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Analysis of Heavy Metal, Aflatoxin, Pesticide Residue, Microbial Contamination And Phytochemical Analysis of Siddha Herbal Drug Peenisathirkku Chooranam

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ABSTRACT

BACKGROUND

The Siddha tradition advocates a holistic approach that integrates physical, mental, and spiritual health. Despite its ancient origins, there is growing interest in formalizing its practices to guarantee safety, effectiveness, and uniformity. This article examines modern techniques used to standardize Peenisathirkku chooranam (PSC), as outlined in Sarabandhira vaithiyamuraigal (sirarogasigichaigal) Part-3, for treating various sinusitis-related conditions.

Aim and objective: The purpose of this study was to assess the presence of heavy metals, aflatoxins, pesticide residues, and microbial contamination in the Siddha herbal formulation Peenisathirkku Chooranam (PSC). The formulation was prepared following Good Manufacturing Practices (GMP) and subjected to various analyses including heavy metal testing, aflatoxin assay, pesticide residue screening, microbial contamination evaluation, and phytochemical profiling at Noble Research Solutions in Chennai.

Result and discussion: Results showed detectable amounts of lead (4.86 PPM) and arsenic (2.22 PPM), with cadmium and mercury below detectable levels. No aflatoxins (B1, B2, G1, G2) or pesticide residues (organochlorine, organophosphorus, organocarbamate, pyrethroids) were found. Microbial tests revealed no pathogens or microbial growth, meeting AYUSH safety standards. Phytochemical screening identified alkaloids, carbohydrates, saponins, flavonoids, diterpenes, gum, and mucilage in PSC.

Conclusion: Overall, PSC contained heavy metals well within permissible limits, was free from toxins and microbes, and contained bioactive compounds supporting its therapeutic use, confirming it as a safe and reliable herbal medicine.

Keywords: Peenisathirk kuchooranam, Siddha, Sinusitis, Heavy metal, Aflatoxins, Pesticides, Phytochemicals.

INTRODUCTION

Peenisathirukku Chooranam is a traditional Siddha herbal formulation used in Indian medicine. While direct data on its contamination is limited, research on similar Siddha herbal products highlights the importance of thorough testing to guarantee safety and effectiveness. Heavy metals like lead, arsenic, mercury, and cadmium may contaminate herbal medicines due to environmental pollution or production methods. Studies on comparable Siddha powders often show these metals at safe or undetectable levels when proper quality controls are applied, but regular monitoring is necessary to prevent contamination. Aflatoxins, which can develop during improper storage in humid conditions, are harmful and cancer-causing. Additionally, pesticide residues from cultivation and microbial contamination by bacteria and fungi pose risks to product safety and quality. Analyzing the phytochemical components is also vital, as these natural compounds are responsible for the medicinal benefits and help ensure consistent quality across batches. Overall, comprehensive testing of Peenisathirukku Chooranam for heavy metals, aflatoxins, pesticides, microbes, and phytochemicals is crucial to meet safety and quality standards, protect public health, and promote its acceptance in contemporary medicine.[1]

MATERIALS AND METHODS

The herbal formulation called Peenisathirkkuchooranam is described in the classical Siddha text SarabendhiraVaithiyamuraigal (Sirarogasigichai), Part 3, authored by Sri K. Vasudeva Sasthiri and Dr. S. Venkatrajan. The ingredients used in this preparation are,[6]

Table-1:Ingredients of PSC

S.NO	INGREDIENTS	BOTANICAL NAME
1	Kadukurogini	Picorrhizakurroa
2	Karkkadagasingi	Rhus succedanea
3	Kandangathariver	Solanum xanthocarpum
4	Sadamanjil	Nardostachysjatamansi
5	Siruthekku	Clerodendrumserratum
6	Indhuppu	Rock salt

PURIFICATION OF THE DRUGS

The drug mentioned was purified according to the methods described in Siddha literature. All impurities, including sand and dust, were thoroughly removed to ensure the drug's purity.

PREPARATION OF THE DRUG PROCEDURE

The purified raw drugs, as listed in Table 1, were meticulously ground into a fine powder using a mortar and pestle. This powdered form, named PeenisathirkkuChooranam, was then stored in an airtight container to preserve its quality and prevent contamination.

RESULTS AND DISCUSSION

1.HEAVY METALANALYSIS OF PSC

The heavy metal test for MRC showed cadmium and mercury were not detected. Lead was found at 4.86 ppm and arsenic at 2.22 ppm, both below their maximum allowed limits of 10 ppm and 3 ppm, respectively. These levels being under the limits suggest that the drug is safe concerning heavy metal contamination[3].

