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ANALYSIS OF HEAVY METAL, AFLATOXIN, PESTICIDE RESIDUE, MICROBIAL CONTAMINATION AND PHYTOCHEMICAL ANALYSIS OF SIDDHA HERBAL DRUG YAZHPANATHENNAI

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ABSTRACT:

Introduction:

The Siddha system provides a holistic approach that integrates physical, mental, and spiritual health. Although Siddha medicine has an ancient legacy, there is now a growing need to standardize its practices to guarantee their safety, effectiveness, and uniformity. This paper examines modern techniques employed to standardize Yazhpanathennai(YPT), as outlined in Koshayi anuboga vaithya bramma ragasiyam ,part-2, for the treatment of Bala karappan.

Aim and objective: The study aimed to assess the presence of Heavy metal, Aflatoxin, Pesiticide residue and Microbial contamination of Siddha herbal formulation Yazhpanathennai(YPT).

Materials and methods: The Siddha formulation Yazhpanathennai was prepared following Good Manufacturing Practices (GMP) guidelines, and tests for heavy metals, aflatoxins, pesticide residues, microbial contamination, and phytochemical content were carried out at Noble Research Solutions, Kolathur, Chennai – 99. **Results and Discussion:** The heavy metal analysis of Yazhpanathennai (YPT) revealed the presence of lead,arsenic, cadmium, and mercury were below the detection limit (BDL). The aflatoxin assay indicated the absence of Aflatoxin B1, B2, G1, and G2. Pesticide residue testing confirmed no detectable levels of organochlorine, organophosphorus, organocarbamates, or pyrethroids. Microbial contamination analysis showed no presence of specific pathogens such as E. coli, Salmonella, Staphylococcus aureus, or Pseudomonas aeruginosa. Additionally, sterility tests found no bacterial or fungal growth, meeting AYUSH specifications. Phytochemical screening identified the presence of alkaloids, carbohydrates, saponins, flavonoids, diterpenes, gums, and mucilages in YPT. **Conclusion:** Based on the results, it can be concluded that the study medicine YPT contains heavy metals below the permissible limits set by the AYUSH PLIM guidelines. The sample was free from aflatoxins, pesticides, microbes, and specific pathogens. Additionally, the comprehensive identification of phytochemical components confirms that Yazhpanathennai (YPT) is therapeutically safe.

KEYWORDS: Yazhpanathennai(YPT), Siddha, Atopic Dermatitis, Heavy metal, Aflatoxins, pesticide, phytochemicals.

INTRODUTION:

Atopic dermatitis (AD), a chronic inflammatory skin disorder characterized by intense itching, erythema, and recurrent eczematous lesions, significantly impairs quality of life for affected individuals. [1]In traditional systems of medicine such as Siddha , AD is closely associated with conditions like BalaKarappan, which are managed through holistic approaches involving herbal formulations and dietary modifications. Yazhpanathennai, a Siddha herbal drug, is traditionally utilized for skin ailments including atopic dermatitis. However, concerns regarding the safety and quality of herbal medicines persist due to potential contamination by heavy metals, aflatoxins, pesticide residues, and microbial agents. Such contaminants can arise from environmental exposure, agricultural practices, or improper storage, posing risks to patient safety and undermining therapeutic efficacy. Moreover, the phytochemical profile of herbal formulations is crucial for their pharmacological action and standardization. Comprehensive analysis of Yazhpanathennai for heavy metals, aflatoxins, pesticide residues, microbial contamination, and phytochemical constituents is therefore essential to ensure its safety, efficacy, and quality. This study aims to address these critical aspects, supporting the safe integration of Siddha herbal medicines in the management of atopic dermatitis. The comprehensive analysis of Yazhpanathennai, a Siddha herbal drug, demonstrates that the formulation is safe for use when prepared and administered according to established protocols. The assessment confirmed that levels of heavy metals, aflatoxins, pesticide residues, and microbial contamination were within permissible safety limits, minimizing the risk of adverse effects.

