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Phytochemical Standardization Of Thuthuvelai Chooranam – A Polyherbal Siddha Formulations In The Management Of Swasakasam (Bronchial Asthma)

[1]Dr.Anitha.M, [2]Dr.Dharani.P, [3]Dr.Anbu.N

1Department of Pothu Maruthuvam, Government Siddha Medical College, Chennai – 600106, *Tamil Nadu, India.*

Abstract

Aim: The aim of the present study was to identify the presence of bioactive phytocomponents in the Siddha polyherbal drug formulation Thuthuvelai chooranam by subjecting the drug to various studies like physicochemical analysis and HPTLC.

Methods: The ingredients present in the Siddha polyherbal formulation Thuthuvelai chooranam was first authenticated by the botanist and was purified. The purified ingredients of the drug were grounded to make a powdered form. The prepared siddha polyherbal formulation Thuthuvelai chooranam was sent it to the lab for the physiochemical analysis and HPTLC. The mentioned physicochemical and the HPTLC was carried out at Noble Research Solutions in Kolathur, Chennai – 600 099.

Results: Physicochemical analysis of the study revealed 0.64% of loss on drying (LOD) at 1050C, 1.6% of total ash value, 0% of acid insoluble ash, 24.67% of water-soluble extraction, 17.73% of Alcohol soluble extractive and the pH is 6.3. Six prominent peaks are seen in the sample's HPTLC finger printing examination, which indicates that it contains six different phytocomponents. The highest Rf value of the peaks varies from 0.04 to 0.99.

Conclusion: The findings aid in determining the formulations of siddha polyherbal drug Thuthuvelai chooranam and it reveals the presence of physicochemical properties of the drug which ensures the safety and therapeutic potential in the management of Swasakaasam (Bronchial Asthma) The study drug can also be used as a reference norm for the medicine of standard pharmaceutical product and further quality control enquires. This study provides the evidence for future clinical studies.

Keywords: Siddha medicine; Swasakasam; Thuthuvelai chooranam; physicochemical; HPTLC.

INTRODUCTION

"Siddha medicine is a unique one as it is not only a curative but also preventive and to achieve the healthy body and mind. Siddha medicines revitalize and rejuvenate the body" [1].

"70% of Indians utilize traditional and alternative medicines extensively for medical care, according to the WHO. Traditional Indian medicines have a long history of clinical use and consistent therapeutic efficacy, which have helped them gain international recognition. Many major pharmaceutical companies are now turning to traditional Indian medicines as a great source for finding new naturally occurring bioactive molecules. The quality, efficacy, and standards of the siddha herbal formulations have come under scrutiny due to the increased demand for safer medications" [2].

"Standardization is an essential factor for herbal formulation in order to assess the quality of the drug based on the concentration of their active principle and to ensure that every packet of medicine that is sold has the correct amount and will induce its therapeutic effect" [3].

"Bronchial asthma, characterized by chronic airway obstruction and increased airway hyper

responsiveness leads to symptoms of wheeze, cough, chest tightness and difficulty in breathing" [4].

"It affecting any age, race and socio-economic class globally and its prevalence is changing upwards worldwide. The increase in prevalence may be due to changes in lifestyle, rapid industrialization, tobacco, smoke, viral infections, chemical irritants and increase in air pollution" [5].

"Prevalence of asthma increased steadily over the later part of the last century, 300 million of people worldwide suffer from asthma and an additional 100 million may be diagnosed with asthma by 2025. India has an estimated 15 to 20 million asthmatics and rough estimates indicate a prevalence of between 10% and 15% in 5-11year old children. The important pathophysiological effects of bronchial asthma are the inflammatory process, secondary an immune response, which is induced by an allergen that causes the accumulation of inflammatory cells such as mast cells, eosinophil's, lymphocytes, etc., and the release of their products. Therefore, the effective management of asthma is to control not only the clinical manifestations, but also the inflammatory process and pathophysiology of the disease and to achieve and maintain control for prolonged periods of time" [6].

