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ANALYSIS OF CHEMICAL CONTAMINANTS IN SEVVIYADHI CHOORANAM

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ABSTRACT

Aim: The aim of the study was to analyze the chemical contaminants such as heavy metals, aflatoxins and pesticide residues in Siddha polyherbal formulation Sevviyadhi Chooranam, in powder form is indicated for the treatment and management of Sinusitis. Materials and Methods: Sevviyadhi Chooranam, the Siddha polyherbal formulation was prepared as per GMP (Good Manufacturing Practices) guidelines. The formulation was analyzed for heavy metals such as mercury, arsenic, lead and cadmium, aflatoxins such as aflatoxin B1, B2, G1, G2 and pesticide residues such as organochlorine pesticides, organophosphorus pesticides, organo carbamates and pyrethroids at Noble Research Solutions, Kolathur, Chennai. Results: Heavy Metal analysis of SC showed that the presence of Lead about 0.26PPM, Arsenic about 0.61 PPM, Mercury and Cadmium in below detection level. Aflatoxin assay of SC resulted absence of Aflatoxin B1, B2, G1 and G2. Pesticide residue analysis revealed the absence of organochloride pesticides, organophosphorus pesticides, organo carbamates and pyrethroids. Conclusion: The findings of this study revealed the presence of heavy metals such as lead and arsenic in limited amount and absence of mercury and cadmium, and absence of aflatoxins and pesticide residue in the Siddha polyherbal formulation Sevviyadhi chooranam. The study ensured that Sevviyadhi chooranam was free from chemical contaminants such as heavy metals, aflatoxins and pesticide residue and validated the safety of SC for therapeutic usage in treatment of sinusitis.

Keywords: Siddha medicine, Sevviyadhi Chooranam, Sinusitis, Chemical contaminants, Heavy metals analysis, Aflatoxin assay, Pesticide residues

INTRODUCTION

"The Siddha system of medicine is one of an ancient system that is practiced in Tamil Nadu in South India and in other Tamil speaking regions of the world. Siddha system of medicine focusses on addressing the root cause of the disease rather than treating the disease symptoms and the combination of herbs, medicinal plants, animal and marine resources go on to make the required drugs"[1]. "Despite the remarkable advancements in modern medicine and the development of synthetic drugs, traditional remedies, now referred to as herbal pharmaceuticals or herbal treatments are still advocated and endorsed by World Health Organization (WHO). According to the second WHO global survey, Siddha medicine is a popular form of traditional and complementary medicine, recognized by several member states" [2].

Sinusitis is the inflammation of the sinuses, which are air filled cavities in the skull. It can be acute or chronic. Types of sinuses are maxillary, frontal, ethmoid and sphenoid. The maxillary sinus is most commonly involved. Etiology of sinusitis can arise from both infectious and noninfectious. Infectious etiologies included viral, bacterial and fungal. Noninfectious etiologies are allergic rhinitis (with either mucosal or polyp obstruction), barotraumas (deep sea diving or air travel), exposure of chemical irritants [3].

Mostly plants are contaminated chemically and biologically. Focusing towards chemical contamination are heavy metals, aflatoxins and pesticide residues. "In the nature, heavy metals induce serious contamination because of their persistence, high toxicity and easy transmission through the food chain. Heavy metals have impact on multiple process in the body, resulting in pathological consequences" [4].

"Fungi are the large group of different eukaryotic organisms widely distributed in nature. Herbal plants of medicinal importance get frequently contaminated by toxigenic fungi. Among 300 types of mycotoxins, aflatoxins are a group of structurally related metabolites produced by Aspergillus fungi such as *A. flavus, A. parasiticus, A. nomius,* and *A. niger*. The frequently occurring aflatoxins are B1, B2, G1 and G2. Most commonly occurring are aflatoxin B1" [5]. Among the more powerful carcinogens and mutagens, aflatoxins are more important[6] according to International Agency of Research on Cancer (IARC) which has classified Aflatoxins (AFs) as Group I human carcinogen[7]. "Pesticides are chemical compounds used to control or eradicate pests. The residues of pesticides including their metabolites will remain in plants or in soil that become a notable source of contamination for herbal medicines".[8]

The World Health Organization estimates about 65-80% of population depends on herbal products as their primary form of health care [8]. Hence it is important to ensure the safety of herbal formulations scientifically to provide a strong trust on people and also for the global acceptance of traditional system of medicine.

