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ANALYSIS OF HEAVY METAL, AFLATOXIN, PESTICIDE RESIDUE AND MICROBIAL CONTAMINATION OF SIDDHA HERBAL FORMULATION MUPPIRANDAI CHOORANAM

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Article History	Abstract
Received on: 28-06-2023 Revised on: 14-07-2023 Accepted on: 09-09-2023	Aim: The aim of the study was to evaluate the presence of Heavy metal, Aflatoxin, Pesticide residue and Microbial contamination of Siddha herbal formulation <i>Muppirandai Chooranam</i> (MRC).
	Place of study: Heavy metal analysis, Aflatoxin assay, Pesticide residue, Microbial contamination analysis were conducted at Noble Research Solutions, Kolathur, Chennai -99.
	Methodology: The Siddha formulation <i>Muppirandai Chooranam</i> was prepared as per Good Manufacturing Practices(GMP) guidelines and the Heavy metal analysis, Aflatoxin assay, Pesticide residue, Microbial contamination analysis were conducted at Noble Research Solutions, Kolathur, Chennai -99.
	Results: The results of Heavy metal analysis of <i>Muppirandai Chooranam</i> (MRC) shown the presence of Lead at 3.54 PPM and Arsenic, Cadmium, Mercury at Below Detection Limit (BDL). Aflatoxin assay of MRC shown the absence of Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2. Pesticide residue analysis showed that there were no traces of Pesticide residues such as Organo chlorine, Organo phosphorus, Organo carbamates and Pyrethroids. In Microbial contamination analysis, Test for Specific Pathogen shown the absence of Organisms <i>E-coli</i> , <i>Salmonella</i> , <i>Staphylococcus Aureus</i> , <i>Pseudomonas Aeruginosa</i> and No Bacterial and fungal growth or colonies were observed in the Sterility test of MRC as per the methods of AYUSH specifications.
	Conclusion: From the results, it is concluded that the study medicine MRC has Heavy metal content below the permissible limit as per PLIM guidelines of AYUSH, and the sample were free from Aflatoxins, Pesticides, Microbes and Specific Pathogens which ensures that the study medicine <i>Muppirandai Chooranam</i> was safe therapeutically.
	Keywords: <i>Muppirandai Chooranam</i> , <i>Siddha</i> , <i>Perumbadu</i> , <i>Menorrhagia</i> , <i>Heavy metal</i>

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1. Introduction

A drug is a substance used as a medicines, which are used either directly as crude drug or after undergone some processes. Siddha system of Medicine uses drugs of plant or animal or metal or mineral origin. To deprive of its impurities, crude raw drugs of either origin should undergone respective standard purification processes and after the actual medicine preparation should be done. This gives better results therapeutically and also helps in detoxification and assures

safety. Since Siddha system has multiple combinations of medicines and various preparatory processes like powdering, heating, boiling, drying, grinding, calcinations, sublimation, filtration and so on, chances for impurities, mishandling, is possible which could affect the global acceptance scientifically. This can be rectified by employing proper procedures and can be analysed by standardization techniques through various parameters to get the reproducible standards [1]. Heavy metal test are performed to look for potentially dangerous levels of metals at certain concentrations and some of them includes, Lead, Arsenic, Cadmium, Mercury and Chromium, which are extremely toxic. Pesticide residues which could present in medicines due to usage of pesticides in cultivation process and for economic return [2]. Pesticides and heavy metals can accumulate in the body through biological chains while being persistent and not biodegradable. Thus it is important to monitor their concentration [3].

Aflatoxins are a class of hazardous mycotoxin compounds produced by *Aspergillus flavus* and *Aspergillus parasiticus* that have structural similarities. The aflatoxin group contains about 16 compounds out of which only aflatoxin B1, B2, G1 and G2 are regularly monitored. High moisture content and temperature leads to occurrence of mycotoxins and have adverse health effects on humans [4]. According to research, approximately 80% of people in developing countries use traditional herbal remedies as their primary form of healthcare. Contamination by microorganisms of various types that may be adherent to the leaves, stems, blossoms, seeds, and roots from which herbal medicines are manufactured. Microorganisms can also be added throughout the processes of harvesting, handling, open-air drying, preserving, and manufacturing. Due to consumers uncompromising conditions and microbial infections, the presence of microbial contaminants in herbal products might negatively impact their health status, posing a global health issue. Therefore, it is essential to ensure that users of herbal products are safe [5]. Thus, the present study deals with the analysis of Heavy metal, Aflatoxin, Pesticide Residue and Microbial Contamination of Siddha Herbal Formulation *Muppirandai Chooranam*, indicated for various ailments including Menorrhagia.

2. Materials and Methods

2.1 Collection of Raw Drugs

The Plant *Muppirandai* was collected and the indigenous herbal raw drugs were procured from a reputed raw drug store, identified and authenticated by the Botanist of Government Siddha Medical College, Chennai, (Voucher number GSMC/MB-603 – 607).

