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Analysis Of Heavy Metal, Aflatoxin, Pesticide Residue And Microbial Contamination Of Siddha Herbal Formulation Thuthuvelai Chooranam

1Dr.Anitha.M, 2Dr.Anbu.N, 3Dr.Sathyanandhini
1PG Scholar, 2Head of the Department, 3PG Scholar
1The Tamil Nadu Dr. M.G.R. Medical University ,
2The Tamil Nadu Dr. M.G.R. Medical University ,
3The Tamilnadu Dr.M.G.R. medical university

Abstract:

Aim: The aim of the study was to evaluate the presence of Heavy metal, Aflatoxin, Pesiticide residue and Microbial contamination of Siddha herbal formulation Thuthuvelai Chooranam (TVC).

Place of study: Heavy metal analysis, Aflatoxin assay, Pesiticide residue, Microbial contamination analysis were conducted at Noble Research Solutions, Kolathur, Chennai -99.

Methodology: The Siddha formulation Thuthuvelai Chooranam 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 Thuthuvelai Chooranam shown the presence of Lead at 7.274 PPM and Arsenic, Cadmium, Mercury at Below Detection Limit (BDL). Aflatoxin assay of TVC 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 E-coli, Salmonella, Staphylococcus Aureus, Pseudomonas Aeruginosa and No Bacterial and fungal growth or colonies were observed in the Sterility test of TVC as per the methods of AYUSH specifications.

Conclusion: From the results, it is concluded that the study medicine TVC 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 Thuthuvelai Chooranam was safe therapeutically.

Keywords: Thuthuvelai Chooranam, Siddha, Swasakasam, Bronchial asthma, Heavy metal

INTRODUCTION

A drug is defined as a substance used as medicine, which may be utilized in its raw form or after undergoing various processes. The Siddha system of medicine incorporates drugs derived from plant, animal, metal, or mineral sources. To eliminate impurities, these crude drugs must go through specific purification processes before being transformed into actual medicines. This approach enhances therapeutic efficacy, promotes detoxification, and ensures safety. Given the Siddha system's complex combinations of medicines and diverse preparation methods—such as powdering, heating, boiling, drying, grinding, calcination, sublimation, and filtration—there is a risk of impurities and mishandling, which could hinder its scientific acceptance on a

global scale. These issues can be addressed by following established procedures and utilizing standardization techniques to achieve reproducible quality through various analytical parameters. (1)(2)

Heavy metal testing is conducted to identify potentially harmful levels of metals at specific concentrations, including lead, arsenic, cadmium, mercury, and chromium, all of which are highly toxic. Additionally, pesticide residues may be present in medicines due to pesticide use during cultivation for economic benefits. Both pesticides and heavy metals can accumulate in the body through biological chains, as they are persistent and not biodegradable. Therefore, monitoring their concentrations is crucial. (3)

Aflatoxins are a group of hazardous mycotoxins produced by Aspergillus flavus and Aspergillus parasiticus, with structural similarities among them. This group consists of about 16 compounds, but only aflatoxins B1, B2, G1, and G2 are regularly monitored. High moisture and temperature levels contribute to the formation of mycotoxins, which can have serious health effects on humans. (4) Research indicates that approximately 80% of people in developing countries rely on traditional herbal remedies as their main form of healthcare. However, contamination by various microorganisms can occur at multiple stages, including the harvesting, handling, drying, preserving, and manufacturing processes, with microorganisms adhering to the leaves, stems, flowers, seeds, and roots used in herbal medicines. This microbial contamination poses significant health risks to consumers, making it a global health concern. Therefore, ensuring the safety of herbal products is essential. The current study focuses on analysing heavy metal content, aflatoxins, pesticide residues, and microbial contamination in the Siddha herbal formulation Thuthuvelai Chooranam, which is indicated for various ailments, including bronchial asthma.(5)

MATERIAL AND METHODS

Collection of raw drugs

The Plant Thuthuvelai and Thippili were procured from a reputed raw drug store, identified and authenticated by the Botanist of Government Siddha Medical College, Chennai,(Voucher number GSMC/MB- 560-564).