Phytochemical screening also verified the presence of beneficial bioactive compounds, supporting the drug's therapeutic potential for conditions such as atopic dermatitis.

It is important to emphasize that the safety and efficacy of Siddha medicines depend on strict adherence to guidelines for raw material collection, purification, preparation, and dosing. Deviations from these protocols or improper use can lead to adverse reactions, as highlighted in Siddha literature and modern pharmacovigilance practices. Therefore, Yazhpanathennai, when properly standardized and quality-controlled, can be considered safe for therapeutic use. Continued monitoring and adherence to pharmacovigilance are essential to maintain patient safety and uphold the credibility of Siddha herbal medicines

MATERIALS AND METHODS

The herbal preparation Yazhpanathennai, was idendified in the canonical text Koshayi anuboga vaithya bramma ragasiyam[6] ,part-2,Author R.C.Mohan. The ingredients for this formulation are included in Table -1

Table 1 Ingrediants of YPT.

S.NO	INGREDIENTS	BOTANICAL NAME
1	Nochi	*Vitex negundo*
2	Karunochi	*vitex trifolia*
3	Semmulli	*Barteria prionitis*
4	Kiranthinayagam	*Asystasia gangetica*
5	Sangankuppi	*Clerodentrum inerme*
6	Sangillai	*Azima tetracantha*
7	Murukkilai	*Erythrina varigata*
8	Senthotti	*Tragia involucrate*
9	Castor oil	*Ricinus communis*
10	Poovarasan	*Thespesia populnea*
11	Siruseruppadai	*Mollungo lotoides*
12	Kozhiavarai	*Dolichas gladiators*
13	Katralai	*Aloe barbadensis*
14	Sengatharipattai	*Capparis deciduas*
15	Karunjeeragam	*Nigella sativa*
16	Kadukka	*Terminalia chebula*
17	Kalipakku	*Areca catechu*
18	Vengayam	*Allium cepa*

COLLECTION ,IDENTIFICATION AND AUTHENTICATION OF THE DRUG:

The plant materials were procured from a raw drug shop located at parry's corner in chennai,Tamilnadu. These materials were subsequently verified and confirmed by botanical and pharmacological experts at the Government Siddha Medical College Hospital in Arumbakkam,Chennai -106.

PREPARATION OF THE DRUG PROCEDURE:

1.Take the leaf extract of nochi, karunochi, silanthi nayagam, sangankuppi, sangillai, murukilai, senthotti, poovarasan, siruseruppadai, kozhi avarai, kattralai 35ml of quantity for each.

2.Mixed these leaf extracts into 325ml of caster oil and then added dried and purified powder of sengathari pattai, karunjeeragam, kadukkai, kalipakku, vengayam with 2.1 gm of each in quantity with the boiling oil.

3.Allowed all of the raw drug to boil in low flame until mezhugu padham (non-sticky in nature).

4.The trial drug are stored in clean dry air tight container and its dispensed to the patients.

RESULTS AND DISCUSSION:

1.HEAVY METAL ANALYSIS OF YPT:

Heavy metal screening of MRC shown that it contains , cadmium, Mercury were BDL(Below Detection Limit), Lead was 7.87 ppm, whose maximum limit was upto 10ppm ,and Arsenic was 2.02 ppm, whose maximum limit was upto 3ppm. However, its lower limit indicating the safety of the drug[3].

Table 2: Test report of Heavy metal analysis of YPT.

Name of the Heavy Metal	Absorption Max	Result Analysis	Maximum Limit
Lead	217.0nm	7.87	10ppm
Arsenic	193.7nm	2.02	3ppm
Cadmium	228.8nm	BDL	0.3ppm
Mercury	253.7nm	BDL	1ppm

2.AFLATOXIN ASSAY OF YPT

The results of Aflatoxin assay of YPT by TLC shown that there were no spots were being identified in the test sample loaded on TLC plates when

compare to the standared which indicates that the sample were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2[7].

