), humors (Akhlat), lifestyle regulation (Tadbeer), and therapeutic intervention through drugs (Ilaj bil Dawa). It views the patient as a complete entity rather than addressing symptoms in isolation [3]. Herbal drugs form the core of Unani pharmacotherapy, and the use of well-standardized plant materials is fundamental to preparing classical formulations such as *Joshanda*, *Majoon*, *Tiryaq*, *Sharbat*, *Itrifal*, and various other dosage forms. Classical Unani texts, such as *Al-Qanun fi al-Tibb* and *Kitab al-Hawi*, describe detailed criteria for drug identification, purification (Tasfiya), correction (Tadbeer-i-Advia), and safety monitoring even centuries before modern quality control frameworks were established [4, 5].
Although herbal medicines are often perceived as inherently safe due to their natural origin, but classical Unani texts have long emphasized the potential risks arising from adulteration, improper harvesting, unsuitable storage, environmental contaminants, and incorrect usage. These traditional warnings closely mirror contemporary concerns, as the global surge in herbal medicine consumption has brought renewed attention to issues of purity, quality assurance, and toxic contamination. In alignment with WHO guidelines, rigorous safety

evaluation of herbal raw materials has therefore become indispensable, particularly for widely used Unani drugs [6, 7, 8].

A comprehensive safety assessment includes the examination of heavy metals, microbial load, pesticide residues, and aflatoxins parameters that significantly impact the quality and safety of medicinal plants. Microbial contamination not only reduces therapeutic efficacy but may also lead to the formation of harmful toxins. Hence, evaluating total bacterial count, yeast and mold count, and screening for pathogenic organisms is essential [9]. Similarly, heavy metals such as lead, arsenic, cadmium, and mercury may accumulate in plants through environmental exposure. While trace quantities may be physiologically relevant, elevated levels are associated with carcinogenic, hepatotoxic, neurotoxic, and other systemic adverse effects. Techniques such as Atomic Absorption Spectrophotometry (AAS) are routinely used to ensure these metals remain within permissible limits [10]. Aflatoxins secondary metabolites produced by Aspergillus species pose another major safety concern, as Aflatoxin B1 has been classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC). Their detection requires highly sensitive analytical platforms, such as LC-MS/MS, to ensure consumer protection [11]. Classical Unani scholars also documented how cultivation methods, storage conditions, and environmental impurities influence drug quality, their observations correspond well with modern findings on persistent pesticide residues. These residues may enter plant material during cultivation or post-harvest processes and require advanced techniques such as GC-MS/MS for precise identification and quantification [12].

In Unani literature, Talmakhana is identified as the seeds of *Hygrophila auriculata* (Schumach.) Heine of the family Acanthaceae. The seeds are described as small, elongated, pale brown, slightly broad, and faintly glossy, with mucilaginous properties similar to *Lepidium iberis* and morphological resemblance to *Blepharis edulis*. Classical Unani physicians described the plant as an erect herbaceous species standing 2-4 feet tall, with a quadrangular stem and whorls of six leaves at each node. A sharp spine approximately one inch long is present between the stem and leaves, both of which are covered with fine white hairs. The leaves resemble those of *Cichorium intybus* but are coarser and distinctly hairy **[13]**. The flowers, typically purplish to violet-blue with a white center, appear in whorls of four at each node and are smaller than those of *Cichorium intybus*. Each flower bears four relatively broad petals. The plant's nodes are rigid like those of *Cichorium intybus*, yet each node is armed with spines reminiscent of *Acacia nilotica*, providing a distinguishing characteristic. The root system is composed of multiple thread-like branches that are grooved and ridged [14].

