



Heavy Metal, Aflatoxin, Pesticide Residue, Microbial Analysis of Siddha Polyherbal Formulation *Veppampoo mathirai*

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Authors' contributions

This work was carried out in collaboration between both authors. Author SMC performed the analysis and wrote the first draft of the manuscript. Author NA did the proof correction and supported to write the final manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The polyherbal siddha formulation *veppampoo Mathirai* is effective in regulating blood pressure but its safety is not known. The heavy metal, aflatoxins, pesticide residue, microbial count have not been evaluated so far. The current study evaluated the above parameters. The present study was aimed to evaluate the safety parameters (heavy metal, aflatoxin, pesticide residue and microbial profile) of *Veppampoo Mathirai*.

Materials and Methods: According to AYUSH [Ayurveda, yoga, unani, siddha, naturopathy] Pharmacopoeial laboratory for Indian medicine (PLIM) guidelines, the formulation was evaluated for its safety parameters at Noble research solutions, kolathur, Chennai, accredited with ISO 9001: 2015. Atomic Absorption Spectrometer (AAS) was used for testing heavy metals and aflatoxins were tested using Thin layer chromatography(TLC). The Pesticide residues content was estimated by GC/MS while microbial count by pour plate method.

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Results: The study revealed presence of heavy metals mercury, arsenic, lead and cadmium within the recommended limit as per AYUSH Pharmacopoeial Laboratory for Indian Medicine Guidelines whereas presence of Aflatoxin, pesticide residues and microbes were absent in the sample which showed the formulation *Veppampoo Mathirai* (VPM) was free from toxicity.

Conclusion: VPM showed heavy metal content below the permissible limit as per PLIM guidelines of AYUSH. Aflatoxins and pesticide residue were not detected while the microbes and specific pathogens were absent in the current batch of VPM. Hence, the present study ensures the formulation was safe for therapeutic use.

Keywords: Aflatoxin; Heavy metal analysis; microbial content; *Veppampoo mathirai*.

1. INTRODUCTION

Traditional medicine is widely used for the prevention and treatment of many diseases and are also used to boost energy and improve immunity system [1]. Large sections of population in developing countries still rely on herbal medicines for their primary care [2]. The limits of toxic metals in the form of impurities depend on the nature of the sample and the contaminants or residues [3]. Plants are the main link in the transfer of heavy metals from the contaminated soil to humans. Heavy metals have low excretion rates through the kidney which could result in damaging effects on humans even at very low concentrations [4]. Besides heavy metals, Aflatoxins are mycotoxins produced mainly by *Aspergillus parasiticus* and *Aspergillus flavus* and, though rarely, by *Aspergillus nomius* [5]. Aflatoxins are well known as one of the most powerful carcinogens and mutagens. Other toxic effects of aflatoxins include immunosuppression, teratogenicity and genotoxicity. Its contamination in foodstuff and animal feed is controlled by legal limits [6]. Herbal supplements may also be contaminated with pesticide residues due to excessive use of pesticides during the cultivation and lack of good agriculture practices. Organochlorine pesticide residues were found in a number of Chinese herbal plants cultivated in China and sold in Hong Kong [7].

In addition, contamination with dusts, pollens, molds and fungi have the potential to cause significant adverse effects [8]. A study in 2011 in Hong Kong showed a serious and under-recognized problem of adulteration of Chinese herbal anti-diabetic products with undeclared pharmaceuticals, including both registered and banned drugs [9]. Furthermore, in the United Arab Emirates, heavy metal concentrations in local and imported herbal plants rendered the plants unsafe for human use [10]. Quality and safety parameters of herbal medicines based on the heavy metal contents and microbial load

have been an important concern for health authorities and health professionals. The contamination of these herbal products reduces their effectiveness and also poses serious health hazards to consumers [11]. Hence, it is very important to standardize Siddha medicines using scientific techniques to prove its safety and quality which might help in building confidence for their possible use as a therapeutic medicine, among people and for their global acceptance. The present study had been done as a measure of the above purpose to ensure its safety.

The national limit for toxic metals and microbial contaminants in various types of herbal products are different for each country and depend on herb type and whether it is raw material or a finished product [3]. According to AYUSH Pharmacopoeial laboratory for Indian medicine (PLIM) guidelines, the formulation was evaluated for its safety parameters.

