WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Pre-Clinical Evaluation of Adathodai Girutham - Evaluating Heavy Metals, Aflatoxin and Microbial Load

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ABSTRACT

Background: Adathodai Girutham, a traditional herbo-mineral formulation in Siddha medicine, is widely prescribed for managing respiratory disorders such as cough, asthma, and bronchitis. Ensuring its safety and quality is essential for therapeutic efficacy.

Aim: This study is done to detect the microbial contamination, aflatoxin , heavy metal content in Adathodai Girutham.

Materials and Methods: Microbial load analysis was performed using the standard pour plate method, while aflatoxins (B1, B2, G1, G2) were quantified using a VICAM fluorometer (Series 4EX). Heavy metal concentrations, including lead, cadmium, mercury, and arsenic, were assessed through Atomic Absorption Spectrometry (AAS).

Results: The results demonstrated that microbial counts and aflatoxin levels were within permissible limits prescribed by pharmacopoeial standards. Additionally, heavy metal concentrations were found to be within acceptable safety thresholds.

Conclusion: These findings confirm that Adathodai Girutham is free from harmful levels of contaminants and heavy metals, supporting its safety and continued therapeutic use in Siddha practice.

Keywords: Adathodai Girutham; Aflatoxins; Atomic Absorption Spectrometry; Heavy metals; Microbial contamination; Respiratory disorders; Siddha medicine; VICAM fluorometer.

INTRODUCTION

Adathodai girutham is a traditional herbo-mineral drug that is used to treat various respiratory ailments.Heavy metals at lower concentration does not cause any health hazards. Certain metals like iron, zinc, copper and manganese are essential for human. Heavy metals are described in various ways by different sources. From a health perspective, these are naturally occurring elements that tend to accumulate and exert harmful effects on the environment and living organisms, including humans. The category also includes semimetals or metalloids that exhibit similar toxic properties. Human exposure to heavy metals occurs through inhalation, ingestion, or skin contact. Environmental contamination with these elements can pollute air, water, sewage, marine ecosystems, and waterways, leading to their accumulation in plants, agricultural produce, seafood, and meat, thereby indirectly impacting human health. Certain occupations pose a higher risk of exposure and related toxicity. [1] In developing countries, particularly in rural areas, the issue of mycotoxins contamination presents a significant challenge, leading to serious consequences about food hygiene and quality. Certain fungi, especially those in the genera Aspergillus, Alternaria, Fusarium, and Penicillium, synthesize secondary metabolites known as mycotoxins, which are small and highly toxic compounds (Iqbal, 2021). [2] "Mycotoxin" originates from the Greek language, combining *mycos* (fungus) and *toxin* (poison). Favorable environmental factors, such as high humidity and temperature, promote the contamination of various crops and food products by these compounds (Akinyemi et al., 2022). Contamination of food by toxin-producing fungi remains a critical yet often overlooked problem, causing significant economic losses by reducing food safety and quality. Over 400 mycotoxins have been reported so far, of which aflatoxins, zearalenone (ZEA), ochratoxin A (OTA), fumonisins, deoxynivalenol (DON), and patulin pose the greatest risk because of their harmful effects on mammals and significant economic impact (Liu et al., 2022). Microorganisms play an vital role in many ecosystems as well as found on a extensive range of indoor and outdoor surfaces. They thrive in device screens and public grab handles, which can negatively impact health, hygiene, and the environment. Microbial presence and activity on surfaces are both dynamic and complex, shaped by human interactions, environmental parameters, cleaning practices, and surface characteristics. Measuring the number and types of microbes on surfaces is key to evaluating human exposure risk from pathogenic organisms. Surfaces can host bacteria, viruses, fungi, and archaea, which may be deposited via direct hand contact, airborne particles, or contamination by infected individuals. Microorganisms'

ability to adhere to, survive on, and sometimes multiply on surfaces raises serious concerns about transmission through direct contact, aerosols, or transfer to hands and mucous membranes. Harmful microbes on surfaces can thus represent significant public health risks—for example, respiratory viruses, foodborne bacteria, and allergenic fungi. Therefore, it is critical to evaluate not only the microbial load but also aflatoxins and heavy metal contamination in Adathodai Girutham.

Aim

To evaluate the safety of Adathodai Girutham by quantifying **aflatoxin** contamination, heavy metal levels, and microbial load, and to assess its suitability for therapeutic use.

Material and method

Heavy Metal Analysis

The heavy metal analysis of Adathodai Girutham was conducted using atomic absorption spectrometry (AAS). For the determination of arsenic and mercury, the sample was treated with 1 mol/L hydrochloric acid (HCl), while for lead and cadmium, 1 mol/L nitric acid (HNO_3) was used. This aligns with standard heavy-metal analysis protocols, in which specific acids are used to reliably extract metals from complex sample matrices. For instance, hydrochloric acid is commonly used for mercury analysis due to its ability to stabilize mercury ions, whereas nitric acid is preferred for lead and cadmium because of its strong oxidizing properties that facilitate the dissolution of these metals .