Ingredients

1. Piper longum (thipilli) - 3 tolas (24gms)
2. Solanum trilobatum (thuthuvelai) - 6 tolas (48gms)
3. Saccharum officinalis (Sugar) - 9 tolas (72gms)

Purification

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

Piper longum (Thippili) - It was purified by soaking it in lemon juice and drying it in sunlight until it dries off.

Solanum trilobatum (thuthuvelai)- the whole plant was cleaned and dried under sunlight.

Sample preparation

All the above purified ingredients were powdered and then sieved by using a sieving cloth. Then the obtained powder was stored in a clean air tight dry container.

MATERIALS AND METHODS

The Heavy metal analysis, Aflatoxin assay, Pesiticide residue, Microbial contamination analysis was conducted at Noble Research Solutions, Kolathur, Chennai -99. The project Id was NRS/AS/1247/11/2023

Heavy Metal Analysis by Atomic Absorption Spectrometry

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. In order to determination the heavy metals such as mercury, arsenic, lead and cadmium concentrations in the test item.

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.

Standard reparation

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

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

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

Procedure

Standard aflatoxin was applied on to the surface to pre coated TLC plate in the volume 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 has moved not less than 15 cm from the origin. Remove the plate from the developing chamber, mark the solvent from and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 365 nm.(8)

Pesticide Residue Analysis

Parameter analysed was Organochlorine pesticides, Organophosphorus pesticides, Oragno carbamates,Pyrethroids

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 40ºC 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)

Test for Specific Pathogen

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 37ºC 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.

Detail of Specific Medium and their abbreviation

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

STERILITY TEST BY POUR PLATE METOD

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).

Methodology

Test sample was inoculated in sterile petri dish to which about 15 mL of molten agar 45ºC 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.

Results and Discussion

Heavy metal screening of TVC 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.

. Test report of Heavy metal analysis of TVC

Name of the Heavy Metal	Absorption Max Λ max	Result Analysis	Maximum Limit
Lead	217.0 nm	7.274	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 TVC 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 and Aflatoxin G2.

Aflatoxin	Sample TC	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)

Pesticide residue analysis of TVC Showed that there were no traces of pesticides residues such as Organo chlorine, Organo phosphorus, Organo carbamates and pyrethroids in the sample provided for analysis.

Test Result Analysis of the Sample TC

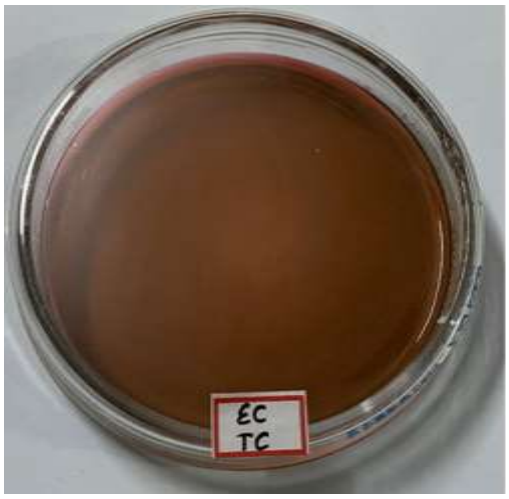
Pesticide Residue / **I.Organo Chlorine Pesticides**	**Sample TC**	**AYUSH Limit (mg/kg)**
Alpha BHC	BQL	0.1mg/kg
Beta BHC	BQL	0.1mg/kg
Gamma BHC	BQL	0.1mg/kg
Delta BHC	BQL	0.1mg/kg
DDT	BQL	1mg/kg
Endosulphan	BQL	3mg/kg
II.Organo Phosphorus Pesticides		
Malathion	BQL	1mg/kg
Chlorpyriphos	BQL	0.2 mg/kg
Dichlorovos	BQL	1mg/kg
III. Organo carbamates		
Carbofuran	BQL	0.1mg/kg
III.Pyrethroid		
Cypermethrin	BQL	1mg/kg