Talmakhana plant is widely distributed across India, Sri Lanka, Myanmar, Indonesia, Malaysia, Nepal, and tropical regions of Africa, particularly thriving in moist or marshy habitats. Regionally, it is known by several vernacular names, including *Prwamoli* (referring to its reddish root), *Rachoora* (due to its resemblance to sugarcane), *Patoka* or *Asthool Kantaka* (indicating its long spines), and *Kokla Jhka* (relating to its thin, pale brown seeds). The shelf life of Talmakhana is approximately three years, after which the seeds develop bitterness and begin to degrade [13, 15]. Unani classical literature attributes numerous therapeutic properties to Talmakhana, and these uses continue in traditional practice. It is described as *Musammin-e-Badan* (adipogenous), *Muqawwi-e-Badan* (general tonic), *Muqawwi-e-Aza* (organ tonic), *Mufarreh* (exhilarant), *Muqawwi-e-Bah* (aphrodisiac), *Moallid-e-Mani* (semen promoter), *Musakkin* (analgesic), *Mudir-e-Bol* (diuretic), *Mubarrid* (refrigerant), and *Mulayyin* (laxative). Traditionally, it is widely incorporated into formulations for genito-urinary conditions, sexual debility, and general weakness. Modern pharmacological studies further report hepatoprotective, anticancer, aphrodisiac, hypoglycemic, diuretic, antioxidant, and free radical scavenging activities, among others [14, 16, 17, 18, 19, 20, 21].

Despite its extensive therapeutic relevance, ensuring its safety remains essential for clinical acceptance. Thus, the present study aims to conduct a comprehensive safety evaluation of Talmakhana (seeds) by analyzing heavy metals, microbial contamination, pesticide residues, and aflatoxins in accordance with WHO and ASU guidelines. Such systematic assessments are crucial to support evidence-based Unani medicine and ensure the availability of safe, high-quality herbal therapeutics.

2. Materials and Methods

2.1 Collection and Authentication

The test sample, Talmakhana seeds of *Hygrophila auriculata* (Schumach.) Heine, was procured from the Dawakhana, Tibbiya College, Aligarh Muslim University (A.M.U.), Aligarh, Uttar Pradesh, India, and utilized as the study material. Proper identification of the drug was carried out through classical Unani descriptions and established botanical criteria. Authentication was performed in the Pharmacognosy Section, Department of Ilmul Advia (Unani Pharmacology), Faculty of Unani Medicine, Ajmal Khan Tibbiya College, as well as in the Department of Botany, A.M.U., Aligarh. Following authentication, the specimen was deposited in the Ibn-e-Baitar Museum of the Department of Ilmul Advia for future reference under Voucher No. SC-369/23.

2.2 Preparation of Powder

Prior to analysis, the raw drug was thoroughly cleaned to remove extraneous matter and shade-dried. The dried seeds were then pulverized using an electric grinder. The resulting powder was passed through sieve no. 80 to ensure uniform particle size. The finely sieved powder was stored in airtight containers under suitable conditions for future experimental study.

2.3 Safety Study

To assess whether the test drug is free of contaminants like microbial load, heavy metals, aflatoxins, or pesticide residues, a comprehensive safety evaluation was performed at AGSS Analytical and Research Lab Pvt. Ltd., Lawrence Road Industrial Area, Delhi-35, India, under URL No. TC121152400009270F and Report No. AGSS/AP/24071300011, following the standards prescribed by the WHO and ASU guidelines.

2.3.1 Determination of Microbial Load

The microbial quantity in the test drug was examined as per WHO approved procedures **[22]**.

Sample Preparation

Before microbial analysis, the test sample was subjected to appropriate pre-treatment to ensure that any intrinsic antimicrobial components did not interfere with the results. Depending on the physicochemical nature of the material, dilution or neutralization steps were applied. Buffered Sodium Chloride-Peptone Solution (pH 7.0; MM1275-500G, HiMedia Laboratories, Mumbai, India) was used as the primary diluent.

1. Water-soluble materials

A quantity of 10 g of the powdered sample was dispersed in lactose broth (M1003-500G, HiMedia Laboratories, Mumbai, India), a medium confirmed to have no inhibitory effect on bacterial growth. The mixture was adjusted to a final volume of 100 ml, and the pH was standardized to approximately 7.0.