Microbial Load Determination

The bioburden of commercial and homemade phytomedicines was evaluated following WHO standards (2007). [6]Pathogenic bacteria were isolated and identified, and the total number of aerobic bacteria and fungi present in volume of one millimeter of each sample was quantified. The obtained samples were uniformly mixed using polysorbate-20, and 1 mL of the mixture was subsequently transferred into an aliquot of 9 ml of peptone broth. Successive dilutions were prepared to reach the desired concentrations, and entire tests were conducted in triplicate.

The pour plate method was used to evaluate microbial viability, with Casein Soybean Digest Agar for bacterial enumeration and Sabouraud Dextrose Agar for fungal detection. The Media served as per the manufacturer's guidelines, and was inoculated, and maintained at 37 °C for incubation for for bacterial analysis(24–48 hours) and at 25 °C for to support fungal growth. (48–72 hours)

After incubation, number of viable microorganisms per gram was computed by multiplying the mean colony count by the corresponding dilution factor. These values were evaluated by comparing with WHO standards, with herbal medicine samples showing bacterial counts above 10^5 CFU/g deemed unsatisfactory in accordance with WHO guidelines for aerobic bacteria.

Identification of Bacteria

To facilitate bacterial identification and isolation samples were diluted with either water or Tween, depending on their degree of dissolution, and thoroughly homogenized by vigorous mixing. A 1 mL aliquot was subsequently added to 9 mL of peptone broth and incubated under the specified time and thermal conditions. All microbial strains has been repeated three times.

Selective media were employed for pathogen detection: EMB agar and MacConkey agar for

Escherichia coli, Deoxycholate Citrate Agar for *Salmonella* spp., Cetrimide agar for *Pseudomonas aeruginosa*, and Mannitol Salt Agar for *Staphylococcus aureus*. Followed by incubation, identification of bacterial isolates were carried out based on colony morphology, Gram staining, and biochemical tests such as oxidase, gas production, and catalase activity.

Aflatoxin test using afla-test fluorometer

Aflatoxins are naturally occurring mycotoxins synthesized by *Aspergillus flavus* and *Aspergillus parasiticus*. The AflaTest method provides a quantitative approach for detecting aflatoxins B1, B2, G1, G2, M1, and M2.

For analysis, 1 mL of the Siddha formulation (AG) was combined with 0.4 g sodium chloride and a solvent mixture of methanol with 2% Tween 20 or phosphate buffer (60:40, v/v). The mixture was vortexed vigorously for 3 minutes and passed through fluted filter paper. From the filtrate, 10 mL was transferred to a measuring cylinder, mixed with 20 mL of purified water, and vortexed again for duration of a minute. The resulting prepared extract was then passed through a pre-wetted glass fiber filter (1.5 µm).

Subsequently, 10 millimeter of the diluted solution was transfered through an AflaTest WB column at a flow rate of 1–2 drops per second. The column was then rinsed with 10 mL of 2% Tween 20, followed by two washes with 10 mL of purified water. The column was eluted using 1 mL of HPLC-grade methanol at a rate of 1 drop per second, and the eluate was then gathered in a sterile VICAM cuvette. To this eluate, a volume of 1 mL of Afla Test Developer was added, mixed thoroughly, and immediately inserted into a VICAM Series 4EX fluorometer. The aflatoxin concentration was measured after 60 seconds.

Results

Heavy metal analysis

The results showed the trace metals Lead,Cadmium,Arsenic And Mercury were within the permissible limits as per WHO guidelines.Heavy metals results are tabulated in table 1.

Heavy/Toxic metals	**Result**	**WHO Permissible limit (ppm)[7]**
Lead	Nil	10
Cadmium	0.0048 mg/L	0.3
Arsenic	Nil	3
Mercury	Nil	1

Table 1. Results for Heavy metals

Aflatoxin

The total Aflatoxin B1+B2+G1+G2 were performed by using Vicam Aflatest Fluorometer Instruction manual.It was found within below limits.Results are given in the table 2.

S.No.	**Parameters**	**Method / Reference**	**Results**
1.	Total Aflatoxin B1+B2+G1+G2	Vicam Aflatest Fluorometer Instruction Manual	1ppb

(Note: Detection Limit - 1ppb)

Table 2. Results for Aflatoxins

Microbial load

Microbial load parameters was found within the permissible limits. Total bacterial count(TBC) and fungal count was found less than 5cfu/g and 3 cfu/g respectively. The disease causing species of Enterobacteriaceae, E.coli, Salmonella, Staphylococcus, Pseudomonas aeruginosa were absent.The results are displayed in the table 3.