In Siddha system of medicine, there are many unique medicines available for treatment and management of Sinusitis. One among them is Sevviyadhi chooranam (SC), mentioned in Siddha literature "Anupava Vaidhya Dheva Ragasiyam- Moondram paagam" [9]. The method of preparation is easy and cost effective. The formulation has been explored for the presence of chemical

contaminants such as heavy metals, aflatoxins and pesticide residues. Therefore, the current study was to ensure the formulation is free from chemical contaminants and validate the safety of drug SC.

MATERIALS AND METHODS

Selection of Drug: The Siddha literature "Anupava Vaidhya Dheva Ragasiyam- Moondram paagam" [9] contains many effective formulations for the treatment of various diseases. One among that is Sevviyadhi Chooranam, which is indicated for an effective management and treatment of sinusitis. The author's aim is to explore the effectiveness of SC in sinusitis. Hence the aim was to collect data for further pre-clinical and clinical evaluations.

Ingredients: The composition of Sevviyadhi chooranam contains twelve herbal ingredients [10]. (Table 1)

Table 1: Ingredients in Sevviyadhi Chooranam

Name	Botanical name	Family
Sevviyam	*Piper nigrum* (Black pepper root)	Piperaceae
Chukku	*Zingiber officinale*	Zingiberaceae
Milagu	*Piper nigrum*	Piperaceae
Thippili	*Piper longed*	Piperaceae
Thalisapathiri	*Abies spectabilis*	Pinaceae
Seeragam	*Cuminum cyminum*	Apiaceae
Nellivatral	*Phyllanthus embilica*	Euphorbiaceae
Chithiramoolam	*Plumbago indica*	Plumbaginaceae
Lavangapattai	*Cinnamomum verum*	Lauraceae
Lavangapathiri	*Cinnamomum tamala*	Lauraceae
Elakkai	*Eletteria cardamomum*	Zingiberaceae
Moongiluppu	*Bambusa arundinaceae*	Poaceae

Collection of Raw drugs: The required raw drugs were obtained from indigenous raw drug store and were identified and authenticated by the botanist, Department of Medicinal botany, Government Siddha Medical College, Arumbakkam, Chennai-106. (Voucher no. GSMC/MB- 579 -590).

Sample preparation

Purification of raw drugs: All the herbal ingredients of SC were purified initially as per Siddha literature "Sikitca Ratna Deepam Ennum Vaidhya Nool" respectively.[10]

Black pepper root (Sevviyam): Purified by peeling out the outer skin and sun dried.
Dried ginger (Chukku): A part of dried ginger was treated and bleached with 2 parts of limestone solution (kal sunnambu) for 3 hours, washed, dried and external scale leaf was peeled off.
Long pepper (Thippili): Soaked and treated in leaf juice of *P. indica* for 24 minutes and sun dried.
East Himalayan Fir (Thalisapathiri): Purified by washed and sun dried.
Cumin (Seeragam): Soil and dust particles were removed and dried in sun light.
Indian gooseberry (Nellivatral): Boiled in cow milk and then seeds were removed and sun dried.
Indian leadwort (Chithiramoolam): Outer bark was removed, powdered and boiled with steaming method in cow milk and dried.
Cinnamon (Lavangapattai): Unwanted dust particles were removed and dried under sunlight.
Indian bark (Lavangapathiri): Cleaned and dried under sunlight.
Cardamom (Elakkai): Unwanted soil and dust were removed, and sun dried.
Black pepper (Milagu): Soaked and treated with buttermilk (sour) for 3 hours and dried under sunlight.
Bamboo salt (Moongiluppu): Dissolved in clear water and dried under sunlight to obtain the salt principate.

Sample preparation: The polyherbal Siddha formulation Sevviyadhi Chooranam was prepared as per Siddha literature.

- After purification, all the ingredients were grounded individually in an iron mortar by using a pestle and sieved with sieving cloth.
- And then all the grounded ingredients were mixed together and stored in an airtight container.

