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Standardization of Gandhaga Thailam: A Traditional Siddha Formulation for Skin Disorders

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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

Standardization is a part of Drug development process. Identifying the quality of a Siddha formulation is essential before it taken up for In vivo, In vitro studies and Clinical trials. Gandhaga thailam a Mineral based Siddha medicine to treat various ailments like Eczema, Ulcer, Peptic ulcer diseases etc. Test samples were extracted with acetone and briefly homogenized. Further filtration followed by addition of acetone to the test mixture. The test samples were heated using a rotary evaporator at a temperature not exceeding 40°C until almost complete evaporation of the solvent. HPTLC analysis reveals the presence of thirteen prominent peaks corresponds to the presence of

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thirteen versatile phytocomponents present within it. Rf value of the peaks ranges from 0.02 to 0.80. Study indicates that the Gandhaga Thailam is in standard quality, hence the preparation and purification processes of ingredients were done as per the literature. With these results Gandhaga thailam may be subjected to invitro and invivo toxicity studies in future. Results reveal the quality of the Medicine Gandhaga Thailam and may be the fingerprint for the future analysis on Gandhaga Thailam.

Keywords: Pharmacology; traditional medicine; Ghandhaga Thailam; siddha; standardization.

1. INTRODUCTION

Siddha system of Medicine a traditional Indian holistic system to cure various diseases since ancient days. Siddha formulations are prepared from the raw drugs originated as herbal, Mineral and metal based. Developing a new drug molecule and the availability of that new drug in the market takes almost 12-15 years [1]. Drug creation using the modern approach is expensive and time-consuming, but using the tried-and-true reverse pharmacology approach based on traditional medicine saves money and reduces time. Standardization is a part of Drug development process. Identifying the quality of a Siddha formulation is essential before it taken up for In vivo, In vitro studies and Clinical trials. Gandhaga thailam a Metal based Siddha medicine to treat various ailments like Eczema, Ulcer, Peptic ulcer diseases etc. [2] It is a thailam based medicine prepared by a special preparatory procedure Sudar thailam method. Hence it is also called as Gandhaga Sudar thailam [3]. Gandhagathailam is a popular medicine practiced mostly in Tamil Nadu state, meanwhile the quality of Gandhaga thailam remains unexplored. Pharmacopoeial laboratory for Indian medicine published a guideline, volume 1 to standardize the Siddha Preparations on 2018. [4]. Guidelines provided parameters to standardize the thailam preparations. Aim of the study is to identify the quality of Gandhaga thailam by applying PLIM guidelines.

2. MATERIALS AND METHODS

2.1 Process of Preparation of Gandhagathailam [2]

Purified Gandhagam (Sulphur) [3] Purified Vediuppu, (Potassium Nitrate) [4] and Purified Manosilai (Arsenic diSulfide) [5] 35 gram each are Powdered. Purified Navacharam (Ammonium chloride) [6], Purified Veeram (Mercuric Per Chloride) [7] 2.5 gram each are then added. Then they are triturated with Cow's butter (140 gram) to get the Thailam by doing the Sudar Thailam Procedure [8]. Dosage is about 488mg, Twice a day for 10 days.

2.2 Physico Chemical Evaluation of Gandhagathailam [9,10,11]

Physico chemical characters evaluated by following parameters, Organoleptic characters, Solubility test, Iodine value, saponification value, Viscosity value, Refractive Index, Acid Value, Peroxide value.

2.3 Thin Layer Chromatography / High Performance Thin Layer Chromatography Analysis [12]

TLC Analysis: With the use of silica gel 60F254 and a 7 X 6 cm (Merck) cut using regular household scissors, the test sample was submitted to TLC in accordance with the traditional one-dimensional ascending procedure. Soft pencil was used to mark plates. Sample volume 10 microliters was applied using a micro pipette spaced 1 cm apart over 5 tracks in order to spot the sample for TLC. Following the run plates' drying and observation using visible light, they were placed in the twin trough chamber equipped with the designated solvent system. Light long-wave UV light (365 nm) and short-wave UV light (254 nm).

HPTLC Analysis: It was a TLC-derived approach. For the efficient and economical examination of botanical materials, HPTLC proved a useful quality assessment instrument. High levels of selectivity, sensitivity, and speed are provided by the HPTLC approach in addition to one-step sample preparation. As a result, using this approach is convenient.

