***Original Research Article***

# Heavy metals analysis and Estimation of Aflatoxin, pesticide residue, microbial content of Siddha poly herbal formulation Veppampoo Mathirai


## Abstract

The polyherbal siddha formulation veppampoomathirai is effective in regulating blood pressure but its quality assessment like heavy metal content, aflatoxin, pesticide residue, microbial content have not been evaluated so far. The current study evaluated the above parameters.

**Aim:**The present study was aimed to evaluate the safety parameters of polyherbal Siddha formulation VeppampooMathirai used for hypertension to regulate blood pressure.

**Materials and methods:**According to AYUSH [Ayurveda, yoga, unani, siddha, naturopathy]Pharmacopieal laboratory for Indian medicine guidelines, the formulation was evaluated for its safety parametersat Noble research solutions, kolathur, Chennai, accrediated with ISO 9001: 2015.

**Result:**The study revealed presence of heavy metals mercury,arsenic, lead and cadmium within therecommendedlimit as per AYUSH Pharmacopieal Laboratory of Indian Medicine Guidelines whereaspresence of Aflatoxin, pesticide residue and microbial content were absent in the sample which showed the formulationVeppampooMathirai was free from toxicity.



**Key words** :aflatoxin, Heavy metal analysis, microbial content, VeppampooMathirai,


## 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 Aspergillusparasiticus and Aspergillusflavus and, though rarely, by Aspergillusnomius[5].Aflatoxins are well known as one of the most powerful carcinogens and mutagens. Other toxic effects of aflatoxins includeimmunosuppression, teratogenicity and genotoxicity. Its contamination in foodstuff and animal feed is controlled by legal limits[6].
Pesticides are used to protect crops against insects,weeds, fungi and other pests.Pesticides are potentially toxic to humans and can have both acute and chronic health effects, depending on the quantity and ways in which a person is exposeSome of the older, cheaper pesticides can remain for years in soil and water[7].Medicinal herbal products have been reported to be contaminated with microorganisms indigenous to the soil and plants where they are grown[8]. Poor conditions during harvesting and postharvest handling of the herbs and herbal products predispose them to contamination[9].

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]. In the current study, according to AYUSH PharmacopeiaI laboratory for Indian medicine guidelines, the formulation was evaluated for its safety parameters.

The Siddhapolyherbal formulation VeppampooMathirai (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 *Azadirachtaindica, Phyllanthusamarus, Solanumtrilobatum, Eclipta prostate, Zingiberofficinalis, Piper nigrum, Piper longum, Terminaliachebula, Terminaliabellerica, Emblicaofficinalis, Eugenia caryophyllata, Cinnamomzeylanicum, Elatteriacardamomum, Coeusvettiveroides*[10].

## 2. Materials and methods

### 2.1 Sample preparation

The herbal ingredients were procured from reputed indigeneous 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 sikitcharathnadeepamsarakusuthimuraigal. 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 about 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 medicine was prepared at pharmacology department, Government siddha medical college,Chennai.The above sample was studied to evaluate heavy metal content, aflotoxins, pesticide residue, microbial content at Noble research solutions, kolathur, Chennai, accrediated with ISO 9001: 2015.

### 2.2Heavy metals

Atomic Absorption Spectrometry (AAS) is a very common and reliable technique for detectingmetals and metalloids in environmental samples. The total heavy metal content of the sampleVPM was performed by Atomic Absorption Spectrometry (AAS) Model AA 240 Series in orderto determine the heavy metals such as mercury, arsenic, lead and cadmium concentrations inthetest sampleVPM. 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 ofHNO3.

### 2.3Aflatoxins

Standard samples was dissolved in a mixture of chloroform and acetonitrile(9.8:0.2)toobtain 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 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. The plate wasremoved 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 UVlight at 365nm[11].