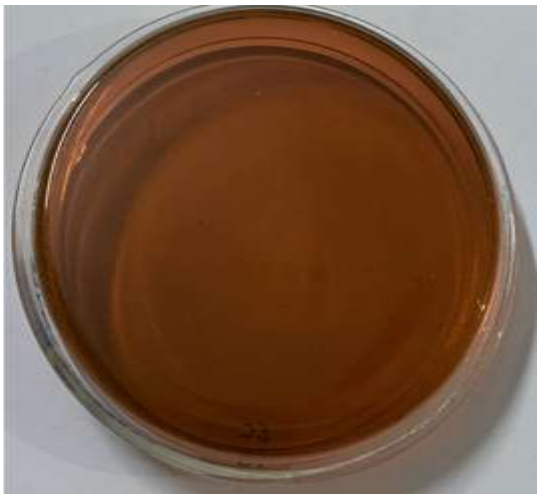
BQL- Below Quantification Limit

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

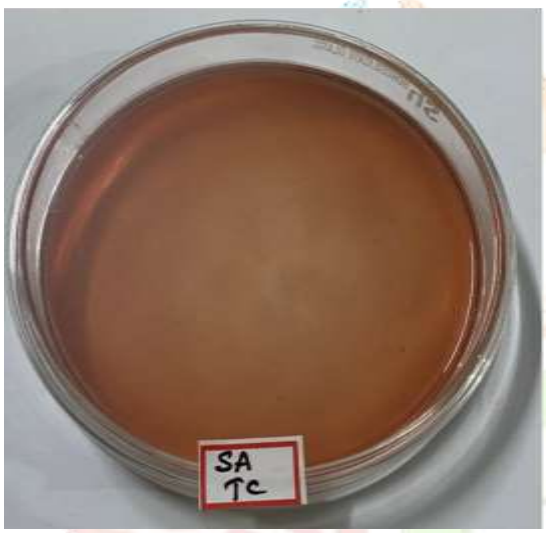
Organism	**Specification**	**Result**	**Method**
E-coli	Absent	Absent	As per AYUSH specification
Salmonella	Absent	Absent	
Staphylococcus Aureus	Absent	Absent	
Pseudomonas Aeruginosa	Absent	Absent	