It was done in chambers with CAMAG Twin Troughs. The method of sample elution was chosen based on the component to be analysed's capacity for adsorption. Plates were removed from the chamber and dried after elution.

Plates were scanned at 366 nm under UV light. Software called CAMAG was used to integrate the data that were scanned. To identify the phytoconstituents in each sample, a chromatographic fingerprint was created, and the corresponding Rf values were recorded.

2.4 Atomic Absorption Spectroscopy (AAS) Analysis [12]

AAS analysis was a common technique for detecting metals and metalloids. The total heavy metal content of the sample was analysed by AAS Model AA 240 Series**.**

2.5 Sterility Test- Pour Plate Method [12]

A sterile petri dish was used to inoculate the test sample, and approximately 15 millilitres of melted agar were added at 45 degrees Celsius. The dish was tilted and swirled to properly mix the agar and sample. The agar was left undisturbed until it fully gelled. (about ten minutes). After that, plates were turned over and incubated for a further 48 hours at 37 °C before being left open for a further 72 hours to observe fungal development. The number of grown organism colonies was then determined for CFU.

2.6 Test for Specific Pathogen [12]

“Sample was directly inoculated in to the specific pathogen medium (EMB, DCC, Mannitol, Cetrimide) using pour plate method” [13]. For observation, the plates were incubated for 24 to 72 hours at 37°C. Existence of a particular pathogen recognised by its unique hue in relation to the pattern of colony formation in every type of media [14].

2.7 Pesticide Residue [12]

“Test samples were extracted with acetone and briefly homogenized. Further filtration followed by addition of acetone to the test mixture. The test samples were heated using a rotary evaporator at a temperature not exceeding 40 °C until almost complete evaporation of the solvent. Add a few milliliters of toluene to the residue and heat again until the acetone is completely removed. The resulting residue is dissolved in toluene and filtered through a membrane filter” [12].

2.8 Aflatoxin Assay [12]

“Standard aflatoxins were applied to the surface of precoated TLC plates in amounts of 2.5 µL, 5 µL, 7.5 µL, and 10 µL. Just like this, dry off the test sample and then use an unsaturated chamber with a solvent system of chloroform, acetone, and isopropyl alcohol (85: 10: 5) to record the chromatogram. This was achieved at a distance not less than15 cm from the origin. Remove the plate from the development chamber, remove the solvent, and air dry the plate. Locate the spot on the plate by inspecting it under 365 nm UV light” [12].

3. RESULTS AND DISCUSSION

HPTLC finger printing analysis of the sample reveals the presence of thirteen prominent peaks corresponds to the presence of thirteen versatile phytocomponents present within it. Rf value of the peaks ranges from 0.02 to 0.80.

Results of the present investigation have clearly shows that the sample has no traces of heavy metal such as Arsenic, Lead, Cadmium and Mercury.

A product's quality is a crucial factor, particularly in the pharmaceutical sector. Though there is a significant chance of patients' life and health could be harmed, the regulatory bodies have given extra attention and have created numerous criteria to guarantee a proper degree of quality in the medicinal preparations. Gandhaga thailam is a internal medicine to treat various ailments. As the oil form preparation is an internal medicine, standardization is essential to prove its quality. The amount of potassium hydroxide milligram’s needed to neutralise the free acids in a one-gram sample of fat or oil is known as the acid value. Thus the acid value and the free fatty acid content have a direct relationship. Acid value is an indication of rancid state. Greater the acid value, lowering the oil quality. Acid value of Gandhaga Thailam is about 0.9. Thus the lower acid value indicated the good quality of Gandhaga Thailam [15].

The degree of oil unsaturation can be inferred from the iodine value. It is useful in identifying adulterated vegetable oils [15]. “In this current study, iodine value is 12.92.a high iodine value indicates a higher risk of rancidity. Thus, in this study, a low iodine value indicates a lower percentage of unsaturated fatty acids and a lower risk of rancidity” [16].

Saponification Value of Gandhaga thailam was 189.06. The fatty acid salts won't be strong enough to extract or saponify the fat or oil, resulting in reduced soapiness and a low saponification rating [15].

