

Original Research Article

Standardization of GandhagaThailam - A Traditional Siddha formulation for skin disorders

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

Keywords: Pharmacology, Traditional medicine, GandhagaThailam, Siddha, Standardization

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. Gandhagathailam 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 Sudarthailam method. Hence it is also called as GandhagaSudar thailam.³ Gandhagathailam is a popular medicine practiced mostly in Tamil Nadu state, meanwhile the quality of Gandhagathailam 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 thailampreparations. Aim of the study is to identify the quality of Gandhagathailam by applying PLIM guidelines.

Materials and Methods

I 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 SudarThailam Procedure.⁸ Dosage is about 488mg , Twice a day for 10 days.

II 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.

III 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.

IV 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 .

V 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.

VI Test for Specific Pathogen:¹²

Sample was directly inoculated in to the specific pathogen medium (EMB, DCC, Mannitol, Cetrinide) using pour plate method. 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.

VII 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.

VIII 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 than 15 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.

Results

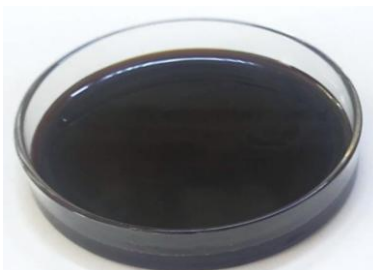
II a) Organoleptic characters:

Table 1: Organoleptic characters of GandhagaThailam

State	Liquid
Nature	Viscous

Odour	Strong Characteristic
Touch	Greasy
Flow Property	Free Flowing
Appearance	Dark Brownish

Figure 1: Sample GandhagaThailam



II b) Solubility Profile:

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

II b) Physico chemical parameters:

Table 3: Physico chemical of GandhagaThailam

S.No	Parameter	Gandhaga Thailam	Values as per CCRAS quality check manual ¹⁴
1	Viscosity at 50°C (Pa s)	81.99	-
2	Refractive index	1.84	1.452
3	Weight per ml (gm/ml)	0.983	-

4	Iodine value (mg I ₂ /g)	12.92	12.12
5	Saponification Value (mg of KOH to saponify 1 gm 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	-

III TLC/HPTLC:

Figure 2: TLC Visualization of GandhagaThailam at 366 nm



Figure 3: GandhagaThailam- 3D – Chromatogram

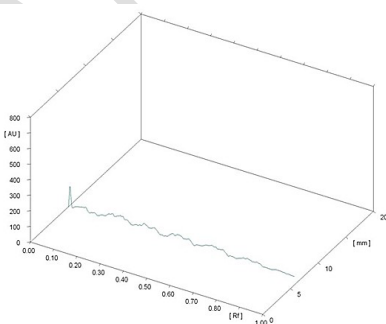
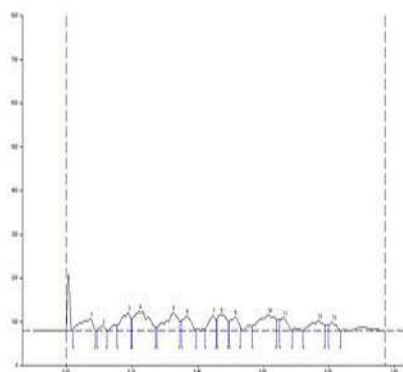
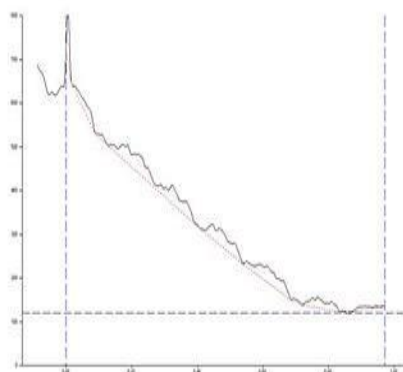


Figure 4: HPTLC Finger printing of GandhagaThailam

Table
Peak



4: HPTLC-
table of

GandhagaThailam

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

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

IV AAS Analysis:

Table 5: Heavy metal concentration of GandhagaThailam

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

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

V Sterility Test:

Figure 5: GandhagaThailam Plates for Microbial contamination

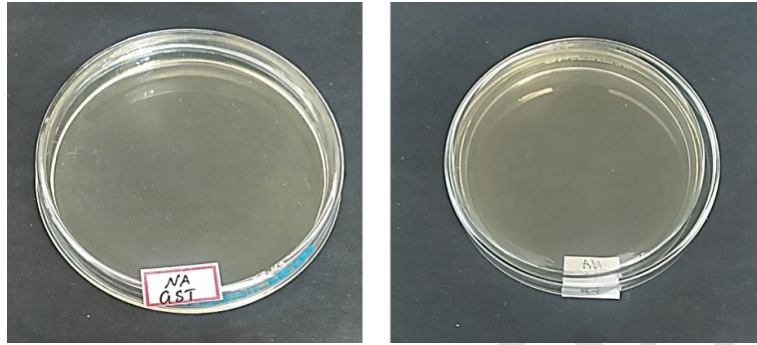


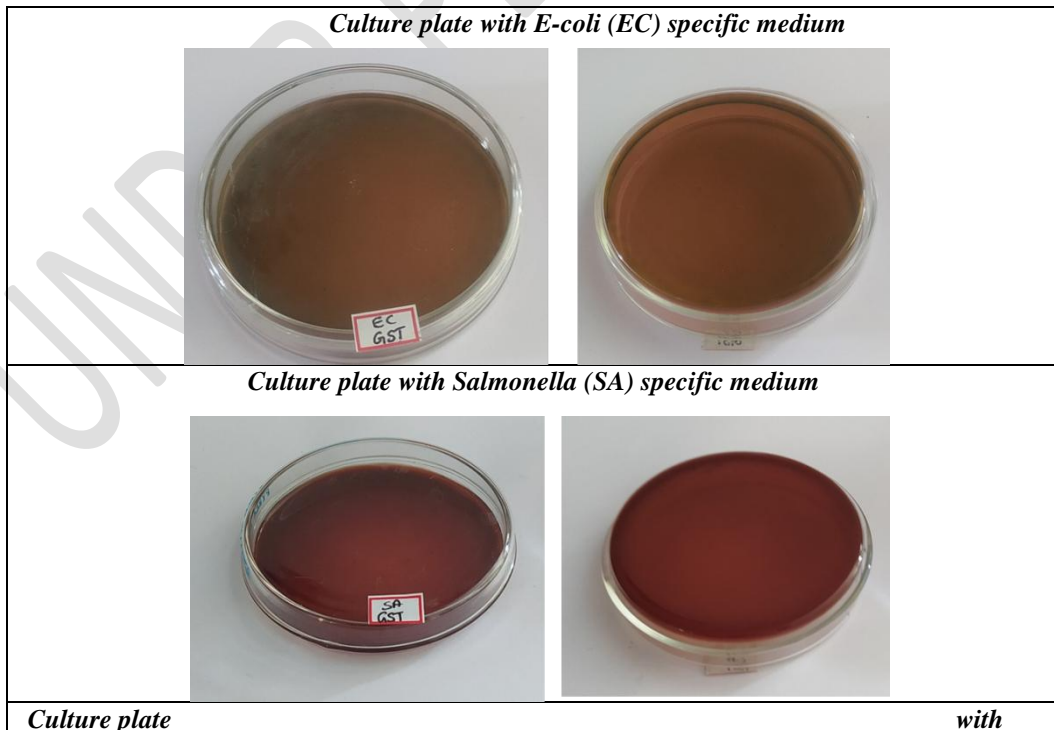
Table 6: Microbial contamination of GandhagaThailam

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

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

VI Test for Specific Pathogens:

Figure 6: Culture plate with specific pathogens



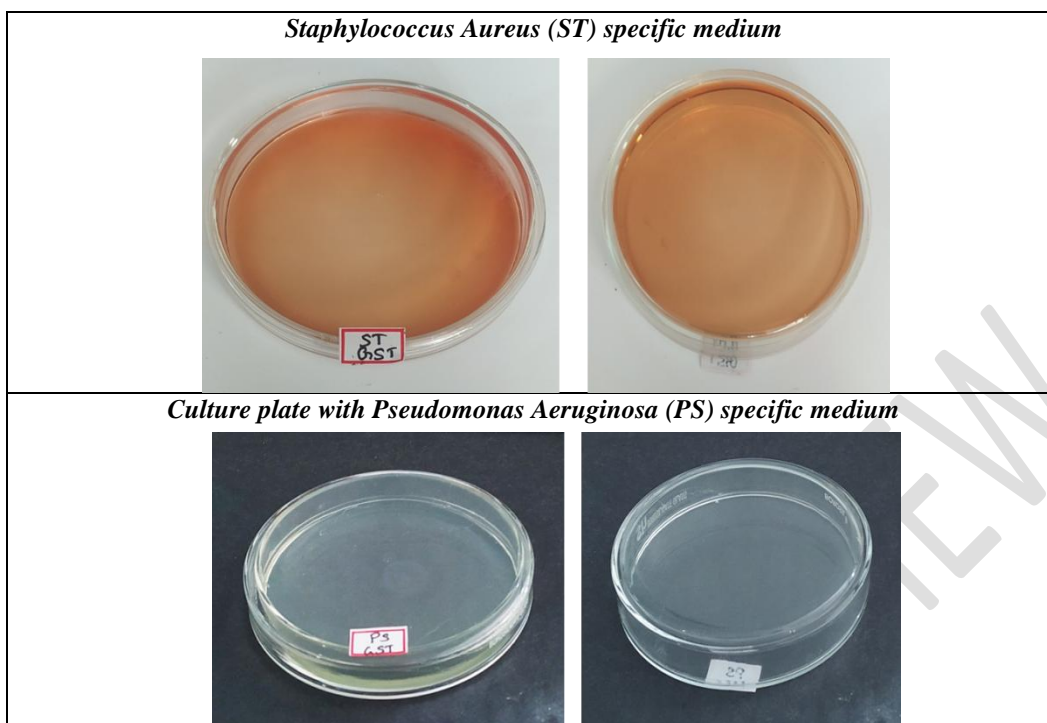


Table 7: Specific pathogens growth on GandhagaThailam

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

VII Pesticide Residue:

Table 8 : list of the Pesticide Residue use for the study

Pesticide Residue	Sample GandhagaThailam	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

VIII Aflatoxin:

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 GandhagaThailam 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.

