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Research Article

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Standardization of Polyherbal Siddha formulation - Pippalyathi Mathirai

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Keywords

Pippalyathi mathirai, Physiochemical Analysis, Phytochemical analysis, HPTLC, Clinical management.

Abstract

Background :
In recent years, the traditional system of medicine has gained popularity, due to its holistic approach in treating ailments. The Siddha system of medicine is an indigenous system of medicine, flourished and practiced in Tamil Nadu. Major Siddha formulation involved in herbal formulations are metals and minerals. They possess unique therapeutic values, but there is a lack in standardization. In this study, classical Siddha formulation "Pippalyathi mathirai'', mentioned in Anuboga vaithiya devaragasiyam was selected and screened for standardization technique. The drug is a poly herbal formulation comprising of six herbal ingredients such as thippilli (Piper longum), Pushkaramoolam (Costus specious), Kadukaithool (Teminalia chebula), Chukku (Zingiber officinale), Kichilikilangu (Curcuma zeodaria), Koraikilangu (Cyperus rotundus). These drugs possess potent bronchodilator and anti inflammatory properties. They proves to be effective in the treatment of Bronchial Asthma.

Methods:
The drug was screened for physiochemical, phytochemical analysis and HPTLC toestimate the quality of the drug.

Results:
Further we need to proven the medicinal uses and phytochemical and bioactivity studiesto prove their therapeutic properties. The results of phytochemical analysis indicates the formulation Pippalyathi mathirai contains alkaloids, coumarins, saponins, tannins, glycosides, triterpenoids, anthocyanin, carbohydrate, protein.The results of HPTLC analysis shows phytoconstituents present in the sample and has no traces of heavymetals metals Cadmium, whereas the sample shows the

presence of Lead at 6.43 ppm, Arsenic at 2.13 ppm and Mercury at 1.04 ppm. The Results obtained from standardization and physiochemical analysis clearly reveals that the Poly herbal formulation possess the values, Loss on drying value was 0.9367 % , total ash value was 2.2 % and acid soluble ash is 0.29% . The alchol extractive value was 6.333% and water soluble extract was 11.47%. 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, Aflatoxin G2. The results showed that there were no traces of pesticides residues such as Organo chlorine, Organophosphorus, Organo carbamates and pyrethroids in the sample provided for analysis. The trail drug is free from microbial contamination.

Conclusion:

From the results of the study it was evident that the Siddha formulation Pippalyathi Mathirai complies with the standard and may be used for clinical management of Swasakasam (Bronchial Asthma). But further studies need to carry out to exact role of phytotherapeutics present in the formulation might responsible for the expected pharmacological action in animals and human as well.

Introduction

Traditional system of medicine(TMS) is practiced, since centuries across the world in casting out disease from mankind. TMS serves as a boon in developing countries. WHO, recommends the practice of traditional system of medicine is affordable, safe and culturally acceptable[1]. In recent years ,the alternate system of medicine has gained popularity, since no holistic approach in treating ailments and no complete cure is obtained. Nearly 25,000 potent plant based formulations are available in Indian system of medicine[2].

The Siddha system of medicine is an indigenous system of medicine, flourished and practiced in Tamil Nadu. They were compiled by wise people known as Siddhars. Its basic principle, “food itself is a medicine” and also based on three humors, whose derangements leads to pathological conditions called as disease. In tamil literature, the word maruntu (drug) itself means or denotes scented root or leaf[3]. Nearly 4444 diseases are described in Bogar Nikandu. Though both metals and minerals are used in Siddha system of medicine, the plant based medicines plays a predominant role. It has been described in Siddha as “Verparu thazai paru minginikal mella parpam chenduram paru”[4].

The pharmacological qualities and safety issues of the herbal based medicines are deeply assessed for the acquisition of reliable data for health care giver and patients. In recent times, Siddha pharmacopeia have taken measures of to standardize many formulations. Each invidual step involved in Siddha formulation has its own quality check evaluation starting from preparatory phase to storage phase. Major Siddha formulation involved in herbal formulations are metals and minerals. They possess unique therapeutic values, but there is a lack in standardization. It is necessary to develop the standardization technique[11].