The heavy metal analysis, aflatoxin assay and pesticide residue test were carried out at Noble Research Solutions, Kolathur, Chennai.

Heavy Metal Analysis: Heavy metal analysis for Sevviyadhi chooranam was carried out at Noble Research Solutions and the project ID was NRS/AS/0896/09/2022. An instrument used for the method of analysis was Atomic Absorption Spectrometry (AAS) model AA 240 series and Extraction solvent used were HCl and HNO_3.

Atomic Absorption Spectrometry (AAS) ia a very common and reliable technique for detecting metals and metalloids in environmental samples. "AAS is a technique for measuring quantities of chemical elements present in environmental samples by measuring the absorbed radiation by the chemical element of interest. In analytical chemistry, AAS is a technique used mostly for determining the concentration of particular metal element in the sample. It can be used to used to analyze the concentration of over 62 different metals in a solution" [11].

Total heavy metal content of the sample was performed by Atomic Absorption Spectrometry (AAS) Model AA 240 Series. In order to determine the heavy metals such as mercury, arsenic, lead and cadmium concentration in Sevviyadhi Chooranam.

Sample Digestion: Test sample was digested with 1 mol/L HCl for determination of arsenic and mercury. Similarly, for the determination of lead and cadmium the sample was digested with 1 mol/L of HNO_3.

Sample preparation: The sample drug Sevviyadhi Chooranam was prepared as follows.
As & Hg- 100 ppm sample in 1mol/L HCl
Cd & Pb- 100 ppm sample in 1 mol/L HNO3

Aflatoxin Assay: Aflatoxin assay was carried out at Noble Research Solutions and project ID was NRS/AS/0896/09/2022. Analysis was done by TLC (B1, B2, G1 and G2). "Several chromatographic methods for the determination of aflatoxins in agricultural and food products are reviewed. In past two decades, identification and determination of aflatoxins were done by thin layer chromatography (TLC) because it was easy, fast and inexpensive" [12]. It was first used by De longh *et al.*[13] and has been regarded as by the Association of Official Analytical Chemist (AOAC) as the method of choice since 1990.[14] The advantage of using TLC method is that it can detect several types of mycotoxins in single test sample [15,16].

Standard: Aflatoxin B1, Aflatoxin B2, Aflatoxin G1 and 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 micro gram per ml each of aflatoxin B1 and

aflatoxin G1 and 0.1 micro gram 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 micro liter, 5 micro liter, 7.5 micro liter and 10 micro liters. Similarly, the test sample was placed and allow the spots to dry and develop the chromatogram, 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, marked and allowed the plate to air-dry. Thew spots were located on the plate by examining under UV light at 365 nm.[17]

Pesticide Residue Analysis: Pesticide residue analysis was carried out at Noble Research Solutions and project ID was NRS/AS/0896/09/2022. There are many reports detected and published the presence of pesticides in medicinal plants such as organochlorides [18-20], organophosphorus [21,22] and their preparations [23]. Due to the presence of these pesticide it leads to several health hazards. Hence pesticide residue analysis plays an important role for the detection of pesticides in medicinal plants.

Parameters analyzed in SC: Organochlorine pesticides, Organophosphorus pesticides, Organa carbamates and Pyrethroids.

Extraction: Test sample SC 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 degrees centigrade until the solvent has almost completely evaporated. To a residue few milliliters of toluene was added and heated again until the acetone is completely removed. Resultant residue was dissolved using toluene and filtered through membrane filter.[24,25]

RESULTS

Results for the heavy metal analysis of sevviyadhi chooranam is described on Table 2.

Report for the heavy metal analysis for sevviyadhi chooranam showed that the presence of lead and arsenic at 0.26 ppm and 0.61 ppm which is under permitted limit and absence of cadmium and mercury in the sample of sevviyadhi chooranam.

Result for the aflatoxin assay of sevviyadhi chooranam is explained on Table 3.

The results for aflatoxin assay showed that there were no spots identified in the test sample loaded on TLC plates when compared to the standard, which indicates that the sample SC were free from aflatoxin B1, aflatoxin B2, aflatoxin G1 and aflatoxin G2.

Result for the pesticide residue analysis is described on Table 4.