Table 1. Organoleptic characters of Gandhaga Thailam

State	Liquid
Nature	Viscous
Odour	Strong Characteristic
Touch	Greasy
Flow Property	Free Flowing
Appearance	Dark Brownish

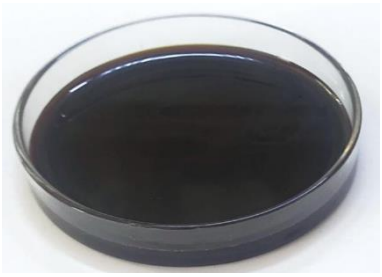


Fig. 1. Sample gandhagathailam

Table 2. Solubility profile of gandhagathailam

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

Table 3. Physico chemical of GANDHAGATHAILAM

S. No	*Parameter*	*Gandhaga Thailam*	Values as per CCRAS quality check manual [14]
1	Viscosity at 50^{o}C (Pa s)	81.99	-
2	Refractive index	1.84	1.452
3	Weight per ml (gm/ml)	0.983	-
4	Iodine value (mg I2/g)	12.92	12.12
5	Saponification Value (mg of KOH to saponify 1gm of fat)	189.06	257.2
6	Acid Value(mg KOH/g)	0.953	1.62
7	Peroxidase Value (mEq/kg)	5.22	10
8	Rancidity	Nil	-

Fig. 2. TLC Visualization of Gandhaga Thailam at 366 nm

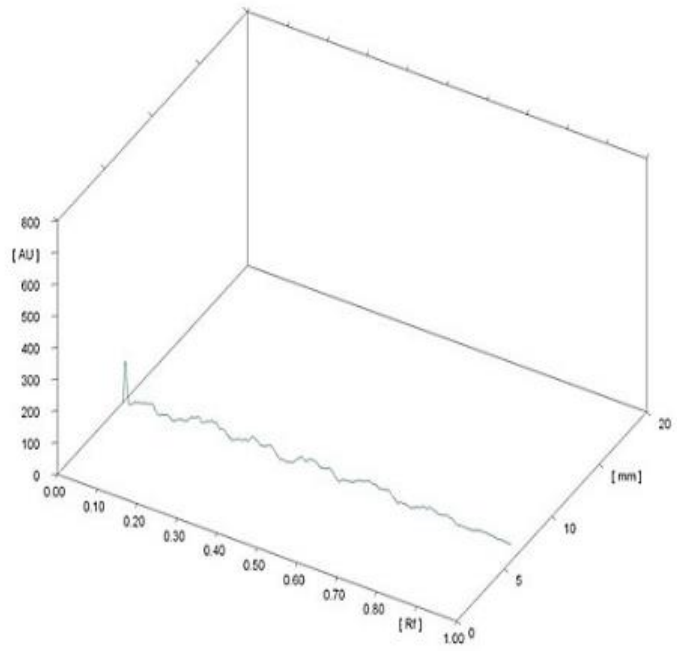


Fig. 3. Gandhaga Thailam- 3D – Chromatogram

Table 4. HPTLC- Peak table of Gandhaga Thailam

Peak	Start Rf	Start Height	Max Rf	Max Height	Max%	End Rf	End Height	Area	Area %
1	0.02	1.5	0.07	28.3	6.82	0.09	1.1	606.3	7.96
2	0.09	1.8	0.11	11.5	2.77	0.12	0.7	108.5	1.42
3	0.15	12.3	0.19	41.0	9.87	0.20	27.4	667.0	8.76
4	0.20	27.5	0.22	44.5	10.70	0.27	8.0	1166.4	15.31
5	0.28	8.7	0.32	42.0	10.12	0.35	22.1	906.7	11.90
6	0.35	24.6	0.37	33.9	8.16	0.40	2.6	506.0	6.64
7	0.42	4.3	0.45	34.8	8.39	0.46	24.7	417.6	5.48
8	0.46	26.2	0.47	37.0	8.91	0.50	22.1	609.6	8.00
9	0.50	22.3	0.51	31.9	7.67	0.53	9.9	453.0	5.95
10	0.57	12.0	0.62	37.0	8.90	0.64	24.4	1036.2	13.60
11	0.65	26.4	0.66	30.0	7.23	0.69	1.4	385.1	5.05
12	0.72	0.3	0.77	23.9	5.74	0.79	12.2	514.5	6.75
13	0.80	13.2	0.81	19.7	4.73	0.84	0.0	241.0	3.16