In this study, classical Siddha formulation Pippalyathi mathirai, mentioned in Anuboga vaithiya devaragasiyam was selected and screened for standardization technique . The drug is a poly herbal formulation comprising of 6 herbal ingredients such as thippilli (Piper longum), Pushkaramoolam (Costus specious), Kadukaithool (Teminalia chebula),Chukku (Zingiber officinale), Kichilikilangu (Curcuma zeodaria), Koraikilangu (Cyperus rotundus). These drugs possess potent bronchodilator and anti inflammatory properties. They proves to be effective in the treatment of Bronchial Asthma.

Materials and Methods

2.1 Selection of drug:

The drug Pippalyathi mathirai was collected from the classical Siddha literature.

2.2 Collection and authentication of the drug:

The raw materials included in the formulation are were thippilli (Piper longum), Pushkaramoolam (Costus specious), Kadukaithool (Teminalia chebula),Chukku (Zingiber officinale), Kichilikilangu (Curcuma zeodaria), Koraikilangu (Cyperus rotundus) procured from the country drug shop at chennai, tamilnadu. They were identified and authenticated by the Botanist, Govt. Siddha Medical College, Arumbakkam, Chennai - 106.

2.3 Purification of drug:

The purification of drugs was done by procedures mentioned in classical Siddha literature. The drug Pippalyathi mathirai is polyherbal medicine .The aim of this study was carried out to standardize the drug Pippalyathi mathirai by evaluating physiochemical properties.

2.4 Preparation of the drug:

The purified raw drugs thippilli (Piper longum), Pushkaramoolam (Costus specious), Kadukaithool (Teminalia chebula),Chukku (Zingiber officinale), Kichilikilangu (Curcuma zeodaria), Koraikilangu (Cyperus rotundus) are taken in the equal quantity and made in to fine powder. Then sieved using a sieve cloth. The obtained powder is grounded with jaggery. Then it is made into Sundai size (500 mg) pills. Then stored in a clean air tight container.

Physio chemical evaluation

State	Solid
Nature	Hard Solid with smooth surface- Crush upon stress
Odour	Mild Characteristic
Touch	Rigid and Hard
Flow Property	Free flowing
Appearance	Blackish

Solubility Profile

S.No	Solvent Used	Solubility / Dispersibility
1	**Chloroform**	Insoluble
2	**Ethanol**	Soluble
3	**Water**	Soluble
4	**Ethyl acetate**	Insoluble
5	**DMSO**	Soluble

Percentage Loss on Drying

Test drug was accurately weighed in evaporating dish. The sample was dried at 105^{o}C for 5 hours and then weighed.

Determination of Total Ash

Test drug was accurately weighed in silica dish and incinerated at the furnace a temperature 400 ^{o}C until it turns white in color which indicates absence of carbon. Percentage of total ash will be calculated with reference to the weight of air-dried drug.

Determination of Acid Insoluble Ash

The ash obtained by total ash test will be boiled with 25 ml of dilute hydrochloric acid for 6mins. Then the insoluble matter is collected in crucible and will be washed with hot water and ignited to constant weight. Percentage of acid insoluble ash will be calculated with reference to the weight of air-dried ash.

Determination of Alcohol Soluble Extractive

Test sample was macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105^{o}C, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

Determination of Water Soluble Extractive

Test sample was macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand and for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105^{o}C, to constant weight and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug[5].

Final Test report

S.No	Parameter	Mean (n=3) SD
1.	Loss on Drying at 105 °C (%)	0.9367 ± 0.005773
2.	Total Ash (%)	2.2 ± 0.3
3.	Acid insoluble Ash (%)	0.29 ± 0.03
4.	Water soluble Extractive (%)	11.47 ± 1.15
5.	Alcohol Soluble Extractive (%	6.333 ± 0.9292

Phytochemical analysis

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:

To the test sample about 5 ml of dilute ammonia solution were been added followed by addition of few drops of conc. Sulfuric acid. Appearance of yellow color indicates the presence of Flavonoids.