Table-2: Test report of Heavy metal analysis of PSC

Name of the heavy metal	Absorption Max<Max	Result Analysis	Maximum Limit
Lead	217.0nm	4.86	10ppm
Arsenic	193.7nm	2.22	3ppm
Cadmium	228.8nm	BDL	0.3ppm
Mercury	253.7nm	BDL	1ppm

2.AFLATOXIN ASSAY OF PSC

The TLC aflatoxin test of the PSC sample showed no visible spots corresponding to aflatoxins B1, B2, G1, or G2 when compared to standard references. This means the sample does not contain detectable levels of these aflatoxins. TLC works by separating compounds on a plate and identifying them based on their position and fluorescence under UV light. Since no matching spots appeared, it indicates that the PSC sample is free from these toxins. This is a common and reliable method used to screen for aflatoxin contamination[4].

Table 3: Test report of Aflatoxin assay of PSC

Aflatoxin	Sample of PSC	AYUSH Specification Limit
B1	Not Detected Absent	0.5ppm(0.5mg/kg)
B2	Not Detected Absent	0.1ppm(0.1mg/kg)
G1	Not Detected Absent	0.5ppm(0.5mg/kg)
G2	Not Detected Absent	0.1ppm(0.1mg/kg)

3.PESTICIDE RESIDUE ANALYSIS OF PSC

The pesticide residue analysis of MRC for organochlorine, organophosphorus, organocarbamate, and pyrethroid pesticides showed no detectable residues. In other words, no traces of these pesticides were found in the tested MRC samples. This means the samples were free from pesticide contamination within the detection limits of the analysis[2].

Table 4:Test report of pesticide residue of PSC

Pesticide Residue **I.Organo chlorine pesticides**	**Sample PSC**	**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	0.2mg/kg
Dichlorovos	BQL	1mg/kg
III. Organo carbamates		
Carbofuran	BQL	0.1mg/kg
III.Pyrethroid		
Cypermethrin	BQL	1mg/kg

4.MICROBIAL CONTAMINATION ANALYSIS OF PSC

The microbial contamination analysis of PeenisathirkkuChooranam was conducted by testing for specific pathogens. No microbial growth was detected after the incubation period, indicating the absence of these specific pathogens. The results are presented below[5]:

Table 5: Test report of specific pathogen of PSC

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

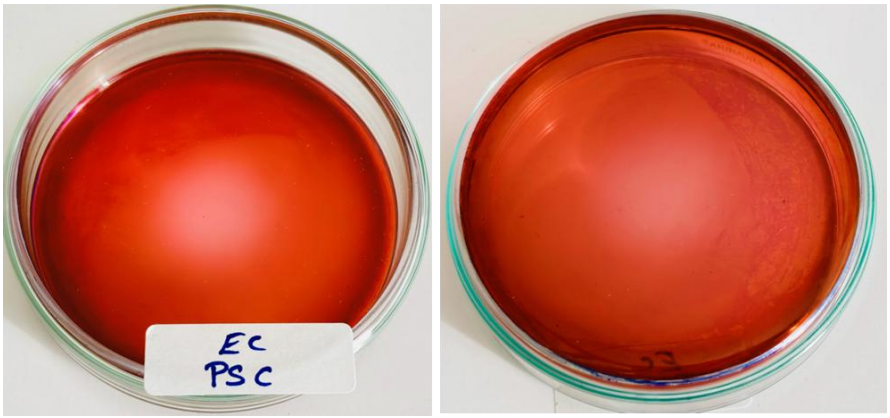

Figure:1 Culture plate with E-coli (EC) specific medium

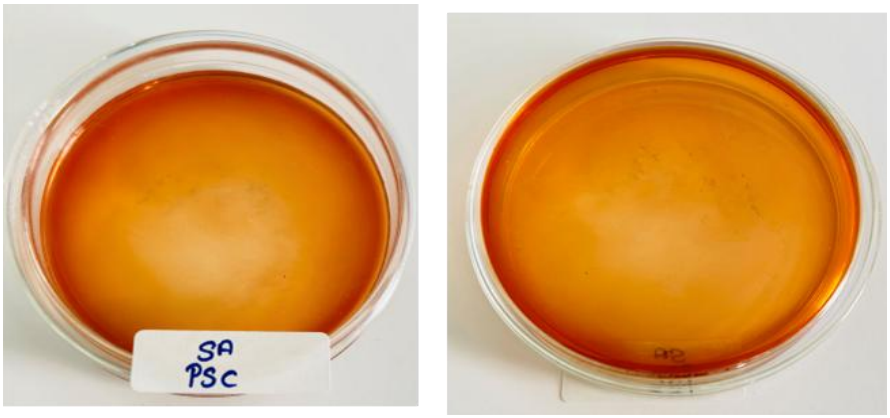

Figure2: Culture plate with Salmonella (SA) specific medium

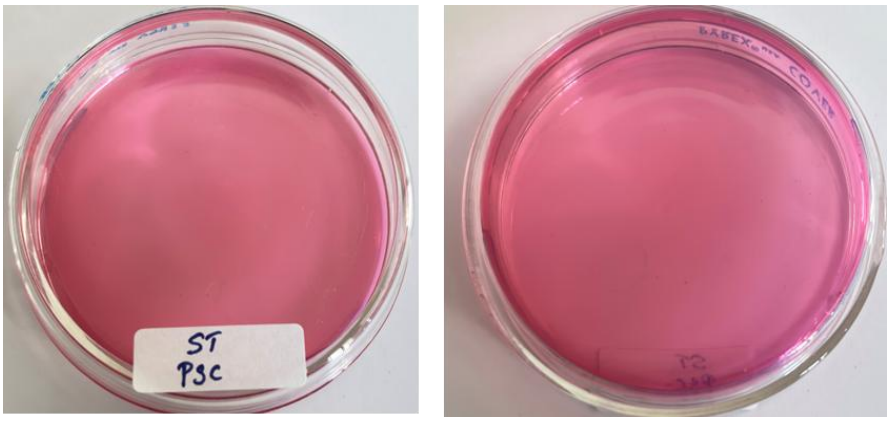

Figure 3:Culture plate with Staphylococcus Aureus (ST) specific medium

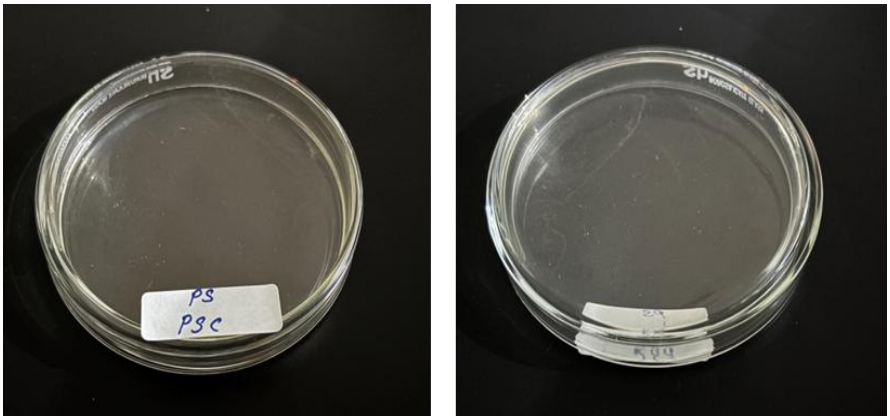

Figure 4: Culture plate with Pseudomonas Aeruginosa (PS) specific medium

The sterility test of the PSC revealed that no growth or colonies were observed on any of the plates inoculated with the test sample, indicating the absence of microbial contamination in both tests.

Table 6: Sterility test report of PSC

No growth / colonies was observed in any of the plates inoculates with the test sample.

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	

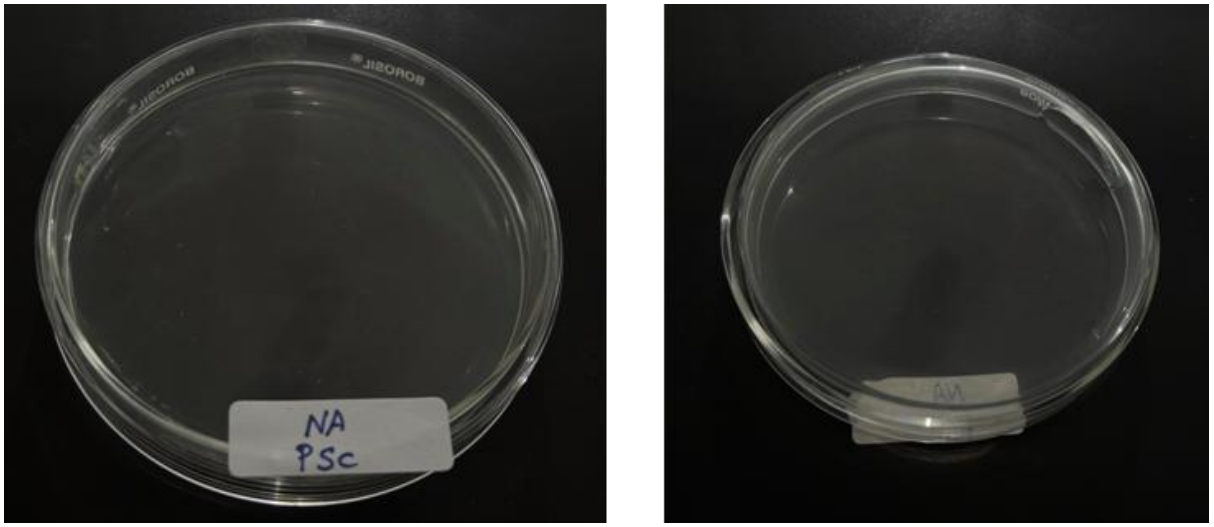

Figure 5: colony plate for Total bacterial and fungal count of PSC.

