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Standardization of *Gowthamar chooranam* – A Polyherbal Siddha Formulations in the Management of *Swasakasam* (Bronchial Asthma)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: 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. Bronchial asthma, characterized by chronic airway obstruction and increased airway hyper responsiveness leads to symptoms of wheeze, cough, chest tightness and difficulty in breathing. 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. Siddha, an ancient system of Indian Medicine has recommended a number of drugs for indigenous plant sources for the treatment of Bronchial Asthma. One among them is Siddha polyherbal formulation *Gowthamar chooranam.*

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Aim: The aim of the present study was to identify the presence of bioactive phytocomponents in the Siddha polyherbal drug formulation *Gowthamar chooranam* by subjecting the drug to various studies like physicochemical analysis, phytochemical screening, and HPTLC.
Methods: The ingredients present in the Siddha polyherbal formulation *Gowthamar chooranam* was first authenticated by the botanist and was purified. The purified ingredients of the drug was grounded to make a powdered form. The prepared siddha polyherbal formulation *Gowthamar chooranam* was sent it to the lab for the physiochemical analysis, phytochemical screening and HPTLC. The mentioned physicochemical and phytochemical analysis was conducted at The Tamil Nadu Dr. MGR Medical University, located at No.69, Anna Salai, Guindy, Chennai – 600 032. 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 105^0C, 2.41% of total ash value, 0.8394% of acid insoluble ash, 1.65% of water soluble ash, 62.30% of water soluble extraction, 11.25% of Alcohol soluble extractive. Phytochemical screening showed the presence of bioactive compounds such as carbohydrates, saponin, phenols, tannin, flavonoids, diterpenes and Gum and mucilage. Seven distinct peaks are seen in the sample's HPTLC finger printing examination, which indicates that it contains seven different phytocomponents. The highest Rf value of the peaks varies from 0.05 to 0.75.
Conclusion: The findings aid in determining the formulations of siddha polyherbal drug *Gowthamar chooranam* and it reveals the presence of physicochemical and phytochemical 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 norms 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; Gowthamar chooranam; physicochemical; phytochemical, HPTLC.

1. 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 5-11 year 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 *Gowthamar chooranam*, a polyherbal formulation that was taken from siddha literature The Pharmacopeia of Siddha Research Medicine Page No. 113 [7].

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

2. MATERIALS AND METHODS

2.1 Selection of Drug

The Siddha formulation *Gowthamar chooranam* which was choosen from the Siddha literature The Pharmacopeia of Siddha Research Medicine Pg.No.113 for the management of *Swasakasam.*

2.2 Ingredients

1. *Terminalia chebula (Kadukkai thole*) - 2 tolas (24gms)
2. *Piper longum (Arisi thipilli*) - 2 tolas (24gms)
3. *Alpinia officinarum* (*Chitharathai*) - 2 tolas (24gms)
4. *Piper cubeba (Valmilagu*) - 4 tolas (48gms)
5. *Myristica fragans (Jathikkai)* - 2 tolas (24gms)
6. *Saccharum officinalis* (Sugar) - 12 tolas (144gms)

2.3 Source of Raw Drugs

The necessary raw medicinal drug were 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).

2.4 Sample Preparation

2.4.1 Purification of siddha raw drugs:

Siddha drugs were purified as mentioned in *Sikitcha Ratna Deepam Sarakku Suthi Muraigal* [8][9]:

- *Terminalia chebula (Kadukaithool*) - It was purified by soaking in rice water filtrate (*kazhuneer*), the seed is removed and dried completely.
- *Piper longum* (*Thippili*) - It was purified by soaking it in lemon juice and drying it in sunlight until it dries off.
- *Alpinia officinarum (Chithrathai*) **–** The epidermal layer of the rhizome was peeled off and cut into pieces and insolate it to get the purified form.
- *Piper cubeba (Vaalmilagu)* **–** The tail like stalk of *Piper cubeba* was removed and sun dried.
- *Myristica fragrans (Jathikkai)* **–** The outer shell of *Myristica fragrans* was removed and the seed kernel was cut into pieces and dried under sunlight to get the purified form.
- *Saccharum officinalis* (Sugar) – The sugar was grounded and powdered.

2.4.2 Sample 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.

2.5 Physicochemical Analysis of *Gowthamar choornam*

The physico chemical analysis and phytochemical screening of *Gowthamar chooranam* was carried out at, The Tamilnadu Dr.MGR Medical University, Guindy, Chennai. The preliminary physicochemical screening test was carried out for *gowthamar choornam* as per the standard procedures mentioned hereunder.

2.5.1 Loss on Drying:1g sample of *Gowthamar choornam* formulation, which was accurately weighed, was placed in a glass bottle and heated at 105 degrees Celsius for six hours. Its moisture content was then calculated based on the shade dried materials [10].