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^{o}C. Formation of bluish green colour indicates the presence of anthocyanin.

Test for Carbohydrates - Benedict's test

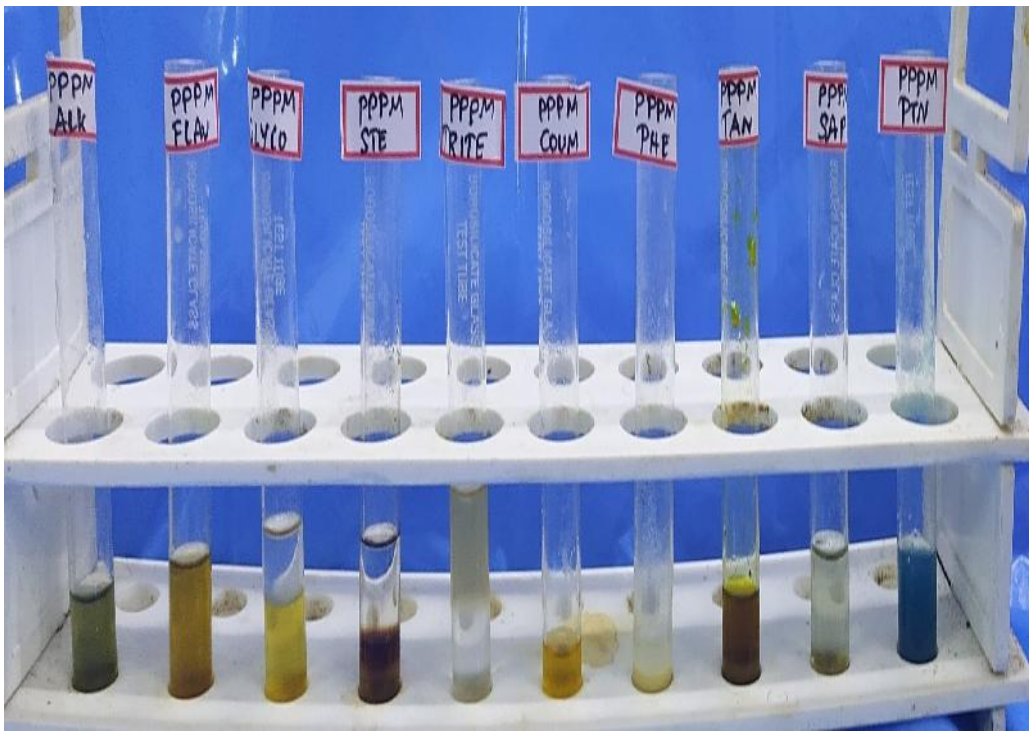
To the test sample about 0.5 ml of Benedic's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

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[6].

Results

Qualitative Phytochemical Investigation



TLC Analysis

Test sample was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Micro pipette were used to spot the sample for TLC applied sample volume 10-micro liter by using pipette at distance of 1 cm at 5 tracks. In the twin trough chamber with the specified solvent system After the run plates are dried and was observed using visible light Short-wave UV light 254nm and light long-wave UV light 365 nm.

High Performance Thin Layer Chromatography Analysis

HPTLC method is a modern sophisticated and automated separation technique derived from TLC. Pre-coated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. Thus this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprint of phytochemicals which is suitable for confirming the identity and purity of phytotherapeutics.

Chromatogram Development

It was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analyzed. After elution, plates were taken out of the chamber and dried.

Scanning

Plates were scanned under UV at 366nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of phytoconstituents present in each sample and their respective Rf values were tabulated[7].

