Research Article

Heavy Metal, Pesticide Residue, Aflatoxins, and Microbial Analysis of Siddha Medicine Ashwagandha Chooranam

Ishwarya R[1*], Poovarasi M[2], Arul Mozhi P[3], Meenakshi Sundaram M[4], Meenakumari R[5]

[1]PG scholar, Department of Kuzhanthai Maruthuvam, National Institute of Siddha, Chennai-600047, India.

[2]PG scholar, Department of Kuzhanthai Maruthuvam, National Institute of Siddha, Chennai-600047, India.

[3]Associate Professor, Department of Kuzhanthai Maruthuvam, National Institute of Siddha, Chennai-600047, India.

[4]Professor, The head of the Department, Department of Kuzhanthai Maruthuvam, National Institute of Siddha, Chennai-600047, India.

[5]Director, National Institute of Siddha, Chennai-600047, India.

***Corresponding author's E-mail:** ishwaryaraj1997@gmail.com

Received: 03-06-2024; **Revised:** 30-08-2024; **Accepted:** 08-09-2024; **Published on:** 15-09-2024.

ABSTRACT

This study evaluated the safety and quality of Ashwagandha Chooranam, a traditional Siddha herbal preparation, by analyzing its content of heavy metals, aflatoxins, pesticide residues, and microbial contaminants. Atomic Absorption Spectrometry, Thin Layer Chromatography, and microbiological tests were employed to assess the presence of these contaminants. The results revealed that Ashwagandha Chooranam was free from heavy metals, pesticide residues, and microbial contaminants, with Aflatoxin B1 present at 0.03 mg/kg, within the permissible limit. The study concludes that Ashwagandha Chooranam is safe for therapeutic use, meeting the recommended limits set by AYUSH. This research highlights the importance of quality control measures in ensuring the safety and efficacy of herbal preparations.

Keywords: Ashwagandha Chooranam, Heavy metal, Aflatoxins, Pesticide residue, Microbial Contaminants.

INTRODUCTION

Siddha medicine, a traditional Indian medical system, has its roots in ancient Tamil civilization (around 4000-1900 BCE)[1]. This holistic approach to health and wellness aims to balance the three humors (tridoshas) - vata, pitta, and kapha - and harmonize the seven elements that constitute the human body[2]. With a focus on natural remedies, spiritual growth, and self-realization, Siddha medicine seeks to achieve physical, mental, and spiritual perfection[3]. This ancient system has been practiced for centuries, offering a unique perspective on health and wellness that continues to influence modern medicine.

Ashwagandha is commonly available as a chooranam, a fine sieved powder that can be mixed with water, ghee or honey. It enhances the function of the brain and nervous system and improves the memory. It improves the function of the reproductive system promoting a healthy sexual and reproductive balance. Being a powerful adaptogen, it enhances the body's resilience to stress. Ashwagandha improves the body's defense against disease by improving the cell-mediated immunity. It also possesses potent antioxidant properties that help protect against cellular damage caused by free radicals[4].

Using a range of methods, this study sought to evaluate the heavy metals, aflatoxins, pesticide residue and microbial analysis of Ashwagandha Chooranam (AC), a Siddha herbal preparation. The results offer insightful information on the possible quality, safety, and future paths of study for this conventional medication.

MATERIALS AND METHODS

Ingredient

Ashwagandha root- *Withania somifera*- 500 grams.[5]

Source and authentication of raw drug

The raw drug was collected from Devaraj raw drug store, Tambaram. The drug was authenticated by Medicinal Botanist at National Institute of Chennai.

Purification and preparation

The drug was placed in a cloth and steamed above the milk for 1 saamam and then it was dried under the sunlight. The purified *Ashwagandha* was powdered and filtered using fine cloth. Then it was stored in airtight container.

METHODOLOGY

Heavy metal analysis by atomic absorption spectrometry

Standard: Hg, As, Pb and Cd – Sigma

Atomic Absorption Spectrometry (AAS) is a very common and reliable technique for detecting metals and metalloids in environmental samples. The total heavy metal content of the sample was performed by Atomic Absorption Spectrometry (AAS) Model AA 240 Series. In order to determination the heavy metals such as mercury, arsenic, lead and cadmium concentrations in the test item.

