IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

HEAVY METAL ANALYSIS, PESTICIDE RESIDUE AND AFLATOXIN ASSAY OF SIDDHA POLYHERBAL FORMULATION *ELATHI MATHIRAI*

Dr. Sreekala. K S.[1], Dr. M. D. Saravana Devi

1. PG Scholar 2. Professor and Head, PG Department of Gunapadam

Government Siddha Medical College, Chennai-106

ABSTRACT

The *Siddha* system is a traditional medical system that provides preventive, curative and rejuvenating healthcare through scientific and holistic approach. *Elathi mathirai* is one among the medicines indicated for *Neerizhivu* (Type 2 Diabetes Mellitus) in *Siddha* system, which is not yet standardized. The present study aims to establish the safety profile of *Elathi mathirai* (EM), a polyherbal *Siddha* formulation. Heavy metal analysis, pesticide residue and aflatoxin assay of the drug EM were assessed. Heavy metal analysis was done using Atomic Absorption Spectrometry (AAS), pesticide residue content using GC/MS and TLC (Thin layer chromatography) estimated aflatoxin assay according to PLIM guidelines. Heavy metal analysis revealed the presence of heavy metals arsenic, cadmium and mercury under the below detection limit as per the guidelines. Pesticide residues of organochlorines, organocarbamates, pyrethroids and aflatoxins are absent in the drug EM. These results contribute to establishing the nature of the formulation's composition and its safety profile for therapeutic use.

Keywords: Siddha system, Elathi mathirai, Aflatoxins, Heavy metal analysis, PLIM

1. INTRODUCTION

Diabetes Mellitus is a chronic heterogeneous metabolic disorder characterized by elevated blood glucose levels or hyperglycemia, which results from abnormalities in either insulin secretion or insulin action, or both [1]. India earned the name ‘Diabetes capital of the world’ with its largest number of diabetic subjects. 537 million adults are living with Diabetes (1 in 10 adults) worldwide. This number is predicted to rise to 643 million by 2030 and 784 million by 2045 [2]. Several categories of drugs that have been currently used for the treatment of diabetes, can act by multiple different mechanisms, such as stimulation of the release of insulin (e.g., sulfonylureas), reduction of hepatic glucose output and enhancement of the peripheral uptake of glucose (e.g. biguanides), etc. The two major concerns in the usage of presently available synthetic anti-diabetic drugs

are the side effects caused and the drug resistance on prolonged usage. Hence, there is a demand for new dimensions in filling the gap of lack of scientific explanations in traditional medicines thereby identifying newer healthcare strategies to combat this multifactorial disease [3]. This has led to an increase in the demand of herbal medicines with antihyperglycemic activity having lesser side effects [4].

The Siddha system of medicine is one of the oldest medical systems in the world, which is believed to have evolved in 10,000- 4,000 B.C. [5]. *Elathi mathirai (EM)* is a polyherbal *Siddha* formulation mentioned in classic *Siddha* literature *Sarabendira Mega Nivarana Bodini*, for the management of *Neerizhivu* (Diabetes Mellitus). It is very important to standardize *Siddha* medicines using scientific techniques to prove their safety and quality, which might help in building confidence for their possible use as a therapeutic medicine, among people and for their global acceptance [6]. The present study has been done to evaluate the safety profile of the drug EM.

2. MATERIALS AND METHODS

2.1. Selection of the drug

In the present study, a polyherbal formulation ELATHI MATHIRAI is taken as the compound drug preparation for *Neerizhivu* (Type 2 Diabetes Mellitus), mentioned in the classical *Siddha* literature "*Sarabendira Mega Nivarana Bodini*," page no. 202, written by Hakkim B Muhammad Abdulla Sahib, published by Chennai Muslim Abimani Press in 1908, 2nd edition.

2.2. Ingredients

1. Elam *(Elettaria cardamom)* - 1 palam (35g)
2. Seenthil kizhangu *(Tinospora cordifolia)* - 1 palam (35g)
3. Thamarai valayam *(Nelumbo nucifera)* - 1 palam (35g)
4. Thanneervittan kizhangu *(Asperagus razimosus)* - 1 palam (35g)
5. Aavarm ver *(Cassia auriculata)* -1 palam (35g)
6. Aavaram mel thol (*Cassia auriculata*) - 1 palam (35g)
7. Aavaram poo (*Cassia auriculata*) - 1 palam (35g)
8. Aavaram kozhunthu (*Cassia auriculata*) - 1 palam (35g)
9. Aavaram pinju (*Cassia auriculata*) - 1 palam (35g)
10. Aavaram vithai (*Cassia auriculata*) - 1 palam (35g)
11. Etti ver pattai *(Strychnus nux vomica)* - 1 palam (35g)
12. Thetran kottai seeval *(Strychnus potatorum)* - 1 palam (35g)

2.3. Collection of plant materials

All the ingredients were bought from M.S.S. Aasan and son's herbal house, Nagercoil, and Ramasamy Mudhaliar Store, Parry's Corner, Chennai.