Heavy metal analysis by AAS

Standard: Hg, As, Pb and Cd – Sigma

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

Test Report

Name of the Heavy Metal	Absorption Max Λ max	Result Analysis	Maximum Limit
Lead	217.0 nm	5.54 PPM	10 ppm
Arsenic	193.7 nm	2.13 PPM	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	1.04 PPM	1 ppm

BDL- Below Detection Limit

Report and Inference

Results of the present investigation have clearly shows that the sample has no traces of heavy metal Cadmium, whereas the sample shows the presence of Lead at 5.54 ppm, Arsenic at 2.13 ppm and Mercury at 1.04 ppm

Sterility test by pour plate method

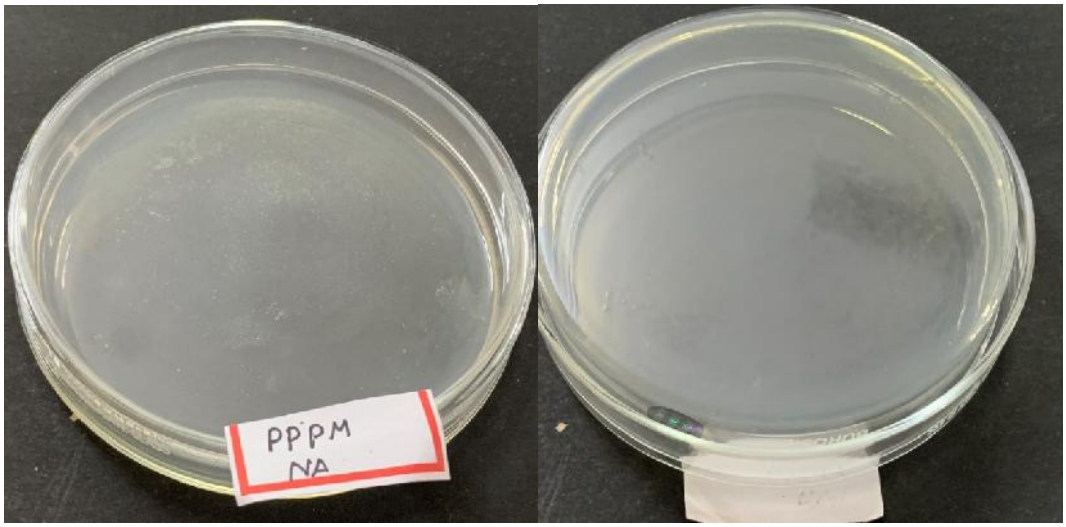
Objective

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 45^{o}C 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^{o} 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.



Observation

No growth was observed after incubation period. Reveals the absence of specific pathogen

Result

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	

Test Report

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 37^{o}C for 24 - 72h for observation. Presence of specific pathogen identified by their characteristic color 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***

Observation

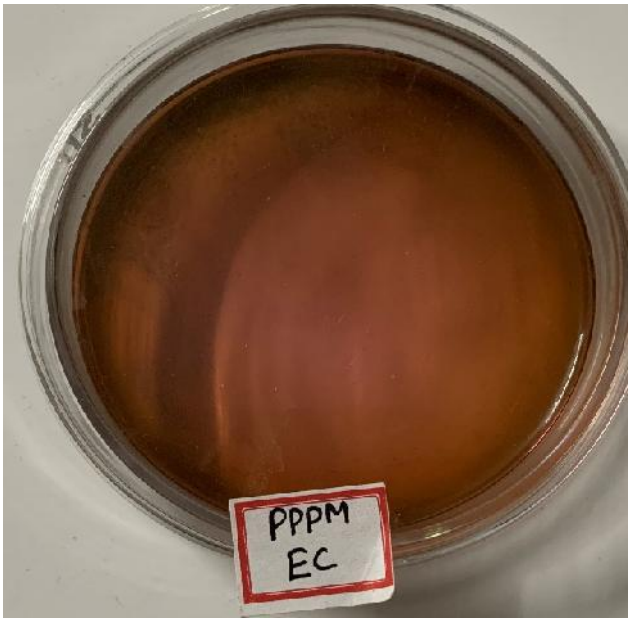
No growth was observed after incubation period. Reveals the absence of specific pathogen