The Siddha polyherbal formulation *Veppampoo Mathirai* (VPM) is indicated for regulating blood pressure, as per Siddha classical text, Noigaluku Siddha Parigaaram, part I, by Dr.M.Shanmugavelu. It contains fourteen herbal ingredients which is presented in tablet form grounded with lime juice. The herbal ingredients are *Azadirachta indica*, *Phyllanthus amarus*, *Solanum trilobatum*, *Eclipta prostrata*, *Zingiber officinalis*, *Piper nigrum*, *Piper longum*, *Terminalia chebula*, *Terminalia bellerica*, *Emblica officinalis*, *Eugenia caryophyllata*, *Cinnamom zeylanicum*, *Elatteria cardamomum*, *Coeus vetiveroides* [12].

2. MATERIALS AND METHODS

2.1 Sample Preparation

The herbal ingredients were procured from reputed indigenous raw drug store Chennai, in the Month of February 2021 and were identified and authenticated by the Botanist, Government

siddha medical college, Chennai (voucher number GSMC/MB-89/21 -- 100/21) The herbals were purified according to the siddha classical text Sikittha rathna deepam saraku suthi muraigal [13]. Each of the herbal ingredients was made in to fine powder separately. All the powdered drugs were mixed in the stone mortar and grounded with required quantity of lime juice for 72 hours and made in to 500 - gram tablets [10]. The tablets were dried well in shade and stored in a clean dry air tight container. The above tablets were prepared at pharmacology lab, pharmacology department, Government siddha medical college, Chennai and was evaluated for heavy metal content, aflatoxins, pesticide residues, microbial content at Noble research solutions, kolathur, Chennai, accredited with ISO 9001: 2015.

2.2 Heavy Metals

Atomic Absorption Spectrometry (AAS) is a very common and reliable technique for detecting metals and metalloids in environmental samples [10]. The total heavy metal content of the sample VPM was performed by Atomic Absorption Spectrometry (AAS). Model AA 240 Series was used to determine the heavy metals content such as mercury, arsenic, lead and cadmium concentrations. The sample VPM was digested with 1mol/L HCl for determination of arsenic and mercury. Similarly for the determination of lead and cadmium the sample was digested with 1mol/L of HNO₃. Standard reparation was As & Hg (arsenic and mercury) -100ppm sample in 1 mol /L Hcl, Cd & Pb (Cadmium and Lead) - 100ppm sample in 1 mol /L HNO₃.

2.3 Aflatoxins

Standard samples was dissolved in a mixture of chloroform and acetonitrile (9.8:0.2) to obtain 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. Test solution concentration was 1µg per ml. 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 the spots were allowed 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. The plate was removed from the developing

chamber, the solvent was marked and the plate was allowed to air-dry. The spots were located on the plate by examination under UV light at 365nm [14].

2.4 Pesticide Residues

Pesticide residue analysis was carried out using GC/MS (Gas chromatography – Mass spectrometry) technique. About 10 gm of test sample was extracted with 100ml of acetone followed by homogenization for breif period. Further filtration was allowed and subsequent addition of acetone with other 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 added a few milliliters of toluene R and heated again until the acetone is completely removed. Resultant residue was dissolved using toluene and filtered through membrane filter [15].

2.5 Microbial Count

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). 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.

2.6 Test for Specific Pathogen

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.

3. RESULTS

Table 1. Heavy metal content in Veppampoo Mathirai

Name of the Heavy Metal	Absorption Max (λ_{max})	Result Analysis	Permissible Limit
Mercury	253.7 nm	0.4667 ppm	1 ppm
Lead	217.0 nm	0.9769 ppm	10 ppm
Arsenic	193.7 nm	0.0593 ppm	3 ppm
Cadmium	228.8 nm	0.0195 ppm	0.3 ppm

Table 2. Aflatoxin Veppampoo Mathirai

Aflatoxin	Sample VPM	LOQ	AYUSH Specification Limit
B ₁	Not detected	0.001	0.5ppm
B ₂	Not detected	0.001	0.1ppm
G ₁	Not detected	0.001	0.5ppm
G ₂	Not detected	0.001	0.1ppm

Ayush – Ayurveda, yoga, unani, siddha, naturopathy. ppm – parts per million. LOQ – Limit of quantification