Table 5. Heavy metal concentration of GandhagaThailam [13]

Heavy Metals	Absorption Max λ max	Result Analysis	MaximumLimit [13]
Lead	217.0 nm	BDL	10 ppm
Arsenic	193.7 nm	BDL	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	BDL	1 ppm

BDL- Below Detection Limit

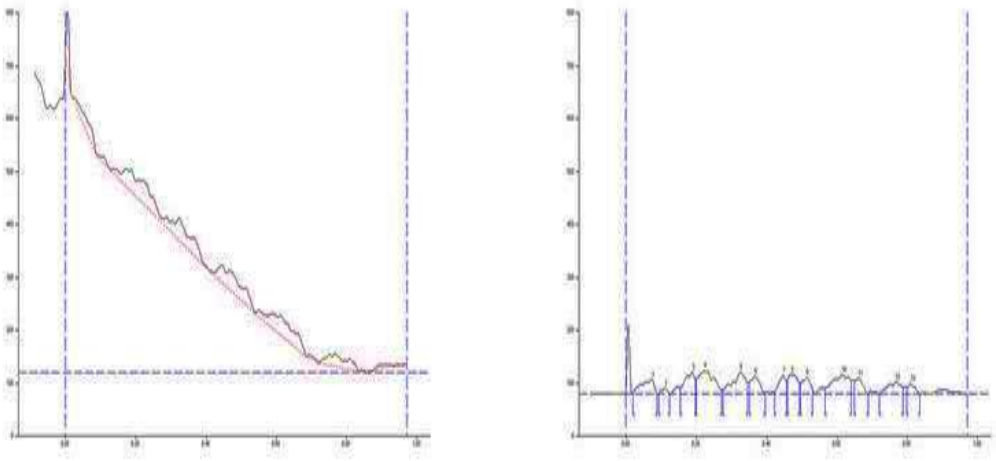


Fig. 4. HPTLC finger printing of gandhagathailam

Table 6. Microbial contamination of GandhagaThailam

Test	Result	Specification	As per AYUSH/WHO
Total Bacterial Count	Absent	NMT 10^5CFU/g	As per AYUSH
Total Fungal Count	Absent	NMT 10^3CFU/g	specification

No growth / colonies was observed in any of the plates inoculates with the GandhagaThailam samples

Table 7. Specific pathogens growth on GandhagaThailam

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

Table 8. list of the pesticide residue use for the study

Pesticide Residue	Sample Gandhaga Thailam	AYUSH Limit (mg/kg) [12]
I.Organo Chlorine Pesticides		
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	0.2 mg/kg
Dichlorovos	BQL	1mg/kg
III. Organocarbamates		
Carbofuran	BQL	0.1mg/kg
III.Pyrethroid		
Cypermethrin	BQL	1mg/kg

BQL – Below quantification limit

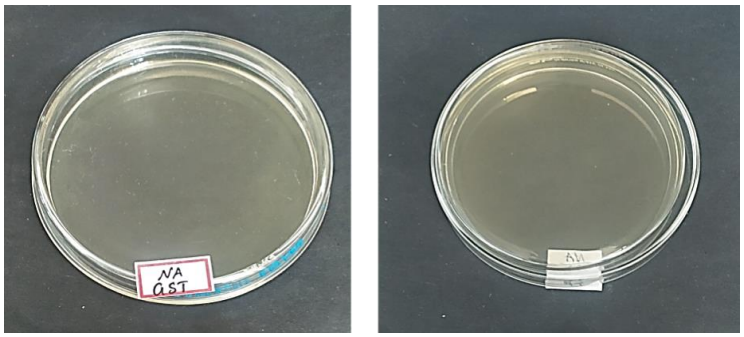
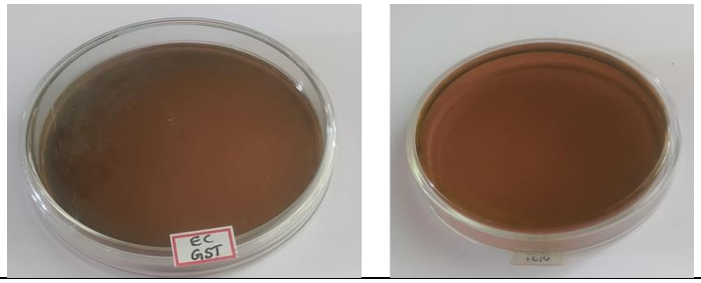