2.5.2 Determination of total ash: Accurately weighing at a temperature of 6000C in a muffle furnace, 2g of the *Gowthamar Choornam* formulation was added until carbon-free ash was formed. It's was calculated based on the air dried drug. [10]

2.5.3 Determination of acid insoluble ash: "Ash which was formed above was boiled with 25 ml of 1M hydrochloric acid

for 5 minutes and filtered with a help of ash less filter paper. Insoluble matter remained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble ash was calculated based on the air dried drug" [10].

2.5.4 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]

2.5.5 Determination of water soluble Extractive: "5 g of air dried crude medicine *Gowthamar choornam* is steeped in 100 ml of distilled water in a sealed bottle for 24 hours with frequent shaking. The solution was filtered and 25 ml of the filtrate was evaporated in a tarred flat-bottomed shallow dish, further dried at 1000 °C and weighed. The percentage of water-soluble extract was calculated from the air-dried drugs" [10].

2.5.6 Determination of alcohol soluble extractive: "1 gm. of air dried drug coarsely powdered *Gowthamar choornam* was macerated with 20 ml alcohol in closed flask for 24 hrs. With frequent shaking, it was filtered rapidly taking precaution against loss of alcohol 10ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100^0C and weighted. The percentage of alcohol soluble extractive was calculated with reference to air dried drug". [10]

2.6 Preliminary Phytochemical Screening of *Gowthamar choornam*

The preliminary phytochemical screening test was carried out for each extracts of *Gowthamar Choornam* as per the standard procedure mentioned hereunder.

2.6.1 Detection of alkaloids:

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

- Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide) Yellow color precipitate forms indicates the presence of alkaloids. Such precipitate was not formed.
- Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (Potassium Bismuth Iodide). Red colour precipitate forms that shows the presence of alkaloids. Such precipitate was not formed.
- Wagner's Test: "Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids. Such precipitate formation was not seen".[10]

2.6.2 Detection of carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used for the detection of carbohydrates.

- Molisch's Test: To 2 ml of plant sample extract, two drops of alcoholic solution of α- naphthol are added. The mixture is shaken well and few drops of concentrated sulphuric acid is added slowly along the sides of test tube. A violet ring shows the presence of carbohydrates.
- Benedict's test: "Benedict's reagent were treated with the filtrate and heated gently. Orange red precipitate shows the presence of reducing sugars"[10].

2.6.3 Detection of saponins

- Foam Test: 0.5 gm. of extract was shaken with 2 ml of water. If foam produced persists for 10 minutes it indicates the presence of saponin [10].

2.6.4 Detection of phenols

- Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols [10].

2.6.5 Detection of tannins

- Gelatin Test: "The extract is dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing 10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds" [10].

2.6.6 Detection of Flavonoids

- Alkaline Reagent Test: Extracts were treated with few drops of sodium

hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

- Lead acetate Test: "Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids" [10].

2.6.7 Detection of diterpenes

- Copper Acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes [10].

2.6.8 Test for quinones

- Test for Quinones: Extract was treated with sodium hydroxide. Blue or red precipitate indicates the presence of Quinones. No such precipitate formation was seen. It indicates the absence of quinones [10].

2.6.9 Gum and Mucilage:

- To 1ml of extract add 2.5ml of absolute alcohol and stirring constantly. Then the precipitate was dried in air and examine for its swelling properties. Swelling was observed that will indicates the presence of gum and mucilage [10].

2.7 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" [11].

2.7.1 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" [11].

2.7.2 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"[11].

3. RESULTS AND DISCUSSION

Physico chemical analysis of the current study 0.64% of loss on drying (LOD) at 105 C, 2.41% of total ash value, 0.8394% of acid insoluble ash, 1.65% of water soluble ash, 62.30% of water soluble extraction, 11.25% of Alcohol soluble extractive.

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

S. No	Parameters	Percentage (%)
1	Loss of drying	0.64
2	Total ash value	2.41
3	Acid insoluble ash	0.8394
4	Water soluble ash	1.65
5	Water soluble extraction	62.
6	Alcohol soluble extraction	11.25

The loss of drying value denotes the drug moisture content which was evaluated as 0.64%. 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 GTC was 2.41% indicates the presence of inorganic residues in the formulation. The value of acid insoluble ash was 0.8394% denotes that the drug contains siliceous matter in not significant amount. The water soluble ash is a part of total ash content of the drug which is soluble in water. The water soluble ash value for test drug was 1.65%. Extractive values of the drug give the approximate amount of chemical constituents present in the formulation.

The Phytochemical screening showed the presence of bioactive compounds such as carbohydrates, saponin, phenols, tannin, flavonoids, Diterpenes and Gum and mucilage. It also shows the absence of alkaloids and Quinones.

The Preliminary phytochemical studies of aqueous extract of *gowthamar choornam* were done using standard procedures. The results were presented in tables 2 and 3. The present study reveals that the bioactive compounds were present in all the extracts of *gowthamar choornam***.**

Positive/Negative present or absent if component tested.