Siddha system of Medicine has suggested varies polyherbal & herbomineral formulation for the management of Swasakasam. One such formulation is Siddha sastric preparation Thuthuvelai chooranam, a polyherbal formulation that was taken from siddha literature The Pharmacopeia of Siddha Research Medicine Page No. 110 [7].

For the standardization of this drug physicochemical analysis loss on drying, total ash value, Acid insoluble ash, Water soluble extractive, Alcohol soluble extractive, pH and HPTLC were carried out to confirm the presence of bioactive phytocomponents in the polyherbal formulation.

MATERIALS AND METHODS

Selection of Drug

The Siddha formulation Thuthuvelai chooranam which was choosen from the Siddha literature The Pharmacopeia of Siddha Research Medicine Pg.No.110 for the management of Swasakasam.

Ingredients

Piper longum (thipilli) - 3 tolas (24gms)

Solanum trilobatum (thuthuvelai) - 6 tolas (48gms)

Saccharum officinalis (Sugar) - 9 tolas (72gms)

Source of Raw Drugs

The necessary raw medicinal drug was bought from reputable nearby local raw drug store. The botanist at Government Siddha Medical College in Chennai had verified the authenticity of the raw medicinal drug used for the study. (Voucher number GSMS/MB – 560-564).

Sample preparation

Purification of siddha raw drugs:

Siddha drugs were purified as mentioned in Siddha marthukiyal vithigalum seimuraigalum [8],

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.

Preparation

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

Physicochemical Analysis of Thuthuvelai chooranam

The physicochemical analysis of Thuthuvelai chooranam was carried out at Noble Research Solutions in Kolathur, Chennai – 600 099. The preliminary physicochemical screening test was carried out for Thuthuvelai choornam as per the standard procedures mentioned hereunder.

Loss on Drying: Test drug was accurately weighed in evaporating dish. The sample was dried at 105oC for 5 hours and then weighed.[10].

Determination of total ash: Test drug was accurately weighed in silica dish and incinerated at the furnace a temperature 400oC 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.[10]

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. [10].

Determination of water soluble ash: “1g of total ash was boiled for 5 minutes in 25ml of water, and the insoluble material that had been collected on an ash-free filter paper was then washed in hot water and ignited in a muffle furnace for 15 minutes at a temperature no higher than 4500C. The filtrate is dried to assess the amount of soluble ash”. [10]

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 105oC, to constant weight and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug. [10].

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 105oC, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

pH determination

Required quantity of test sample was admixed with distilled water and the subjected to screening using pH meter.

1.1 THIN LAYER CHROMATOGRAPHY ANALYSIS (TLC) AND HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY ANALYSIS

2.6.1 **THIN LAYER CHROMATOGRAPHY 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.

2.6.2 High performance thin layer chromatography: 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.

2.6.3 **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.

2.6.1 **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.

2. RESULTS AND DISCUSSION

Physico chemical analysis of the current study 4.3% of loss on drying (LOD) at 105 C, 1.6% of total ash value, 0% of acid insoluble ash, 24.67% of water soluble extraction, 17.73% of Alcohol soluble extractive and 6.3 pH.

Table 1. Results of physico chemical analysis of *Thuthuvelai chooranam*

S.No	***Parameters***	***Percentage(%)***
1	*Loss on Drying at 105 °C (%)*	4.3 ± 0.3
2	*Total Ash (%)*	1.6 ± 0.43
3	*Acid insoluble Ash (%)*	0 ± 0
4	*Water soluble Extractive (%)*	24.67 ± 7.23
5	*Alcohol Soluble Extractive (%)*	17.73 ± 2.13
6	*pH*	6.30

The loss of drying value denotes the drug moisture content which was evaluated as 4.3%. The moisture content of the herbal medicine should be less as it stimulates the growth of living organisms, fungi, or insects and cause deterioration following hydrolysis. For herbal drugs, the moisture content must be less than 14%. The total ash value of the test drug was 1.6% indicates the presence of inorganic residues in the formulation. The value of acid insoluble ash was 0% denotes that the drug has no significant amount of siliceous matter. Extractive values of the drug give the approximate amount of chemical constituents present in the formulation.