2. Water-insoluble non-fatty materials

For materials not readily soluble in water, 10 g of the test sample was suspended in lactose broth supplemented with Polysorbate-20 (M1307-500G, HiMedia Laboratories, Mumbai, India) to assist dispersion. The surfactant solution contained potassium tellurite at a concentration of 1 mg/ml (FD052, HiMedia Laboratories). The final volume was made to 100 ml, and the pH was adjusted to nearly neutral.

Microbial Enumeration Procedures

1. Total Bacterial Count

For bacterial enumeration, 1 ml of the pre-treated sample was introduced into sterile Petri dishes (90 mm diameter). Approximately 15 ml of molten casein-soybean digest agar (M290-500G, HiMedia Laboratories) at not more than 45 °C was gently poured into each dish. After solidification, duplicate plates prepared from the same dilution were inverted and incubated at 30-35 °C for 48-72 hours. Colony-Forming Units (CFU) were counted on plates containing up to 300 colonies, and results were calculated accordingly.

2. Total Fungal Count (Yeasts and Molds)

For fungal estimation, 1 ml of the processed sample was transferred into sterile Petri dishes and mixed with approximately 15 ml of molten Sabouraud glucose agar with antibiotics (MI472-500G, HiMedia Laboratories). After the medium solidified, the plates were inverted and incubated at 20-25 °C for five days. Fungal colonies that developed were counted and expressed as CFU, using the plates providing the most reliable count.

2.3.2 Heavy Metal Analysis

The assessment of heavy metals in the test material was undertaken to determine whether the sample is free of any toxic metallic impurities. To ensure compliance with safety standards, the concentration of these metals in the test drug was quantified using an Atomic Absorption Spectrophotometer (AAS). The analytical procedure followed the protocols outlined in the ASU Testing Guidelines, which specify the permissible limits and the standardized approach for detecting metallic contaminants in herbal raw materials [23].

2.3.3 Aflatoxin Analysis

For aflatoxin quantification, 2 g of the sample was homogenized with 20 ml of 60% acetonitrile-water for two minutes to extract the metabolites. The mixture was centrifuged at 1600 × g for ten minutes, and the clear supernatant was collected. An aliquot of 2 ml from this extract was then diluted with 48 ml of phosphate-buffered saline (PBS, pH 7.4), ensuring that the final organic solvent concentration did not exceed 10%.

The diluted sample was passed through an immunoaffinity column at a flow rate of 5 ml/min, allowing selective binding of aflatoxins. After loading, the column was washed with 20 ml of distilled water at the same flow rate and subsequently dried. The bound aflatoxins were eluted and the eluent was brought to volume using 1.5 ml of distilled water.

A 500 µl aliquot of the purified extract was subjected to analysis using LC-MS/MS (LC: Perkin, MS: Applied Biosystems, Model 2000). Chromatographic separation was achieved on a ZORBAX RX C18 column (2.1 × 150 mm, 5 µm) using a mobile phase composed of:

- **Solvent A:** Water (100%)
- **Solvent B:** Acetonitrile (100%)

The system was operated with a column oven temperature of 30 °C and a flow rate of 0.75 ml/min. Aflatoxin levels in the test sample were quantified by comparing the peak area/height with that of certified aflatoxin standards [24].

2.3.4 Pesticide Residue Analysis

For analysis, 2 g of the powdered test sample was extracted with 5 ml of ethyl acetate by vigorous mixing for approximately two minutes. The mixture was then centrifuged at 10,000 rpm for two minutes to obtain a clear extract. From the resulting supernatant, 1 ml was carefully withdrawn and introduced into the GC-MS system for qualitative and quantitative determination of pesticide residues [23].

3. Observations and Results

3.1 Microbial Load Analysis

The microbial quality assessment of *Talmakhana* revealed that the sample complied with all permissible microbiological limits prescribed for raw herbal drugs. As shown in Table 1, the Total Bacterial Count (44,000 cfu/g) and Total Fungal Count (180 cfu/g) were well below the maximum allowable limits of 100,000 cfu/g and 1000 cfu/g, respectively. Importantly, all tested pathogenic microorganisms including *Escherichia coli*, *Salmonella* spp., *Pseudomonas aeruginosa* and *Staphylococcus aureus* were absent, indicating good microbial safety of the sample.