2.2 Ingredients

1. *Cissus quadrangularis* - three sided (*Muppirandai*) - 1 *thooku* (1.75 kg)
2. *Zingiber officinale* Rosc. (Dried ginger) - 1 *palam* (35gm)
3. *Piper nigrum* Linn. (Pepper) - 1 *palam* (35gm)
4. *Piper longum* Linn. (Long pepper) - 1 *palam* (35gm)
5. *Trachyspermum ammi* Linn. (Ajwain / thymol seeds) - 1 *palam* (35gm)

2.3 Purification

Raw drugs were purified as mentioned in *Sikitcha Rathna Deepam Ennum Vaidhiya Nool*[6], *Marundhu Sei Iyalum Kalaiyum*[7].

Muppirandai

Muppirandai was cleaned by removing its *kanu*, outer skin and soaked in buttermilk, added with salt for 3 days and dried in sunlight.

Chukku

One part of dried ginger was bleached with 2 parts of lime stone (*kal sunnambu*) for 3 hours (1 *saamam*), washed, dried and the outer skin was peeled.

Milagu

Soaked in sour buttermilk for 3 hours (1 *saamam*) and sun dried.

Thippili

Soaked in lime juice and dried.

Omam

Omam was washed with lime stone water and dried.

2.4 Sample Preparation

1 *thooku* (1.75 kg) of *Muppirandai*, were taken in a mud vessel, boiled with cow's milk, filtered, squeezed and juice was taken. Dried ginger, Pepper, Long pepper, Ajwain (each 35gms) were roasted, powdered and mixed with the above juice. The juice mixed with the powders, were kept sun dried. After drying, contents were finely powdered.

3. Materials and Methods

The Heavy metal analysis, Aflatoxin assay, Pesticide residue, Microbial contamination analysis were conducted at Noble Research Solutions, Kolathur, Chennai -99. The project Id was NRS/AS/0898/09/2022

3.1 Heavy Metal Analysis By Atomic Absorption Spectrometry(AAS)

3.1.1 Methodology

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, extraction solvent HCl and HNO₃ in order to determine the heavy metals such as mercury, arsenic, lead and cadmium concentrations in the test item. Standard: Hg, As, Pb and Cd – Sigma.

3.1.2 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.

3.1.3 Standard preparation

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

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

3.2 Aflatoxin Assay by Thin Layer Chromatography (TLC)

Standard: Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2
Solvent: Standard samples was 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.

3.2.1 Procedure

Standard aflatoxin was applied on to the surface to pre coated TLC plate in the volume of 2.5 µL, 5µL, 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 front and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 365 nm [8].

3.3 Pesticide Residue Analysis

Parameter analysed was Organochlorine pesticides, Organophosphorus pesticides, Organocarbamates, Pyrethroids.

3.3.1 Extraction

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 40°C 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 [9][10].

3.4 Test for Specific Pathogen

3.4.1 Methodology

Test sample was directly inoculated in to the specific pathogen medium (EMB, DCC, Mannitol, Cetrimide) by pour plate method. The plates were incubated at 37°C 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.

Table 1. Detail of Specific Medium and their abbreviation

Organism	Abbreviation	Medium
<i>E-coli</i>	<i>EC</i>	<i>EMB Agar</i>
<i>Salmonella</i>	<i>SA</i>	<i>Deoxycholate agar</i>
<i>Staphylococcus Aureus</i>	<i>ST</i>	<i>Mannitol salt agar</i>
<i>Pseudomonas Aeruginosa</i>	<i>PS</i>	<i>Cetrimide Agar</i>

3.4 Sterility Test By Pour Plate Method

3.4.1 Objective

The pour plate techniques were adopted to determine the sterility of the product. Contaminated / un sterile sample (formulation) when come in contact with the nutrition rich medium it promotes the growth of the organism and after stipulated period of incubation the growth of the organism was identified by characteristic pattern of colonies. The colonies are referred to as Colony Forming Units (CFUs).

3.4.2 Methodology

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.

4. Results and Discussion

Heavy metal screening of MRC shown that it contains Arsenic, Cadmium, Mercury were BDL (Below Detection Limit), and Lead was 3.54 PPM, whose maximum limit was upto 10PPM. However, its lower limit indicating the safety of the drug.

Table 2. Test report of Heavy metal analysis of MRC

Name of the Heavy Metal	Absorption Max/λmax	Result Analysis	Maximum Limit
Lead	217.0 nm	3.54PPM	10 ppm
Arsenic	193.7 nm	BDL	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	BDL	1 ppm

The results of Aflatoxin assay of MRC by TLC 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 B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2.

Table 3. Test report of Aflatoxin assay of MRC

Aflatoxin	Sample MRC	AYUSH Specification Limit
B1	Not Detected - Absent	0.5 ppm
B2	Not Detected - Absent	0.1 ppm
G1	Not Detected - Absent	0.5 ppm
G2	Not Detected - Absent	0.1 ppm

Pesticide residue analysis of MRC with the parameters Organochlorine pesticides, Organophosphorus pesticides, Organocarbamates, Pyrethroids were found to be that there were no traces of pesticides residues and the results were given below.