Table 3. Pesticide residue in Veppampoo Mathirai

Pesticide Residue	Sample VPM	LOQ	AYUSH Limit (mg/kg)
I. Organo chlorine pesticides			
Alpha BHC	Not detected	0.04	0.1mg/kg
Beta BHC	Not detected	0.04	0.1mg/kg
Gamma BHC	Not detected	0.04	0.1mg/kg
Delta BHC	Not detected	0.04	0.1mg/kg
DDT	Not detected	0.04	1mg/kg
Endosulphan	Not detected	0.04	3mg/kg
II. Organo phosphorus pesticides			
Malathion	Not detected	0.04	1mg/kg
Chlorpyrifos	Not detected	0.04	0.2mg/kg
Dichlorovos	Not detected	0.04	1mg/kg
III. Pyrethroid			
Cypermethrin	Not detected	0.01	1mg/kg

LOQ – Limit of quantification

Table 4. Microbial count in Veppampoo Mathirai

Test	Result	AYUSH Limit
Total Bacterial Count	Absent	NMT 10 ⁵ CFU/g
Total Fungal Count	Absent	NMT 10 ³ CFU/g

CFU/g: colony-forming units per gra

Table 5. Test for specific pathogen in Veppampoo Mathirai

Organism	Specification	Result	Medium	Method
E-coli	Absent	Absent	<i>EMB Agar</i>	As per AYUSH specification
Salmonella	Absent	Absent	<i>Deoxycholate agar</i>	
Staphylococcus aureus	Absent	Absent	<i>Mannitol salt agar</i>	As per AYUSH specification
Pseudomonas aeruginosa	Absent	Absent	<i>Cetrimide agar</i>	

EMB Agar – Eosin methylene blue agar

4. DISCUSSION

Several studies were carried out globally to evaluate heavy metal content and microbial contamination of herbal products. Studies conducted in countries like Ghana, Japan, Nigeria, Brazil, South Africa, and some of the Arabian countries like Saudi Arabia and Egypt showed metals like Lead, Cadmium, Aluminium, Mercury and Arsenic were above the set standard in some of the most commonly used medicinal plants [16,17,18,19]. The presence of heavy metals in a drug beyond the permissible limits would cause serious side effects on brain, kidney, developing foetus, vascular and immune system [20]. Similarly, aflatoxins have emerged as a major threat to human health, because a number of serious side effects such as hepatotoxicity, carcinogenicity and immune suppression are associated with them. Therefore, WHO has set a permissible limit of their concentration in the plant [21].

In India, AYUSH has set a permissible limit in their guidelines to analyze safety parameters for all kinds of herbal formulation. As VPM was a tablet formulation the results were analyzed given for that. Heavy metals content of mercury, lead, arsenic and cadmium of VPM were 0.4667ppm, 0.9769ppm, 0.593ppm, and 0.0195ppm respectively that was found below the permissible limit as per PLIM guidelines listed in Table 1 [22]. Aflatoxins B1, B2, G1, G2 as shown in Table 2 were not detected as per limitations set by AYUSH. Similarly, Pesticide residues like organo chlorine, organo phosphorous, and pyrethroid were also not detected in VPM depicted in Table 3. Table 4 shows the microbial count of bacteria and fungus, that were found to be absent in VPM. (Bacterial, yeast and Mould). Test for specific pathogens E-coli, Salmonella, Staphylococcus aureus and Pseudomonas aeruginosa were analyzed and listed in table 5 confirmed that they were absent in VPM [21,22]. Similarly, a study conducted by Sana Nafees et al (2018) previously, on Unani formulation analyzed for the above safety parameters reported the result within the permissible limit as per AYUSH guidelines [21].

In Siddha system of medicine each ingredient should undergo purification process before the formulation gets prepared. The purification process was followed as per the classical text only. Hence there was a greater possibility for the toxins to get eliminated while undergoing the

purification process. But, still there are chances for contamination of the products while going for further process like drying, storage, etc. So, standardization by practiced scientific techniques is quite necessary.

5. CONCLUSION

The findings of the present study revealed that all the four safety parameters carried out on *Veppampoo mathirai* were found to be within the recommended limit as per PLIM guidelines set by AYUSH. Heavy metal content was found below the permissible limit. Aflatoxins and pesticide residue were not detected while the microbes and specific pathogens were absent in the current batch of VPM. Hence, the present study assures the formulation was safe for therapeutic use.

5.1 Limitation

The level of the toxic metals, mycotoxins, pesticide residues and microbial contaminations depends on various factors like the source, season, and the place of the ingredients obtained and so the result only suggests the safety of the current batch of the VPM for its therapeutic use.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

NOTE

The study highlights the efficacy of "Siddha,AYUSH " which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of

modern medical science and can be utilized partially if found suitable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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