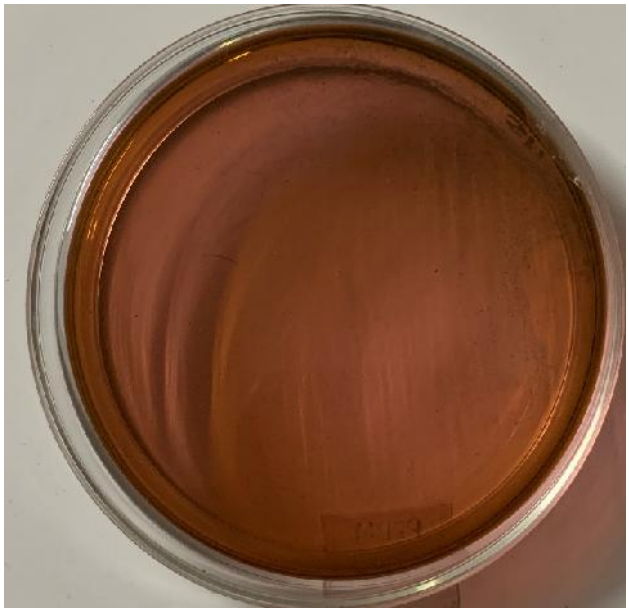
Result

No growth / colonies were observed in any of the plates inoculated with the test sample.

Organism	Specification	Result	Method
E-coli	Absent	Absent	As per AYUSH specification
Salmonella	Absent	Absent	
Staphylococcus aureus	Absent	Absent	
Pseudomonas aeruginosa	Absent	Absent	

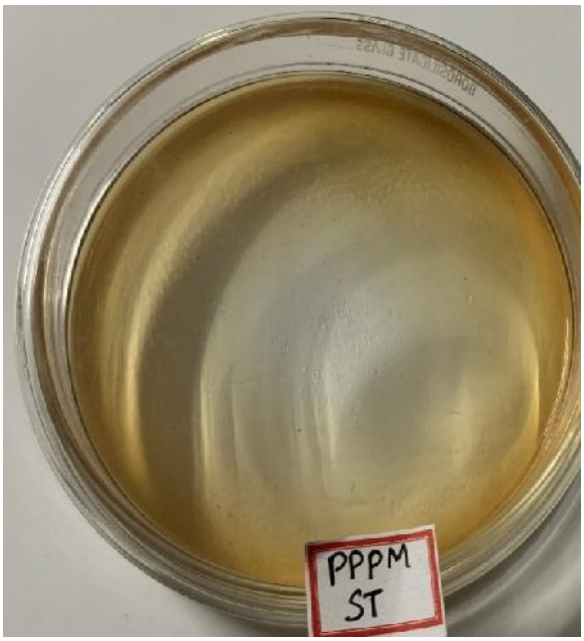
Culture plate with E.coli (EC) specific medium