The results showed that there were no traces of pesticide residues such as organochlorine, organo phosphorous, organo carbamates and pyrethroids in Sevviyadhi chooranam.

Table 2: Heavy metal analysis of Sevviyadhi Chooranam

Name of the heavy metal	Absorption max A max	Result Analysis	Maximum limit
Lead	217.0 nm	0.26 ppm	10 ppm
Arsenic	193.7 nm	0.61 ppm	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	BDL	1 ppm

Table 3: Aflatoxin assay of Sevviyadhi chooranam

Aflatoxin	Sample SC	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

Table 4: Pesticide residue analysis of Sevviyadhi chooranam

Pesticide residue	Sample SC	AYUSH Limit (mg/kg)
I. Organochlorine pesticides		
Alpha BHC	BQL	0.1 mg/kg
Beta BHC	BQL	0.1 mg/kg
Gamma BHC	BQL	0.1 mg/kg
Delta BHC	BQL	0.1 mg/kg
DDT	BQL	1 mg/kg
Endosulphan	BQL	3 mg/kg
II. Organo phosphorous pesticides		
Malathion	BQL	1 mg/kg
Chlorpyriphos	BQL	0.2 mg/kg
Dichlorvos	BQL	1 mg/kg
III. Organo carbamates		
Carbofuran	BQL	0.1 mg/kg
IV. Pyrethroid		
Cypermethrin	BQL	1 mg/kg

BQL- Below Quantification Limit

DISCUSSION

Heavy metal analysis of the present study resulted presence of lead about 0.26 ppm and arsenic about 0.61 ppm which is under permitted limit and absence of cadmium and mercury in sevviyadhi chooranam. The presence of lead and arsenic under permitted limit revealed that sevviyadhi chooranam is safer for administration as therapeutic drug. Aflatoxin assay revealed absence of aflatoxin B1, aflatoxin B2, aflatoxin G1 and aflatoxin G2 in sevviyadhi chooranam. Pesticide residue analysis of sevviyadhi chooranam concluded that the absence of traces of organochlorines pesticides, organophosphorous pesticides, organo carbamates pesticides and pyrethroids.

Most of the prior research papers explored the heavy metal analysis, aflatoxin assay and pesticide residue analysis in some of the specific ingredients of Sevviyadhi Chooranam. The contents of heavy metals namely lead, mercury, cadmium and arsenic are found to be in permissible limit for the three roots of *Plumbago* species, indicating that they are safe to utilize as drugs [26], the aflatoxin below detecting level and absence of pesticides in all the three roots of *Plumbagin* indicates it is safer for internal use[27], Absence of toxic contaminants in *Piper nigrum* [28], *Elettaria cardamomum* usage in inhibition of aflatoxigenic fungi [29], antifungal and antiaflatoxigenic activity of *Cinnamomum tamala* [30], *Phyllanthus emblica* was found free of contaminant when analyzed for pesticides and aflatoxins whereas heavy metals were found in safe limits [31].

The current study for heavy metal analysis, aflatoxin assay and pesticide residue analysis of Sevviyadhi chooranam generated scientific evidence for the presence of lead and arsenic under permitted limit and absence of mercury and cadmium and also absence of aflatoxins and pesticide residues. The purpose of this study was to explore the presence of chemical contaminants such as heavy metals, aflatoxins and pesticides in sevviyadhi chooranam for establishing its purity and safety for therapeutic administration. The present study ensured the purity and validated safety of the polyherbal formulation sevviyadhi chooranam, thus intensifying its value for therapeutic inference. This study concluded that the Siddha polyherbal formulation is safer for internal administration in the treatment and management of sinusitis. However, further in vivo studies and clinical trials are important to ensure its efficacy as preferred treatment for sinusitis.

CONCLUSION

Based on the results and discussion, the study concluded the presence of heavy metals such as lead and arsenic in limited amount and complete absence of mercury and cadmium, addition to that the study revealed the absence of aflatoxins and pesticide residue in the Siddha polyherbal formulation Sevviyadhi chooranam. Hence the present study ensured Sevviyadhi chooranam was free from all these chemical contaminants, this validated the safety of Sevviyadhi chooranam and established its therapeutic usage for the treatment of Sinusitis.

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