Culture plate with E-coli (EC) specific medium

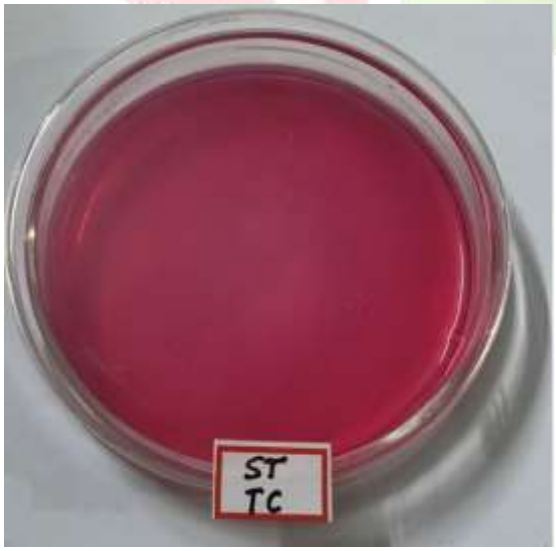




Culture plate with Salmonella (SA) specific medium



Culture plate with Staphylococcus Aureus (ST) specific medium

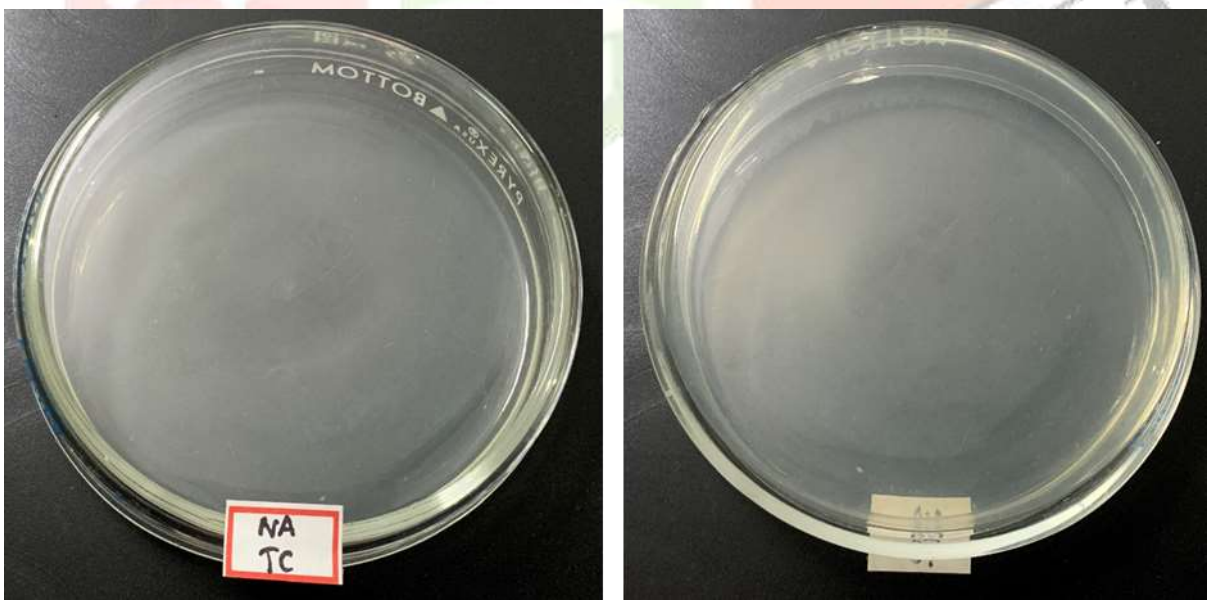


Culture plate with Pseudomonas Aeruginosa (PS) specific medium

Sterility test of TVC 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

Sterility test report of TVC

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



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

Conclusion

Through the present study, it is concluded that the sample Thuthuvelai Chooranam (TVC) 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 TVC in terms of contamination from biological chains. This preliminary standardisation study would assist in further research and clinical trials with the basic quality sustain.

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Consent

It is not applicable.

Ethical Approval and Inform Consent

It is not applicable.

Author Contribution

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

Competing Interests

Authors have declared that no competing interests exist.

References

1. Saraswathy A. (1994). Standardisation of siddha drugs. Ancient science of life, 14(1-2), 53–60.
2. Selvi C, Paramasivam M. Review on pesticide residue analytical methods and residue status in medicinal plants.J Entomol Zool Stud. 2017;5(3):945-50.
3. kumar Das S, singh Grewal A, Banerjee M. A brief review: Heavy metal and their analysis. Organization. 2011 Nov;11(1):003.
4. Siddique NA, Mujeeb M, Ahmad S, Panda BP, Makhmoor M. Determination of aflatoxins in medicinal plants by high-performance liquid chromatography–tandem mass spectrometry. Journal of pharmacy & pharmaceutical sciences. 2013 Jul 26;16(2):321-30.
5. de Sousa Lima, C. M., Fujishima, M. A. T., de Paula Lima, B., Mastroianni, P. C., de Sousa, F. F. O., & da Silva, J. O. (2020). Microbial contamination in herbal medicines: a serious health hazard to elderly consumers. BMC complementary medicine and therapies, 20(1), 17. https://doi.org/10.1186/s12906-019-2723-1
6. Pillai C.Kannusamy. Sikitcha Rathna Deepam Ennum Vaidhiya Nool. Chennai: B. Rathna Nayakkar & Sons; 2018.
7. Samuel D.Asirvadham. Marundhu Sei Iyalum Kalaiyum. Chennai: Directorate of Indian Medicine and Homeopathy; [date unknown].
8. Luciana de CASTRO. Determining Aflatoxins B1, B2, G1 and G2 in Maize Using Florisil Clean Up with Thin Layer Chromatography and Visual and Densitometric Quantification. Ciênc. Tecnol. Aliment. vol.21 no.1 Campinas. 2001.
9. WHO guideline for assessing the quality of herbal medicines with reference to contaminants and residues.WHO Geneva. 2007.
10. Lohar. D.R. Protocol for testing of ASU medicines. Pharmacopoeial Laboratory for Indian Medicines.Ministry of AYUSH. 2007.