Table 1: Microbial Load Analysis of *Talmakhana* (*Hygrophila auriculata* (Schumach.) Heine)

S. No.	Microbiological Parameters	Units	Results	Permissible Limit as per API
1	Total Bacterial Count	cfu/gm	44,000	100,000 Max
2	Total Fungal Count	cfu/gm	180	1000 Max
Detection of Specific Pathogens				
3	*Escherichia coli*	per gm	Absent	Absent
4	*Salmonella* spp.	per gm	Absent	Absent
5	Pseudomonas aeruginosa	per gm	Absent	Absent
6	*Staphylococcus aureus*	per gm	Absent	Absent

*cfu/gm = colony forming units per gram

3.2 Heavy Metal Analysis

Heavy metal profiling of talmakhana shown in Table 2 demonstrated that levels of lead (Pb), arsenic (As), cadmium (Cd), and mercury (Hg) were below the limit of quantification (BLQ; 0.05 mg/kg). All values were significantly lower than the permissible limits recommended in the *Ayurvedic Pharmacopoeia of India (API)* (Pb ≤10 mg/kg, As ≤3 mg/kg, Cd ≤0.3 mg/kg, Hg ≤1 mg/kg). These findings confirm the absence of heavy-metal contamination and indicate that the plant material is safe for medicinal use in accordance with regulatory guidelines.

Table 2: Heavy Metal Profiling of *Talmakhana* (*Hygrophila auriculata* (Schumach.) Heine)

S. No.	Heavy Metal Parameters	Units	Results	Permissible Limit as per API
1	Lead (Pb)	mg/kg	BLQ (0.05)	10.0 Max
2	Arsenic (As)	mg/kg	BLQ (0.05)	3.0 Max
3	Cadmium (Cd)	mg/kg	BLQ (0.05)	0.3 Max
4	Mercury (Hg)	mg/kg	BLQ (0.05)	1.0 Max

*BLQ = Below Limit of Quantification
*(0.05 mg/kg = Limit of Quantification)

3.3 Aflatoxin Estimation

Aflatoxin screening in Table 3 showed that both Aflatoxin B1 and Total Aflatoxins (B1 + B2 + G1 + G2) were below the limit of quantification (BLQ; 1.0 µg/kg). These results fall well within the recommended limits (Aflatoxin B1 ≤ 2 µg/kg; Total Aflatoxins ≤ 5 µg/kg), confirming that *Talmakhana* is free from aflatoxin contamination. This indicates proper post-harvest handling and storage conditions.

Table 3: Aflatoxin Estimation of *Talmakhana* (*Hygrophila auriculata* (Schumach.) Heine)

S. No.	Aflatoxin Parameters	Units	Results	Permissible Limit as per API
1	Aflatoxin B1	µg/kg	BLQ (1.0)	2.0 Max
2	Total Aflatoxins (B1 + B2 + G1 + G2)	µg/kg	BLQ (1.0)	5.0 Max

*BLQ = Below Limit of Quantification
*(1.0 µg/kg = Limit of Quantification)

3.4 Pesticide Residue Analysis

The pesticide residue analysis of *Talmakhana* included 34 commonly evaluated pesticide groups, as listed in Table 4. All residues were found to be below the limit of quantification (BLQ; 0.01 mg/kg). All detected values were significantly lower than the permissible limits defined by the API. This confirms that the sample is free from agricultural and environmental pesticide contamination.