Table 3: Test report of Aflatoxin assay of YPT.

Aflatoxin	Sample YPT	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)

3.PESTICIDE RESIDUE ANALYSIS OF YPT:

Pesticide residue analysis of MRC with the parameters Organochlorine pesticides, Organophosphorus pesticides, Organo carbamates, Pyrethroids were found to be that there were traces of pesticides residues and the results were given below.[2]

Table 4: Test report of pesticide residue of YPT.

Pesticide Residue I.Organo Chloride Pesticides	Sample YPT	AYUSH Limit (mg/kg)
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 phosphorus pesticides		
Malathion	BQL	1 mg/kg
Chlorpyriphos	BQL	0.2 mg/kg
Dichlorovos	BQL	1 mg/kg
III. Organo carbamates		
Carbofuran	BQL	0.1 mg/kg
IV. Pyrethroid		
Cypermethrin	BQL	1 mg/kg

4.MICROBIAL CONTAMINATION ANALYSIS OF YPT:

Microbial contamination analysis of YPT by test for specific pathogen shown that there were no growth was observed after incubation period, reveals the absence of specific pathogen. Results were given below[5].

Table 5: Test report of specific pathogen of YPT.

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

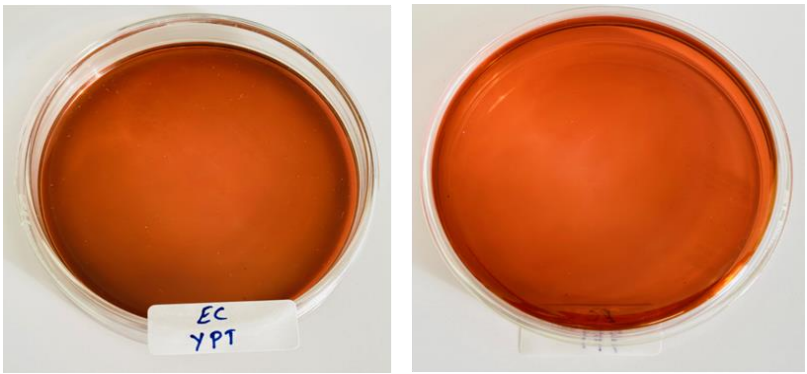

Figure 1:Culture plate with E-coli (EC) specific medium

Figure 2: Culture plate with Salmonella (SA) specific medium

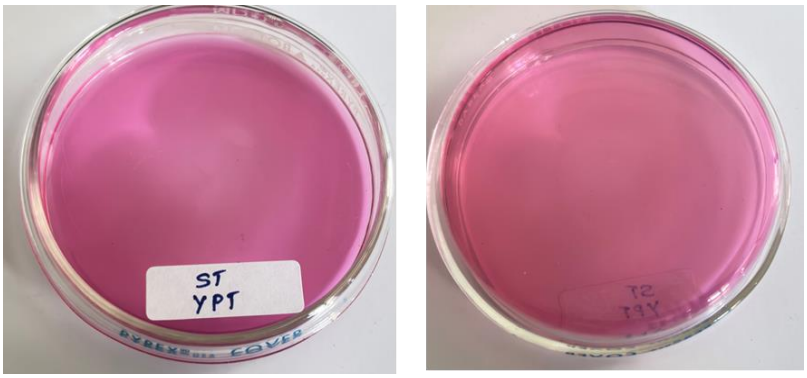

Figure 3: Culture plate with Staphylococcus Aureus (ST) specific medium