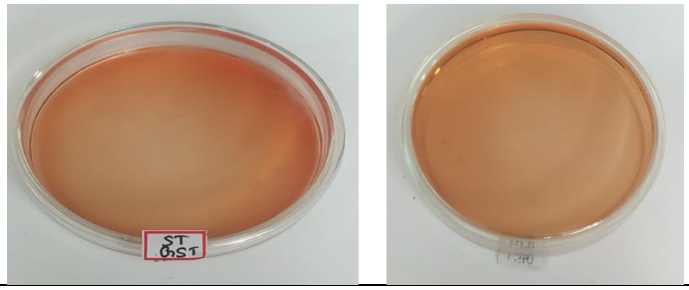
Fig. 5. GandhagaThailam Plates for Microbial contamination

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

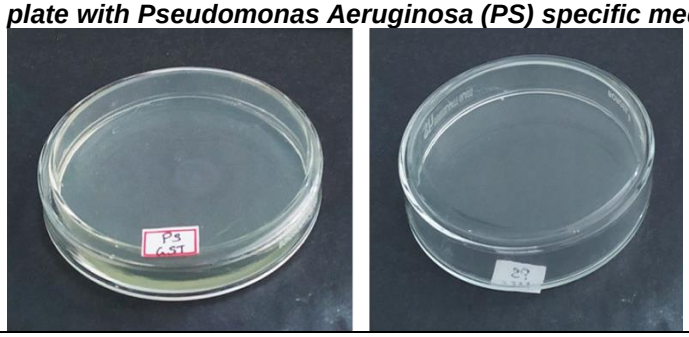


Fig. 6. Culture plate with specific pathogens

Table 9. List of Aflatoxin used for the study

Aflatoxin	Sample GT	AYUSH Specification Limit [12]
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)

The results shown that there were no spots were being identified in the Gandhaga Thailam loaded on TLC plates when compare to the standard which indicates that the sample were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1 and Aflatoxin G2.

"The peroxide value is a measure of the active oxygen in the oil and the potential to go rancid. Elevated initial peroxide values are not good. According to the SLS (Sri Lanka Organic agriculture standard) 1341: 2008 standard, upper limit of the peroxide value of a oil is 10 Meq/Kg. Peroxide value of Gandhaga Thailam is about 5.22 mEq/kg. In general, peroxide levels higher than 10 may mean less stable oil with a shorter shelf life" [17].

"HPTLC analysis reveals the presence of thirteen prominent peaks corresponds to the presence of thirteen versatile phytocomponents present within it. Rf value of the peaks ranges from 0.02 to 0.80. The ingredients of Gandhaga Sudar thailam, Gandhagam, Navacharam, Manosilai and Veeram and were purified with Milk, Cow's urine, Ginger and Tender coconut water respectively. Cow's Ghee is the base for Gandhaga thailam Preparation. Purifying agents and Base material used for the Preparation are responsible for the presence of Peaks" [18,19,20,21].

AAS analysis shows the Heavy metals are below the detection limit, it indicates the Gandhaga Thailam is safe to use for In Vivo models. Sterility study shows the absence of microbial contamination and Test for specific pathogens shows absence of *E. coli*, *Salmonella*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, it indicates the purification and preparatory process done hygienically. Organo Chlorines, Organo Phosphorus, Organo carbamates and Pyrethroids were below quantification limit, and Gandhaga thailam was also free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1 and Aflatoxin G2. Results reveals that Gandhaga thailam is hygienically prepared as per the Siddha literature. Hence the quality of Gandhaga thailam was standard and can be use for further toxicity studies and efficacy studies [22].

4. CONCLUSION

Study indicates that the Gandhaga Thailam is in standard quality, hence the preparation and purification processes of ingredients were done as per the literature. With these results Gandhaga thailam may be subjected to invitro and invivo toxicity studies in future. Results reveals the quality of the Medicine Gandhaga Thailam and may be the fingerprint for the future analysis on Gandhaga Thailam.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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