Sample Digestion

Test sample was digested with 1mol/L HCl for determination of arsenic and mercury.

Similarly, for the determination of lead and cadmium the sample were digested with 1mol/L of HNO3.

Standard reparation

As & Hg- 100 ppm sample in 1mol/L HCl

Cd & Pb- 100 ppm sample in 1mol/L HNO3

Pesticide residue

Test sample were extracted with acetone and followed by homogenization for brief period. Further filtration was allowed and subsequent addition of acetone to the test mixture. Heating of test sample was performed using a rotary evaporator at a temperature not exceeding 40ºC until the solvent has almost completely evaporated. To the residue add a few milliliters of toluene and heat again until the acetone is completely removed. Resultant residue will be dissolved using toluene and filtered through membrane filter[6,7].

Aflatoxins

Standard: Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2

Solvent: Standard samples were dissolved in a mixture of chloroform and acetonitrile (9.8: 0.2) to obtain a solution having concentrations of 0.5 µg per ml each of aflatoxin B1 and aflatoxin G1 and 0.1 µg per ml each of aflatoxin B2 and aflatoxin G2.

Procedure

Standard aflatoxin was applied on to the surface to pre coated TLC plate in the volume of 2.5 µL, 5 µL, 7.5 µL and 10 µL. Similarly, the test sample was placed and allow the spots to dry and develop the chromatogram in an unsaturated chamber containing a solvent system consisting of a mixture of chloroform, acetone and isopropyl alcohol (85: 10: 5) until the solvent front has moved not less than 15 cm from the origin. Remove the plate from the developing chamber, mark the solvent from and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 365 nm[8].

Sterility test

Test sample was inoculated in sterile petri dish to which about 15 mL of molten agar 45°C were added. Agar and sample were mixed thoroughly by tilting and swirling the dish. Agar was allowed to completely gel without disturbing it. (about 10 minutes). Plates were then inverted and incubated at 37° C for 24-48 hours and further extended for 72 hrs for fungal growth observation. Grown colonies of organism was then counted and calculated for CFU.

Specific pathogen test

Test sample was directly inoculated in to the specific pathogen medium (EMB, DCC, Mannitol, Cetrimide) by pour plate method. The plates were incubated at 37oC for 24 - 72h for observation. Presence of specific pathogen identified by their characteristic colour with respect to pattern of colony formation in each differential media.

RESULTS

The report presents the results of a study on the safety and quality of Ashwagandha Chooranam (AC), a Siddha herbal preparation. They are presented in the following tables 2 to 6.

Table 1: Detail of Specific Medium and their abbreviation

Organism	Abbreviation	Medium
E-coli	EC	EMB Agar
Salmonella	SA	Deoxycholate agar
Staphylococcus aureus	ST	Mannitol salt agar
Pseudomonas aeruginosa	PS	Cetrimide Agar

Table 2: Heavy metal analysis

Name of the Heavy Metal	Absorption Max Λ max	Result Analysis	Maximum Limit
Lead	217.0 nm	BDL	10 ppm
Arsenic	193.7 nm	BDL	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	BDL	1 ppm

*BDL- Below Detection Limit

Inference

Results of the present investigation have clearly shown that the sample has no traces of heavy metal such as Mercury, Arsenic, Lead and Cadmium.

Table 3: Pesticide residue analysis

Pesticide Residue	Sample AC	AYUSH Limit (mg/kg)
I. Organo Chlorine Pesticides		
Alpha BHC	BQL	0.1mg/kg
Beta BHC	BQL	0.1mg/kg
Gamma BHC	BQL	0.1mg/kg
Delta BHC	BQL	0.1mg/kg
DDT	BQL	1mg/kg
Endosulphan	BQL	3mg/kg
II. Organo Phosphorus Pesticides		
Malathion	BQL	1mg/kg
Chlorpyriphos	BQL	0.2mg/kg
Dichlorovos	BQL	1mg/kg
III. Organo carbamates		
Carbofuran	BQL	0.1mg/kg
III. Pyrethroid		
Cypermethrin	BQL	1mg/kg

*BQL- Below Quantification Limit

Inference

The results showed that there were no traces of pesticides residues such as Organo chlorine, Organo phosphorus, Organo carbamates and pyrethroids in the sample provided for analysis.