2.4. Identification and Authentication of the drug

All the raw materials were identified and authenticated by the Gunapadam experts & Botanist, department of Gunapadam, GSMC, Chennai. Samples of the ingredients were preserved in the Gunapadam department for future reference.

2.5. Method of preparation of the drug

All the ingredients were taken in equal ratios (1 palam each, 35 g), dried and powdered separately. Then all the powders were mixed and ground together well which favours the homogenous preparation. Then the mixture of the powder was sieved through a thin clean white cloth (*vasthirakayam)* to obtain fine powder. After that, subjected to the pittaviyal process for purification, dried and powdered again. Obtained fine powder was ground with a sufficient quantity of buttermilk and made into karkam, then rolled into pills & dried under the shadow. Preserved in an airtight container.

Heavy Metal Analysis by AAS

The total heavy metal content of the sample was performed by Atomic Absorption Spectrometry (AAS) Model AA 240 Series, to determine the heavy metals such as mercury, arsenic, lead and cadmium concentrations in the test drug EM. The sample drug EM 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 preparation was As & Hg- 100 ppm sample in 1mol/L HCl, Cd & Pb- 100 ppm sample in 1mol/L HNO3.

Pesticides Residue Analysis

Test sample were extracted with acetone and followed by homogenization for a brief period. Further filtration was allowed and subsequent addition of acetone to the test mixture. Heating of the test sample was performed using a rotary evaporator at a temperature not exceeding 40ºC until the solvent had almost completely evaporated. To the residue add a few milliliters of toluene and heat again until the acetone is completely removed. The resultant residue will be dissolved using toluene and filtered through a membrane filter [7,8].

Aflatoxin Assay by TLC

Standard samples of Aflatoxin B1 Aflatoxin B2 Aflatoxin G1 Aflatoxin G2 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. Standard aflatoxin was applied onto the surface of the pre-coated TLC plate in the volumes 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 had moved not less than 15 cm from the origin. Remove the plate from the developing chamber, mark the solvent, and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 365 nm [9].

3. RESULTS AND DISCUSSION

3.1. Heavy metal analysis by AAS

Table 1: Test report of Heavy metal analysis

Name of the Heavy Metal	Absorption Max	Result Analysis	Maximum Limit
Lead	217.0 nm	1.517	10 ppm
Arsenic	193.7 nm	BDL	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	BDL	1 pp

(BDL- Below Detection Limit)

Results of the present investigation have clearly shown that the sample has no traces of heavy metal such as Mercury, Arsenic and Cadmium whereas the sample shows the presence of Lead at 0.517 and 1.517 ppm as listed in the table.

3.2. Pesticides Residue Analysis

Table 2: Test report of Pesticides residue

Pesticide Residue	Sample EM	AYUSH Limit (mg/kg)
I.OrganoChlorine 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.OrganoPhosphorus Pesticides		
Malathion	100 µg/kg	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

(BQL- Below Quantification Limit)

Result: The results showed that there were no traces of pesticides residues such as Organochlorine, Organophosphorus, Organocarbamates and pyrethroids in the sample provided for analysis. Whereas the sample reveals the presence of mild traces of Malathion at 100 µg/kg which belongs to the category of Organophosphorus pesticide.

3.3. Aflatoxin assay by TLC

Table 3: Test report of Aflatoxin assay

Aflatoxin	Sample EM	AYUSH Specification Limit
B1	Not Detected – Absent	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)

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 B1, Aflatoxin B2, Aflatoxin G1 and Aflatoxin G2.

4. CONCLUSION

Heavy metal tests are performed to look for potentially dangerous levels of metals at certain concentrations and some of them include Lead, Arsenic, Cadmium, Mercury and Chromium, which are extremely toxic. Pesticide residues could be present in medicines due to the usage of pesticides in the cultivation process and for economic return. 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 concentrations**.** Besides heavy metals, Aflatoxins are mycotoxins produced mainly by Aspergillus parasiticus and Aspergillus flavus and, though rarely, by Aspergillus nomius . Aflatoxins are well known as one of the most powerful carcinogens and mutagens. Other toxic effects of aflatoxins include immunosuppression, teratogenicity and genotoxicity [10] **.** Heavy metal analysis of the test drug EM by AAS method shows the absence of arsenic, cadmium and

mercury, lead 1.517 ppm is present which is below the maximum limit. Pesticides residue analysis and aflatoxin assay of EM showed the absence of organochlorines, organophosphates, pyrethroids and organocarbamates, and Aflatoxins B1, B2, G1, G2 respectievely.

5. ACKNOWLEDGEMENT

The authors would like to express our heartfelt thanks to The Tamil Nadu Dr. M.G.R medical university for giving permission to carry out these analyses and all the faculties of PG Department of Gunapadam, as well as the Principal of Government Siddha Medical College, Chennai. Also express our special thanks to Noble Research Solutions, Chennai.

Source of funding: None

Ethical Approval: Approved

Conflict of Interest: No conflict of interest.

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