S. No.	**Parameters**	**Results**	**Remarks**
1	**Total Bacterial Count (TBC)**	$2x10^4$cfu/ml	Within permissible limits
2	**Total Fungal Count (TFC)**	Less than 5 cfu/ml	
3	**Enterobacteriaceae**	Absent	
4	***Escherichia coli***	Absent	
5	**Salmonella Spp**	Absent	
6	***Staphylococcus aureus***	Absent	
7	***Pseudomonas aeruginosa***	Absent	

Table 3. Results for Microbial Load

Discussion

The results stated the Heavy metals Lead,Cadmium,Arsenic And Mercury were within the permissible limits as per WHO guidelines .Lead, Arsenic and mercury was absent in Adathodai Girutham. Cadmium level was 0.0048mg/L.The levels were within the WHO permissible limits. Total Aflatoxin B1+B2+G1+G2 were within 1ppb. Total Bacterial Count (TBC) was found $2x10^4$cfu/ml and Total Fungal Count (TFC) was found Less than 5 cfu/ml . Enterobacteriaceae *Escherichia coli* Salmonella Spp *Staphylococcus aureus Pseudomonas aeruginosa* were found absent.Microbial contamination in herbal remedies can arise from the microbiological (bacterial and fungal) load present in medicinal plants. Various biotic and abiotic factors—such as

temperature, soil pH, heavy metals, air quality, and precipitation—can influence microbial presence. The microbial load of these plants is often the primary source of contamination in herbal medicines (de Sousa Lima et al., 2020).[9] Herbal plants are particularly vulnerable to pathogenic microorganisms, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Shigella* spp., *Salmonella* spp., *Escherichia coli*, and fungi like *Aspergillus parasiticus*, *A. flavus*, *A. niger*, and *A. ochraceus*, which can originate from soil, water, and air (Castronovo et al., 2021). These microbes not only reduce the shelf life and quality of herbal drugs but can also pose significant health risks to humans (Yesuf et al., 2016; Taghinasab and Jabaji, 2020). Pathogenic organisms can form mono- and multispecies biofilms on herbal products, diminishing their potency. Climatic conditions and handling practices—spanning pre- and post-harvest stages, as well as storage— further influence contamination levels, with factors such as humidity, temperature, pH, and rainfall playing key roles (Abba et al., 2009; de Freitas Araújo and Bauab, 2012; de Sousa Lima et al., 2020). To enhance the safety, efficacy, purity, and shelf life of these products, strict adherence to cleanliness and sanitization protocols is essential. This includes measures such as pH standardization, moisture control, and durability testing to ensure microbial contamination remains within permissible limits.[10]

Metal toxicity in herbal medicines often results from polluted environments where these plants are cultivated . Increasing concern exists regarding the possible transfer of hazardous metals to humans through the consumption of herbs cultivated in polluted areas. The World Health Organization (WHO) highlights the critical need to ensure the quality of plant-based products, particularly by implementing analytical monitoring of hazardous metal concentrations in medicinal plants. Consuming decayed medicinal plants poses significant health risks to humans. For example, long-term exposure to cadmium (Cd) has been associated with hypertension, lung and prostate cancers, bone and kidney disorders, and emphysema. Similarly, excessive copper intake may result in metabolic disturbances, liver and kidney damage, anemia, and abdominal pain. Lead exposure has been linked to severe impacts on the cardiovascular and nervous systems, renal failure, and reproductive dysfunction. In response, WHO and other regulatory authorities have established guidelines specifying permissible limits of trace metals in herbal plants intended for human use. [11]

Conclusion

The study demonstrates that Adathodai Girutham complies with safety standards for aflatoxins and heavy metals as defined by regulatory guidelines. No detectable levels of aflatoxins (B1, B2, G1, and G2) were found, metal contaminant concentration such as lead, cadmium, mercury, and arsenic remained within permissible limits. These results confirm the formulation's toxicological safety and support its traditional therapeutic use. Nevertheless, ongoing standardization check and maintaining conformity to Good Manufacturing Practices (GMP) are crucial to ensure long-term consumer safety and maintain product effectiveness.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

I would like to express my sincere thanks to lecturers of PG Pothu maruthuvam department at Government Siddha Medical College,Chennai for their Enlightened support. I m also grateful to thank Dr.Sudhamathi and Head of the department of PG Pothu maruthuvam, Government Siddha Medical College, Chennai. Furthermore I express my sincere Gratitude to my family and friends for their encouragement and support throughout this research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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