Table 4: Pesticidal Residue Estimation of *Talmakhana* (*Hygrophila auriculata* (Schumach.) Heine)

S. No.	Pesticide Residue	Units	Results	Permissible Limit as per API
1	Alachlor	mg/kg	BLQ (0.01)	0.02 Max
2	Aldrin & Dieldrin (sum)	mg/kg	BLQ (0.01)	0.05 Max
3	Azinphos-methyl	mg/kg	BLQ (0.01)	1.0 Max
4	Bromopropylate	mg/kg	BLQ (0.01)	3.0 Max
5	Chlordane (cis-, trans-, oxychlordane)	mg/kg	BLQ (0.01)	0.05 Max
6	Chlorfenvinphos	mg/kg	BLQ (0.01)	0.5 Max
7	Chlorpyrifos	mg/kg	BLQ (0.01)	0.2 Max
8	Chlorpyrifos-methyl	mg/kg	BLQ (0.01)	0.1Max
9	Cypermethrin (and isomers)	mg/kg	BLQ (0.01)	1.0 Max
10	DDT (p,p'-DDT, p,p'-DDE, p,p'-TDE)	mg/kg	BLQ (0.01)	1.0 Max
11	Deltamethrin	mg/kg	BLQ (0.01)	0.5 Max
12	Diazinon	mg/kg	BLQ (0.01)	0.5 Max
13	Dichlorvos	mg/kg	BLQ (0.01)	1.0 Max
14	Dithiocarbamates (as CS_2)	mg/kg	BLQ (0.01)	2.0 Max

15	Endosulfan (isomers + sulphate)	mg/kg	BLQ (0.01)	3.0 Max
16	Endrin	mg/kg	BLQ (0.01)	0.05 Max
17	Ethion	mg/kg	BLQ (0.01)	2.0 Max
18	Fenitrothion	mg/kg	BLQ (0.01)	0.5 Max
19	Fenvalerate	mg/kg	BLQ (0.01)	1.5 Max
20	Fonofos	mg/kg	BLQ (0.01)	0.05 Max
21	Heptachlor (and epoxide)	mg/kg	BLQ (0.01)	0.05 Max
22	Hexachlorobenzene	mg/kg	BLQ (0.01)	0.1 Max
23	Hexachlorocyclohexane isomers (except γ)	mg/kg	BLQ (0.01)	0.3 Max
24	Lindane (γ-HCH)	mg/kg	BLQ (0.01)	0.6 Max
25	Malathion	mg/kg	BLQ (0.01)	1.0 Max
26	Methidathion	mg/kg	BLQ (0.01)	0.2 Max
27	Parathion	mg/kg	BLQ (0.01)	0.5 Max
28	Parathion-methyl	mg/kg	BLQ (0.01)	0.2 Max
29	Permethrin	mg/kg	BLQ (0.01)	1.0 Max
30	Phosalone	mg/kg	BLQ (0.01)	0.1 Max
31	Piperonyl butoxide	mg/kg	BLQ (0.01)	3.0 Max
32	Pirimiphos-methyl	mg/kg	BLQ (0.01)	4.0 Max
33	Pyrethrins (sum of isomers)	mg/kg	BLQ (0.01)	3.0 Max
34	Quintozene (and metabolites)	mg/kg	BLQ (0.01)	1.0 Max

*BLQ = Below Limit of Quantification
*(0.01 mg/kg = Limit of Quantification)

4. Discussion

The comprehensive safety evaluation of *Talmakhana* (*Hygrophila auriculata (*Schumach.) Heine) seeds in the present study demonstrates an overall safe profile, with microbial counts, heavy metals, aflatoxins, and pesticide residues all within acceptable regulatory limits. Medicinal plants may accumulate hazardous metals such as arsenic, lead, cadmium, and mercury due to environmental exposure, soil contamination, irrigation water quality, or atmospheric pollutants. Lead (Pb), arsenic (As), cadmium (Cd), and mercury (Hg) were all below the limit of quantification (BLQ), indicating very low risk of heavy-metal exposure. These results contrast with global analyses showing widespread metal contamination in herbal medicines. A multinational survey of more than 1,700 samples reported that heavy metals including Pb, Cd, As, and Hg were frequently detected and often above permissible limits **[25]**. The absence of detectable metals in *Talmakhana* suggests that the samples originated from relatively unpolluted environments and were handled appropriately. This is important because chronic exposure to heavy metals is associated with neurotoxicity, hepatotoxicity, carcinogenicity, and systemic organ failure [26].