Table 4: Aflatoxins

Aflatoxin	Sample AC	AYUSH specification Limit
B1	0.03mg/kg	0.5 ppm (0.5mg/kg)
B2	Not Detected – Absent	0.1 ppm (0.1mg/kg)
G1	Not Detected – Absent	0.5 ppm (0.5mg/kg)
G2	Not Detected – Absent	0.1 ppm (0.1mg/kg)

Inference

The results shown that there were no spots were being identified in the test sample loaded on TLC plates when compare to the standard which indicates that the sample were free from Aflatoxin B2, Aflatoxin G1, Aflatoxin G2. Whereas results indicate the presence of Aflatoxin B1 at 0.03 mg/kg and is below the specification limit.

Table 5: Microbial analysis

Test	Result	Specification	As per AYUSH/WHO
Total Bacterial Count	Absent	NMT 10^5CFU/g	As per AYUSH specification
Total Fungal Count	Absent	NMT 10^3CFU/g	

Inference

No growth / colonies were observed in any of the plates inoculated with the test sample, indicating the absence of microbes.

Table 6: Test for specific pathogen

Organism	Specification	Result	Method
E-coli	Absent	Absent	As per AYUSH specification
Salmonella	Absent	Absent	
Staphylococcus aureus	Absent	Absent	
Pseudomonas aeruginosa	Absent	Absent	

Inference

No growth / colonies were observed in any of the plates inoculated with the test sample indicating the absence of specific pathogen.

DISCUSSION

The study analyzed the presence of heavy metals, aflatoxins, pesticide residues, and microbial contaminants in AC. The results show that:

- Heavy metals (Mercury, Arsenic, Lead, and Cadmium) were not detected.

- Pesticide residues (Organo chlorine, Organo phosphorus, Organo carbamates, and pyrethroids) were not detected.

- Aflatoxins (B2, G1, and G2) were not detected, while Aflatoxin B1 was present at 0.03 mg/kg, within the permissible limit.

- Microbial contaminants (Total Bacterial Count, Total Fungal Count, *E-coli, Salmonella*, *Staphylococcus AUREUS*, and *Pseudomonas aeruginosa*) were absent.

CONCLUSION

The study concludes that Ashwagandha Chooranam is safe for therapeutic use, as all safety parameters were within the recommended limits set by AYUSH. It contributes to the existing body of knowledge on siddha medicine and highlights the need for further studies to explore the potential of Ashwagandha chooranam in promoting health and well-being.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Weiss, R. Siddha Medicine: An Ancient Indian Medical System. Journal of Ayurveda and Integrative Medicine, 2009;1(1):13-19.

2. Srikumar, R. Siddha System of Medicine: An Overview. International Journal of Research in Ayurveda and Pharmacy, 2017;8(2):1-5.

3. Murthy, A. R. V. Siddha Medicine: A Review. Journal of Pharmaceutical and Scientific Innovation, 2018;7(4):351-356.

4. Kumar G, Singh P. *Withania somnifera* (Ashwagandha): A Review. Pharmacognosy Rev. 2012;6(12):13-23.

5. The Siddha Pharmacopoeia of India, Part-1, Volume-1, First Edition 2020.

6. WHO guideline for assessing the quality of herbal medicines with reference to contaminants and residues. WHO Geneva. 2007.

7. Lohar. D.R. Protocol for testing of ASU medicines. Pharmacopoeial Laboratory for Indian Medicines. Ministry of AYUSH. 2007.

8. Luciana de CASTRO. Determining Aflatoxins B1, B2, G1 and G2 in Maize Using Florisil Clean Up with Thin Layer Chromatography and Visual and Densitometric Quantification. Ciênc. Tecnol. Aliment. 2001;21(1):50-56.

9. Kannusamy pillai, Chikicha rathna deepam-Rathna naicker and sons, 2nd edition, Chennai: 2018.