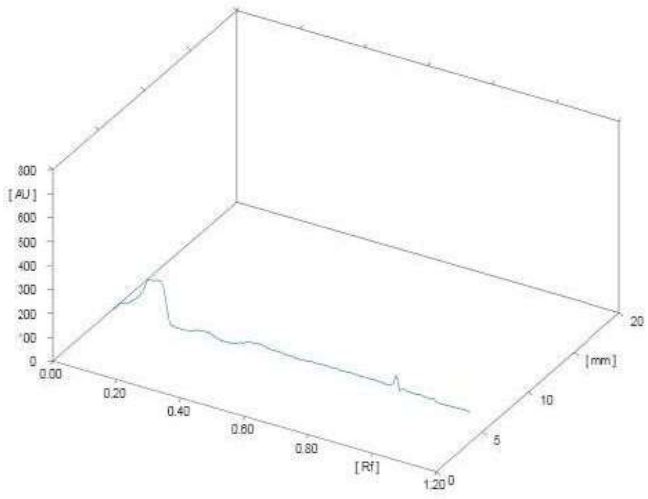
Six prominent peaks are seen in the sample's HPTLC finger printing examination, which indicates that it contains six different phytocomponents. The highest Rf value of the peak varies from 0.04 to 0.99.

There were previous studies done on the individual ingredients present in the polyherbal formulation *Thuthuvelai choornam* for their therapeutic effects. Some of them are anti-inflammatory, anti-oxidant, immunomodulatory activity and anti-microbial activity of *Solanum trilobatum*[11] [12] [13] [14], anti-asthmatic activity, anti- inflammatory, immunomodulatory properties of *Piper longum* [15] [16][17] [18].

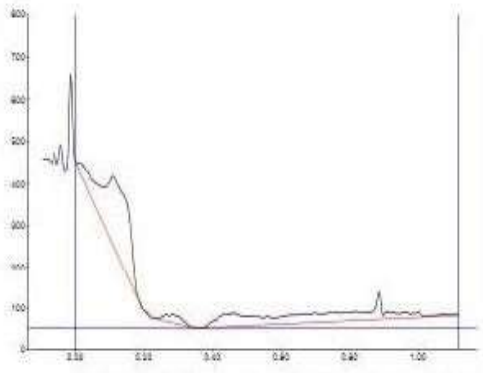
The outcomes of the physicochemical and HPTLC tests will be valuable as a tool for identifying substances and ensuring the existence of bioactive phytocomponents and the efficacy of drug formulations, which adds value to therapeutic interventions.

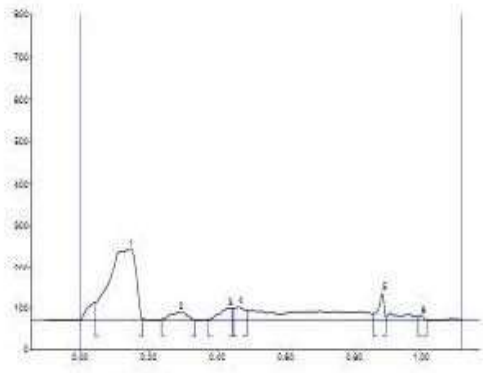
TLC Visualization of TC at 366 nm

3D – Chromatogram



HPTLC finger printing of TC





Peak value

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.04	42.6	0.14	175.9	51.92	0.18	4.8	7194.5	75.06
2	0.24	3.5	0.29	20.3	5.99	0.34	0.4	553.5	5.77
3	0.37	0.5	0.43	30.9	9.11	0.45	29.1	614.6	6.41
4	0.45	28.8	0.46	32.8	9.68	0.49	23.9	550.2	5.74
5	0.86	17.1	0.89	64.8	19.14	0.90	8.4	566.8	5.91
6	0.99	9.4	1.00	14.1	4.16	1.02	2.0	105.2	1.10

3. CONCLUSION

From the above study results, the author concluded that siddha polyherbal formulations *Thuthuvelai chooranam* having biologically active components may help in management of *Swasakasam* (Bronchial asthma). Therefore it's recommended to take formulations to next level of investigations like pharmacological studies and clinical trials. The study drug can also be used as a reference norms for the medicine of standard pharmaceutical product and further quality control enquires.

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