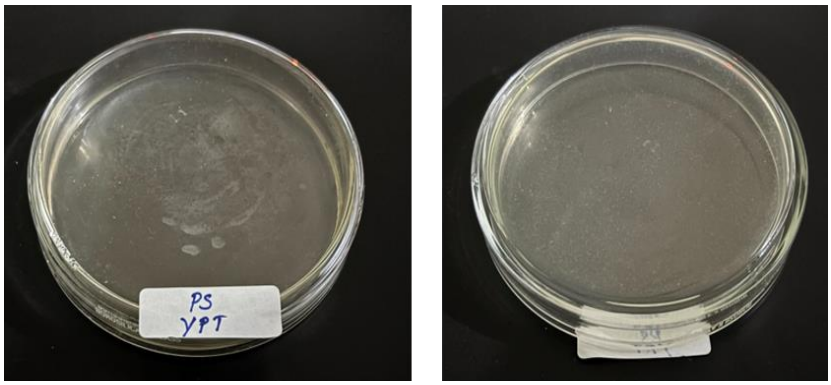

Figure 4: Culture plate with Pseudomonas Aeruginosa (PS) specific medium

Sterility test of YPE also found to be that there were NO growth/ colonies was observed any of the plates inoculates with the test sample which ensures that the sample is devoid of microbial contamination in both the tests.

Table 6: Sterility test report of YPT

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	

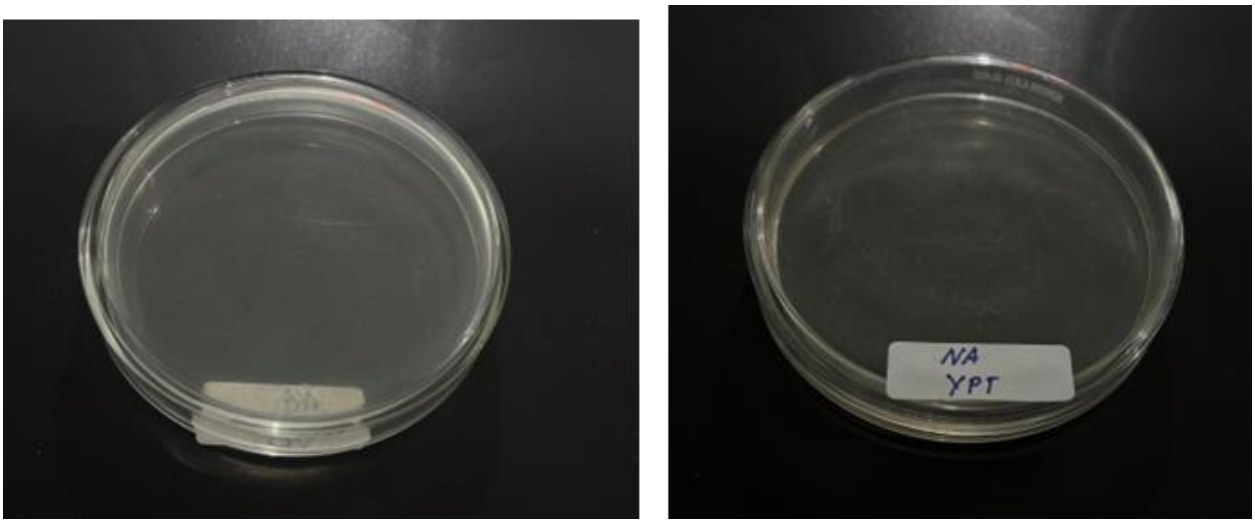

Figure 5: colony plate for Total bacterial and fungal count of YPT.

5. PHYTOCHEMICAL ANALYSIS OF YPT:

The Phytochemical screening of YPT shown the presence of Alkaloids in Wagner test, Carbohydrates in Molisch's test, Benedict test, Saponin in Foam test, Flavanoids in lead acetate test, Diterpenes in Copper acetate test, Gum and Mucilage in Gum and mucilage test of the study sample. Also, shown the absence of Tannins, Phenols and Quinones.

Test for alkaloids:

Mayer's Test: To the test sample, 2ml of mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

Test for coumarins:

To the test sample, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow color.

Test for saponins:

To the test sample, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of Saponins.

Test for tannins:

To the test sample, ferric chloride was added, formation of a dark blue or greenish black color showed the presence of tannins.