Medicinal plant materials are naturally exposed to microbial contamination, including bacteria and fungi, during harvesting, transportation, processing, and storage. These factors may substantially increase microbial growth, making routine microbiological assessment essential **[27]**. In line with global regulatory expectations, the World Health Organization (WHO) recommends that herbal raw materials intended for therapeutic use must undergo microbial load testing [28]. Microbial analysis showed that the total bacterial count (44,000 cfu/g) and fungal count (180 cfu/g) were well within the permissible limits. No pathogenic microorganisms including *Escherichia coli*, *Salmonella* spp., *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were detected. These findings align with recent microbial studies on Unani plant drugs such as *Nardostachys jatamansi*, which also reported absence of pathogens [29]. However, several investigations have documented unacceptable microbial loads in herbal preparations, particularly in products from unregulated markets. Studies from Saudi Arabia and Bangladesh have detected Gram-negative bacteria and toxigenic fungi in herbal formulations **[30,31]**. Such variation underscores the importance of proper post-harvest handling, drying, packaging, and storage principles emphasized in classical Unani pharmaceutics under Tadbeer (processing) and Hifz-i-Advia (drug preservation) [4].

Aflatoxins are potent mycotoxins generated as secondary metabolites by fungi belonging to *Aspergillus* species particularly *A. flavus*, *A. parasiticus*, and *A. nomius*. These fungi frequently contaminate agricultural commodities such as cereals, oilseeds, nuts, and legumes, posing significant health hazards due to their hepatotoxic and carcinogenic properties. The major aflatoxin derivatives relevant to herbal testing include B1, B2, G1, and G2. Aflatoxin B1 and total aflatoxins were below the quantification limit in the present study. This is noteworthy because seeds and underground organs of medicinal plants are particularly vulnerable to Aspergillus contamination under humid conditions. Several studies have documented aflatoxin contamination in stored herbal products, linked to poor drying and improper storage [32]. The absence of aflatoxins in *Talmakhana* indicates ideal handling and storage conditions. Pesticide contamination in medicinal plants may arise from routine agricultural activities such as crop spraying, soil treatment, and post-harvest fumigation. Since herbal formulations are often consumed for prolonged durations, the WHO emphasizes the need to establish acceptable limits and to routinely monitor pesticide residues in plant-based raw materials [27]. The evaluation of pesticide residues in the present study followed these recommended analytical procedures and found that all 34 screened pesticides were below the quantification limit. This contrasts with reports showing significant pesticide residues including organochlorines such as HCH and DDT in medicinal herbs grown in chemically treated agricultural fields **[33]**. The clean profile of *Talmakhana* suggests sustainable or low-pesticide cultivation practices.

The results reinforce that medicinal plants used in the Unani system can meet modern safety standards when subjected to proper collection, purification, and storage. Classical Unani literature has historically emphasized drug identification, Tasfiya (purification), Tadbeer-i-Advia (detoxification/processing), and Hifz-i-Advia (storage) [5]. The clean safety profile of *Talmakhana* is consistent with these principles and demonstrates the benefit of integrating traditional knowledge with modern analytical techniques.

The contrast between these findings and reports of contamination in other herbal materials underscores the variability in herbal drug safety due to environmental pollution, soil heavy-metal content, agricultural practices, and storage conditions. Therefore, consistent batch-wise testing remains essential.

5. Limitations and Recommendations

Although the tested sample showed a strong safety margin, contamination levels in herbal materials may vary with geographical origin, season, and harvesting practices. Previous large-scale studies indicate that more than one-third of herbal samples worldwide may contain heavy metals above recommended limits [25]. Thus, future studies should include multi-location sampling and assessment of additional contaminants such as pesticide metabolites, other mycotoxins, and residual solvents.

6. Conclusion

The present study affirms that *Talmakhana* seeds of (*Hygrophila auriculata (*Schumach.) Heine) possess a favourable safety profile, being free from hazardous levels of heavy metals, microbes, aflatoxins, and pesticide residues. These findings support the continued therapeutic use of *Talmakhana* in Unani medicine and highlight the importance of rigorous quality assessment for ensuring the safety and reliability of herbal formulations.

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