Table 4. Test report of Pesticide residue of MRC

Pesticide Residue	Sample MRC	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 PhosphorusPesticides		
Malathion	BQL	1mg/kg
Chlorpyriphos	BQL	0.2 mg/kg
Dichlorovos	BQL	1mg/kg
III. Organo carbamates		
Carbofuran	BQL	0.1mg/kg
III.Pyrethroid		
Cypermethrin	BQL	1mg/kg

Microbial contamination analysis of MRC by test for specific pathogen shown that there were No growth was observed after incubation period, reveals the absence of specific pathogen. Results were given below.

Table 5. Test for specific pathogen report

Organism	Specification	Result	Method
<i>E-coli</i>	Absent	Absent	As per AYUSH specification
<i>Salmonella</i>	Absent	Absent	
<i>Staphylococcus Aureus</i>	Absent	Absent	
<i>Pseudomonas Aeruginosa</i>	Absent	Absent	

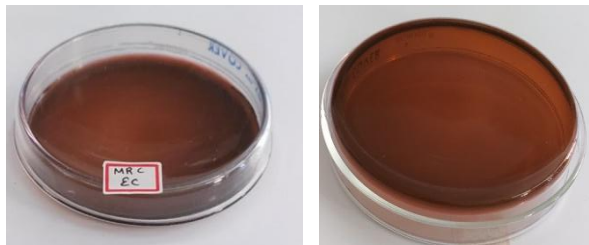


Figure 1. Culture plate with E-coli (EC) specific medium

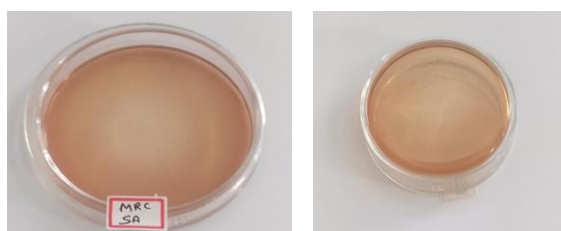


Figure 2. Culture plate with Salmonella (SA) specific medium

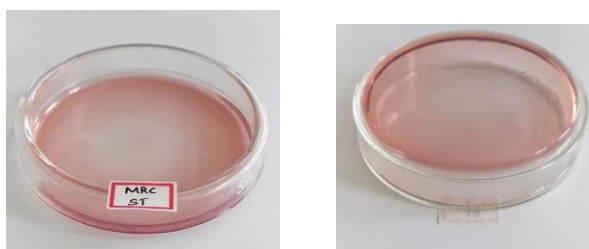


Figure 3. Culture plate with Staphylococcus Aureus (ST) specific medium

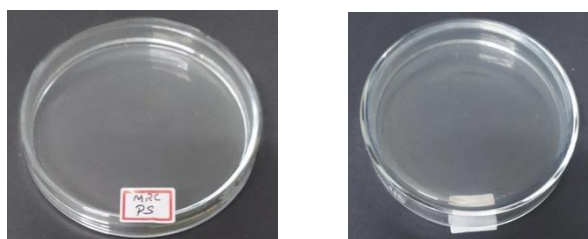


Figure 4. Culture plate with Pseudomonas Aeruginosa (PS) specific medium

Sterility test of MRC also found to be that there were No growth / colonies was observed in any of the plates inoculates with the test sample which ensures that the sample is devoid of microbial contamination in both the tests.

Table 6. Sterility test report of MRC

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

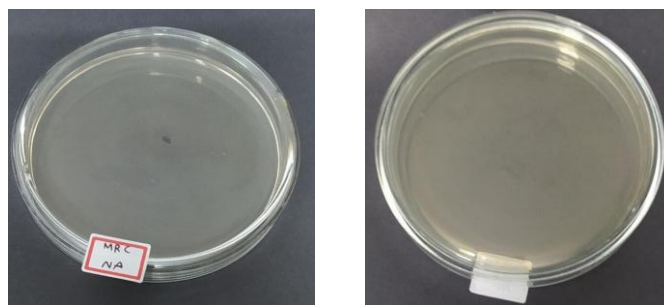


Figure 5. Colony plate for Total bacterial and fungal count of MRC

Apart from this, previous studies of MRC includes, Functional groups identification through FTIR characterization, which identified few organic functional groups [11].

5. Conclusion

Through the present study, it is concluded that the sample *Muppirandai Chooranam* (MRC) was found to be safe with the presence of heavy metals below the detection limit, Devoid of aflatoxins, pesiticide residues, and microbial contamination in specific pathogens, as well as bacterial and fungal counts. This ensures the quality profile of MRC in terms of contamination from biological chains. This preliminary standardisation study would assist in further research and clinical trials with the basic quality sustain.

6. Acknowledgement

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7. Consent

It is not applicable.

8. Ethical Approval and Inform Consent

It is not applicable.

9. Author Contribution

Dr. Shanthini R, performed the study and prepared the manuscript. Dr. Anbu N, guided the study and approved the manuscript.

10. Competing Interests

Authors have declared that no competing interests exist.

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