Discussion

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. Gandhagathailam isa 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 GandhagaThailam 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 Gandhagathailam 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

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 GandhagaThailam 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 GandhagaSudarthailam, 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 Gandhagathailam 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 GandhagaThailam 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, OrganoPhosphorus, Organocarbamates and Pyrethroids were below quantification limit, and Gandhagathailam was also free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1 and Aflatoxin G2. Results reveals that Gandhagathailam is hygienically prepared as per the Siddha literature. Hence the quality of Gandhagathailam was standard and can be use for further toxicity studies and efficacy studies.

Conclusion

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

Reference

1. Deore et al., The Stages of Drug Discovery and Development Process; Asian Journal of Pharmaceutical Research and Development; 2019: 7(6): P.no:62-67.

2. AgasthiarParipooranam 400; Author:R.M.K.Thirumalaisamypillai; Published in:1994; Page no.155,156.
3. Siddha MateriaMedica (Mineral and Animal Kingdom). Published in: 2008, Dr.AnaivaariR.Anandan, First Edition, Page no.252.
4. Siddha MateriaMedica (Mineral and Animal Kingdom). Published in: 2008, Dr.AnaivaariR.Anandan, First Edition, Page no. 363-364.
5. Siddha MateriaMedica (Mineral and Animal Kingdom). Published in: 2008, Dr.AnaivaariR.Anandan, First Edition, Page no.286.
6. Siddha MateriaMedica (Mineral and Animal Kingdom). Published in: 2008, Dr.AnaivaariR.Anandan, First Edition, Page no. 336.
7. Siddha MateriaMedica (Mineral and Animal Kingdom). Published in: 2008, Dr.AnaivaariR.Anandan, First Edition, Page no. 240.
8. Siddha MateriaMedica (Mineral and Animal Kingdom). Published in: 2008, Dr.AnaivaariR.Anandan, First Edition, Page no.36.
9. CCRS_Guidline of Drug_Book-7_CD MATTER.pdf (ccras.nic.in)
10. India Pharmacopeia I Volume I, Government of India, Ministry of Health and Family welfare, Indian Pharmacopeia commission, 2014.
11. Indian standard methods of sampling and test for oils and fats Indian standard institution New Delhi 47-50. 1964.
12. K. Rajeswari et al. Physicochemical Standardisation Of Siddha Tablet Preparation JathikaaiMathirai; Int. J. Res. Ayurveda Pharm;12 (4), 2021,Page no:87-93.
13. Parvathy Mohan et al, Pharmacopoeial Standards Of Snehakalpana; JETIR, December 2020, Volume 7, Issue 12: P.No 80-82
14. R. Arunadevi et al.Preparation and standardization of MathanTailam: A classical Siddha formulation for diabetic ulcerative wound healing; Journal of Ayurveda and Integrative Medicine; 2020: 11: p.no10-15
15. Great Iruoghene Edo et al. Physicochemical and Pharmacological Properties of Palm Oil: an Approach for Quality, Safety, and Nutrition Evaluation of Palm Oil; n Food Analytical Methods;2022:April. DOI: 10.1007/s12161-022-02293-4

16. Hassan F, Edo GI, Nwosu LC et al (2021) An inventory of medicinal plants used as sedative, analgesic and blood tonic in Abeokuta, Ogun State. Nigeria ActaEcol Sin. <https://doi.org/10.1016/j.chnaes.2021.11.003>
17. Hewageegana HGSP et al, Standardization of Vipadikaharaghrithaila: An Ayurvedic medicated oil for common skin lesions; Unique journal of Ayurvedic and Herbal Medicines; 2013, 01(01); P.no 48-51.
18. M.M Srivastava et al., Identification and quantification of amino acids in coconut water using high performance thin layer chromatographic method; Analytical Chemistry an Indian Journal; 2011; 10(7): P.no 440-443.
19. Roopa Rani et al., HPTLC-MS analysis of melamine in milk: standardization and validation; Dairy Sci. & Technol. (2015) 95:257–263. DOI 10.1007/s13594-014-0204-3.
20. Prawez Alam, Densitometric HPTLC analysis of 8-gingerol in Zingiber officinale extract and ginger-containing dietary supplements, teas and commercial creams; Asian Pacific Journal of Tropical Biomedicine; 2013; 3(8): 634-638. doi:10.1016/S2221-1691(13)60128-8.
21. Syed Shariq Mian et al., Physicochemical Analysis of Ginger (Zingiber officinale Rosc.) Rhizome along with its TLC, HPLC and HPTLC Profile; Pharm Methods, 2019; 10(1): 31-36. DOI : 10.5530/phm.2019.1.6