Seven distinct peaks are seen in the sample's HPTLC finger printing examination, which indicates that it contains seven different phytocomponents. The highest Rf value of the peak varies from 0.05 to 0.75.

There were previous studies done on the individual ingredients present in the polyherbal formulation *Gowthamar choornam* for their therapeutic effects. Some of them are Bronchodilator, anti-histamine of *Terminalia chebula* [12], anti-asthmatic activity, anti-inflammatory, immunomodulatory properties of *Piper longum* [13] [14] [15], anti-inflammatory activity of *Alpinia officinarum* [16], anti-inflammatory activity of *Piper cubeba* [17] [18] anti-asthmatic, anti-inflammatory, anti-allergic activity of *Myristica fragrans* [19].

The outcomes of the physicochemical, phytochemical, 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.

Table 2. Results of phytochemical analysis of a *Gowthamar chooranam*

S. No	Phytochemicals	Test Name	H_2O Extract
1	Alkaloids	Mayers Test	Negative
		Dragendroffs Test	Negative
		Wagner Test	Negative
2	Carbohydrates	Molisch Test	Positive
		Benedict Test	Positive
3	Saponin	Foam Test	Positive
4	Phenols	Ferric Chloride Test	Positive
5	Tannins	Gelatin Test	Positive
6	Flavonoids	Alkaline Reagent Test	Positive
		Lead acetate	Positive
7	Diterpenes	Copper Acetate Test	Positive
8	Quinones	Test for Quinones	Negative
9	Gum & Mucilage	Test for Gum & Mucilage	Positive

Fig. 1. TLC Visualization of *Gowthamar chooranam* at 366 nm

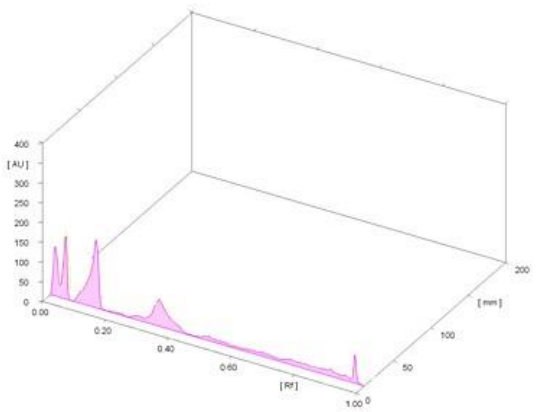

Fig. 2. 3D Chromatogram

Table 3. HPTLC Peak Table of *Gowthamar chooranam*

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.00	42.2	0.03	183.7	24.89	0.04	8.2	2465.2	11.70
2	0.05	6.4	0.08	95.0	12.87	0.08	45.9	943.7	4.48
3	0.08	49.9	0.09	105.0	14.23	0.13	0.2	1113.7	5.29
4	0.19	22.2	0.26	82.9	11.23	0.26	82.0	2569.4	12.20
5	0.26	81.5	0.33	195.9	26.54	0.39	6.0	9640.4	45.77
6	0.63	7.3	0.69	22.9	3.10	0.70	22.2	734.9	3.49
7	0.75	30.8	0.81	52.7	7.14	0.88	15.9	3595.3	17.07

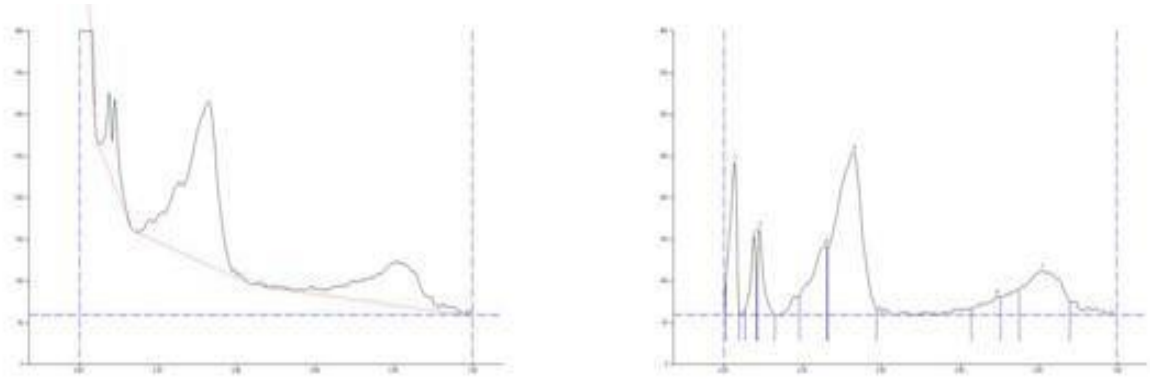

Fig. 3. HPTLC finger printing of sample *Gowthamar chooranam*

4. CONCLUSION

From the above study results, the author concluded that siddha polyherbal formulations *Gowthamar 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.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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