5. PHYTOCHEMICAL ANALYSIS OF PSC

The phytochemical analysis of PSC revealed the presence of alkaloids (via Wagner test), carbohydrates (Molisch's and Benedict tests), saponins (foam test), flavonoids (lead acetate test), diterpenes (copper acetate test), and gum and mucilage. It showed absence of tannins, phenols, and quinones.

Details of individual tests include:

Alkaloids (Mayer's Test): Addition of 2 ml Mayer's reagent to the sample produces a dull white (cream-colored) precipitate, indicating alkaloids. Mayer's reagent is a solution of mercuric chloride and potassium iodide; alkaloids form a coordinate covalent bond with the reagent yielding a characteristic precipitate.

Coumarins: 1 ml of 10% sodium hydroxide added to the sample produces yellow color if coumarins are present.

Saponins: Shaking the sample with 5 ml water produces copious lather indicating saponins.

Tannins: Addition of ferric chloride produces dark blue or greenish-black color if tannins present.

Glycosides (**Borntrager'sTest**): Hydrolyzed sample when shaken with chloroform, then ammonia added, yields pink color indicating glycosides.

Flavonoids (Alkaline Reagent Test): Addition of sodium hydroxide produces an initial deep yellow color which turns colorless on adding dilute HCl, confirming flavonoids.

Phenols (**LeadAcetate** Test): Adding 3 ml of 10% lead acetate solution causes bulky white precipitate in presence of phenols.

Steroids: Addition of chloroform and concentrated sulfuric acid causes red upper layer and yellow fluorescent acid layer indicating steroids.

Triterpenoids (**Liebermann-BurchardTest**): Adding acetic anhydride and sulfuric acid creates a red ring indicating triterpenoids.

Anthocyanins: Addition of 2N sodium hydroxide and heating forms bluish-green color if anthocyanins present.

Carbohydrates (**Benedict'sTest**): Heating with Benedict's reagent produces colored precipitate indicating reducing sugars.

Proteins (Biuret Test): Additionof copper sulfate and sodium hydroxide produces violet-purple color if proteins present.

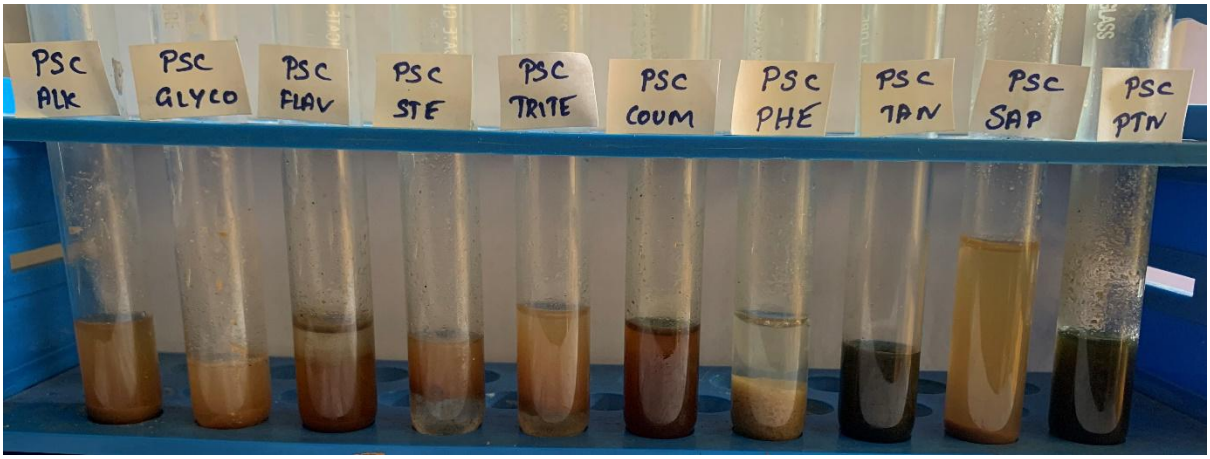

Figure 6: Qualitative Phytochemical analysis of PSC

Conclusion

This study confirms that the Peenisathirkku Chooranam (PSC) sample is safe for use, as it contains heavy metals at levels below detectable limits and is free from aflatoxins, pesticide residues, and microbial contaminants, including specific pathogens as well as bacterial and fungal populations. Additionally, the research offers a detailed analysis of the phytochemical constituents found in PSC. These results validate the product's quality, especially regarding its absence of biological contaminants. This initial standardization effort provides a solid basis for future research and clinical trials, supporting the consistent quality of PSC for subsequent applications.

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