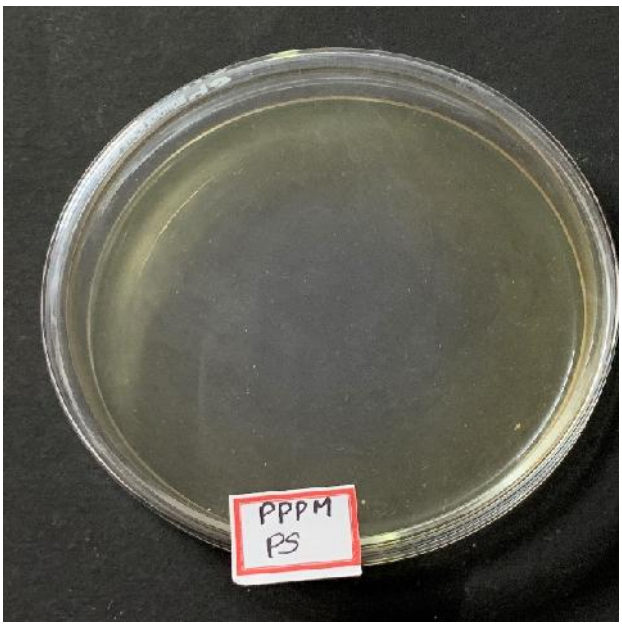


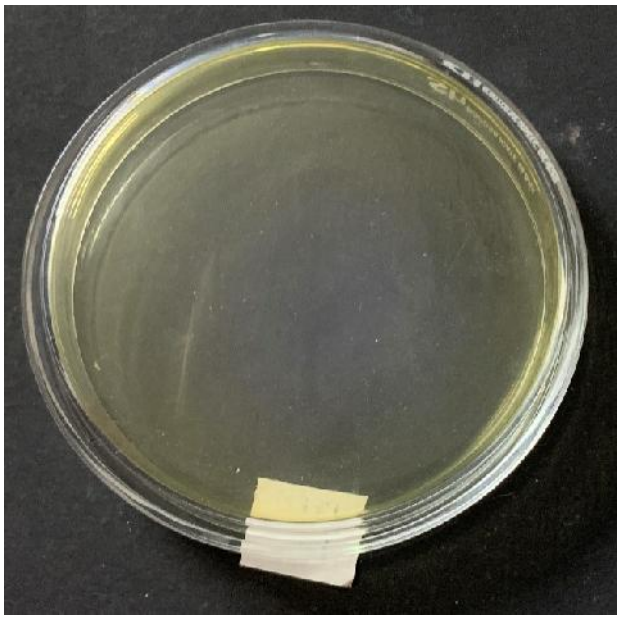
Culture plate with Salmonella (SA) specific medium

Culture plate with Staphylococcus aureus (ST) specific medium



Culture plate with Pseudomonas aeruginosa (PS) specific medium





Pesticide

Extraction

Test sample were extracted with 100 ml of 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[8,9].

Test Result Analysis of the Sample PPPM

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

ND- Not Detected

Result: The results 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.

Aflatoxin

Standard 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[10].

Aflatoxin	Sample PPPM	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

Result: 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, Aflatoxin G2.

Discussion

The traditional Medicine has gained its popularity across worldwide, due to its user friendly nature and intrinsic side effects when compared to modern medicine. The results obtained from standardization and physiochemical analysis of the poly herbal formulation Pippalyathi mathirai clearly reveals that, Loss on drying value was 0.9367 % , total ash value was 2.2 % and acid soluble ash is 0.29% .The alchol extractive value was 6.333% and water soluble extract was 11.47%. Further the physiochemical and phytochemical analysis are to prove their therapeutic properties. The results of phytochemical analysis indicates the formulation Pippalyathi Mathirai contains alkaloids, coumarins, saponins, tannins, glycosides, triterpenoids, anthocyanin, carbohydrate, protein.

The results of HPTLC analysis shows phytoconstituents present in the sample and has no traces of heavymetals metals Cadmium, whereas the sample shows the presence of Lead at 5.54 ppm, Arsenic at 2.13 ppm and Mercury at 1.04 ppm. 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, Aflatoxin G2. The results showed that there were no traces of pesticides residues such as Organo chlorine, Organophosphorus, Organo carbamates and pyrethroids in the sample provided for analysis. No growth\ colonies was observed in any of the plates inoculates with the test sample. This proves the trial drug possess significant microbial activity against *E.Coli, Salmonella, Staphylococcus aureus, Pseudomonas aeruginosa.* Which clearly indicates the trial drug Pippayathi Mathirai is safety for human consumption, by its potent theraupeutic values proves to be beneficial in the treatment of Swasakasam (Bronchial Asthma).

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