### 2.4Pesticide residue

About 10 g of test substance were extracted with 100 ml of acetone and followed byhomogenizationforbriefperiod.Furtherfiltrationwasallowedandsubsequentadditionof acetone other test mixture. Heating of test sample was performed using a rotary evaporator at a temperature notexceeding 40ºC until the solvent has almost completely evaporated. To the residue added a few milliliters oftoluene R and heated again until the acetone is completely removed. Resultant residue was dissolved usingtolueneandfilteredthroughmembranefilter[12].

## 2.5Microbial content

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$^{o}$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$^{o}$ 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$^{o}$C for 24 - 72h for observation.Presenceof specific pathogen identified by their characteristic colour with respect to pattern of colony formation in each differential media.

## 3. Results

The results of this study demonstrated that heavy metals, as listed in Table 1 were found below the recommended limit as per AYUSH guidelines. Aflotoxins as shown in Table 2 were not detected at all.Pesticide residue as depicted in Table 3, were found below quantification limit BQL, while microbial content depicted in Table 4,(Bacterial, yeast and Mould) and test for specific pathogens in Table 5 as listed were not found at all

**Table 1.Heavy metal content in VeppampooMathirai**

| Name of the Heavy Metal | Absorption MaxΛmax | Result Analysis | Maximum 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.Aflotoxinin VeppampooMathirai**

| Aflatoxin | Sample VPM | AYUSH Specification Limit |
|---|---|---|
| B1 | NotDetected-Absent | 0.5ppm |
| B2 | NotDetected-Absent | 0.1ppm |
| G1 | NotDetected-Absent | 0.5ppm |
| G2 | NotDetected-Absent | 0.1ppm |

Ayush – Ayurveda, yoga, unani, siddha, naturopathy.ppm – parts per million.

**Table 3.Pesticide residue in VeppampooMathirai**

| Pesticide Residue<br>1.Organo chlorine pesticides | Sample VPM | AYUSHLimit(mg/kg) |
|---|---|---|
| AlphaBHC | BQL | 0.1mg/kg |
| BetaBHC | BQL | 0.1mg/kg |
| GammaBHC | BQL | 0.1mg/kg |
| DeltaBHC | BQL | 0.1mg/kg |
| DDT | BQL | 1mg/kg |
| Endosulphan | BQL | 3mg/kg |
| **11.Organophosphoruspesticides** | | |
| Malathion | BQL | 1mg/kg |
| Chlorpyriphos | BQL | 0.2mg/kg |
| Dichlorovos | BQL | 1mg/kg |
| **III.Pyrethroid** | | |
| Cypermethrin | BQL | 1mg/kg |

BQL - below quantification limit

**Table 4.Microbial content in VeppampooMathirai**

| 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 | |

CFU/g: colony-forming units per gram

**Table 5.Test for specific pathogen in VeppampooMathirai**

| Organism | Specification | Result | Medium | Method |
|---|---|---|---|---|
| *E-coli* | Absent | Absent | *EMB Agar* | |
| *Salmonella* | Absent | Absent | *Deoxycholate agar* | As per AYUSH specification |
| *Staphylococcus Aureus* | Absent | Absent | *Mannitol salt agar* | |
| *Pseudomonas Aeruginosa* | Absent | Absent | *Cetrimide Agar* | |

*EMB Agar* – Eosin methylene blue agar

## 4. Discussion

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[13]. 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 current study had been done as a measure of the above purpose to prove the safety of the polyherbal formulation VeppampooMathirai.

## 5. Conclusion

The Heavy metal content (mercury, arsenic, cadmium, lead) and pesticide residue of VPM showed within recommended limit as per AYUSH guidelines whereas the aflatoxins (B1, B2, G1and G2) ,microbial content and specific pathogens *(E-coli*, *Salmonella, Staphylococcus Aureus, Pseudomonas Aeruginosa)* were not detected at all. The present study ensures the polyherbal siddha formulation VeppampooMathiraimay be safe for use in the treatment of blood pressure.

## Consent

It is not applicable.

## Ethical approval

It is not applicable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

*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.*

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