Test for glycosides- Borntrager's Test

Test drug is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests. To 2 ml of filtered hydrolysate, 3 ml of choloroform is added and shaken, choloroform layer is separated and 10% ammomia solution is added to it. Pink colour indicates presence of glycosides.

Test for flavonoids:

Alkaline reagent test. Two to three drops of sodium hydroxide were added to 2 mL of extract. Initially, a deep yellow colour appeared but it gradually became colourless by adding few drops of dilute HCL, indicating that flavonoids were present.

Test for phenols:

Lead acetate test: To the test sample; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

Test for steroids:

To the test sample, 2ml of chloroform was added with few drops of conc. Sulphuric acid (3ml), and shaken well. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

Triterpenoids

Liebermann–Burchard test: To the chloroform solution, few drops of acetic anhydride was added then mixed well. 1 ml concentrated sulphuric acid was added from the sides of the test tube, appearance of red ring indicates the presence of triterpenoids.

Test for Cyanins

A.Aanthocyanin:

To the test sample, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100°C. Formation of bluish green colour indicates the presence of anthocyanin.

Test for Carbohydrates - Benedict's test

To the test sample about 0.5 ml of Benedic's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

Proteins (Biuret Test)

To extracts 1% solution of copper sulphate was added followed by 5% solution of sodium hydroxide, formation of violet purple colour indicates the presence of proteins.

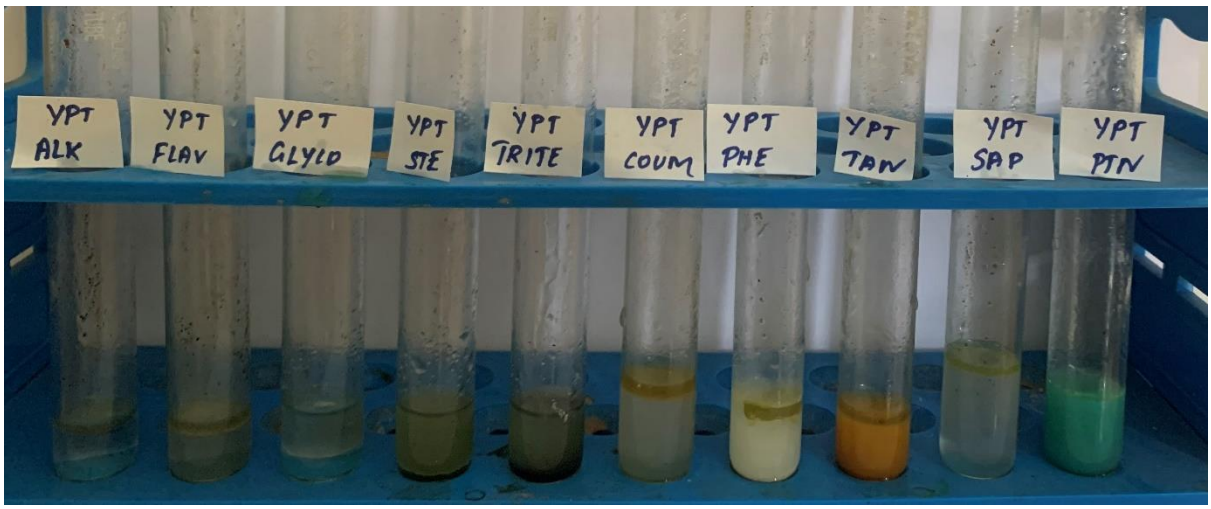

Figure 6: Qualitative Phytochemical analysis of YPT.

CONCLUSION:

The comprehensive analysis of Yazhpanathennai, a Siddha herbal drug, demonstrates that the formulation is safe for use when prepared and administered according to established protocols. The assessment confirmed that levels of heavy metals, aflatoxins, pesticide residues, and microbial contamination were within permissible safety limits, minimizing the risk of adverse effects. Phytochemical screening also verified the presence of beneficial bioactive compounds, supporting the drug's therapeutic potential for conditions such as atopic dermatitis.

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