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ANALYTICAL STANDARDIZATION OF *GANDHAGARASAYANAM- ASIDDHA* POLYHERBO MINERAL FORMULATION

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ABSTRACT

Gandhaga Rasayanam is a *Siddha*drug widely used in the treatment of skin diseases. There are multiple methods and various herbo-mineral combinations abundantin *Siddha* texts to prepare *Gandhaga rasayanam*. The scientific standardization of traditional preparations is essential to ensure their quality, safety and efficacy. The present study aims to evaluate the physiochemical parameters of *Gandhaga rasayanam* prepared by, as per the reference *Anuboga Vaidya Navaneedham- part 6*. It was then subjected to physiochemical and phytochemical evaluation,tests for microbial load, aflatoxins and pesticide residuesas per AYUSH guidelines. The formulation exhibited the presence of characteristic bioactive ingredients in HPTLC profiling and phytochemical evaluation. The safety of this drug is assessed by screening microbial load, identification of aflatoxins and detection of pesticide levels. Thus, the study provides scientific evidence supporting the quality, efficacy, and standardization of the formulation highlighting its potential for further clinical and pharmacological investigations.

Keywords: *Gandhaga rasayanam, Siddha formulation, HPTLC profiling, physicochemical analysis, poly herbo mineral formulation.*

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INTRODUCTION

Siddha system is considered as the mother medicine of ancient Tamils of Indian peninsula [1]. The system employs the use of eight methods of examination *(En Vakai Thervu)* to diagnose the diseases. The concept of *Siddha* medicine deals with the synchronization of three humoral forces *(Mukkutram)* that lies in our body [2]. Five primordial elements of the universe are the basic substances for 3 humors and also for 6 tastes. When the vitiation of the three humors is rectified with herbals or minerals a cure will be attained. Though herbs are fundamental substances in *Siddha* formulations, the evolution caused the use of minerals too for notorious ailments [3]. The synergy of herbs with minerals as herbo-mineral formulations are used for diseases like psoriasis, eczema, alopecia, diabetic ulcer, warts, vitiligo, pemphigus, leprosy etc [4]. One such widely used formulation is *Gandhaga rasayanam*(GRM). *Siddha* literatures mention many formulations under the same name *Gandhaga rasayanam*, each with different ingredients in differing proportions. This study is the analytical standardization of *Gandhaga Rasayanam* (GRM), a herbo-mineral drug mentioned in *Anuboga Vaidya Navaneetham- part 6*indicated for Hemorrhoids, Uterine disorders, Abdominal colic, Anemia, Jaundice, Diabetic Ulcers, and digestive impairment [5]. The aim of the study is to assess the phytochemical, physicochemical, HPTLC (High Performance Thin Layer Chromatography) profiling, microbial load, pesticide residue, aflatoxin levels of GRM.

MATERIALSANDMETHODS

The raw materials were procured from an esteemed raw drug store, Broadway, Chennai and they were authenticated by the experts of Post Graduate department of *Siddha* Pharmacology at Government Siddha Medical College, Chennai. Specimen samples

had been stored with the corresponding register numbers for future reference. 10 *Palam* (350 g) of *Nellikai Gandhagam*(Sulphur) was purified in the steam of milk, fermented rice water, pumpkin juice (*Benincasa hispida*) separately (*aavi pudam/ dhooma pudam*) as per thereference text. All the other ingredients with their ratio of weight as in table 1 werepurified as per the text *Sarakku suthi sei muraigal [*6]. All ingredients were powdered into a *chooranam*. The prepared chooranam (powder) was purified with *pittaviyal* method as mentioned in *Gunapadam Thadhu Jeeva vaguppu [*7]. It was then ground in a stone mortar- pestle for 6 hours with 100 ml of cow's ghee till it attains the desired consistency. The prepared*Gandhaga rasayanam* was stored in an airtight container.

Table 01: Ingredients of *Gandhaga rasayanam* (GRM)

SNO	DRUG	LATIN NAME	PART USED	PROPORTION
01	*Nellikai gandhagam*	*Sulphur*	Mineral	350 g
02	*Jadhikai*	*Myristica fragrans*	Dried seed	17.5 g
03	*Lavangam*	*Syzygium aromaticum*	Flower bud	17.5 g
04	*Elarisi*	*Eletteria cardomomum*	Dried seed	17.5 g
05	*Vaaluzhuvai arisi*	*Celastrus paniculatus*	Dried Seed	17.5 g
06	*Chitrarathai*	*Alpinia officinarum*	Rhizome	17.5 g
07	*Vaividangam*	*Embelia ribes*	Dried Seed	17.5 g
08	*Kodiveli verpattai*	*Plumbago zeylanica*	Root bark	17.5 g
09	*Sukku*	*Zingiber officinale*	Dried rhizome	17.5 g
10	*Thippili*	*Piper longum*	Dried fruit	17.5 g
11	*Milagu*	*Piper nigrum*	Dried fruit	17.5 g
12	*Adhimadhuram*	*Glycyrrhiza glabra*	Dried root	17.5 g
13	*Nei*	*Cow's ghee*		100 ml

Physicochemical analysis

The physicochemical analysis of GRM was conducted at Siddha Central Research Institute, Chennai 106. The total ash, acid insoluble ash, Water soluble extractive, Alcohol soluble extractive, fat content, pH parameters were analyzed. All the above parameters were carried out as per the standard test procedures [8].

Phytochemical analysis

High Performance Thin Layer Chromatography (HPTLC)

Sample preparation for TLC

Sample 10 ml was dissolved in 10 ml of methanol and then sonicated for 15 minutes and filtered. This solution was used for TLC [9].

TLC/ HPTLC methodology

5, 10, 15, 20 µl applied sample extract on TLC plate using Camag's ATS4 applicator and developed by the mobile phase: Toluene: Ethylacetate: Acetic acid (8;2;0.5, v/v) up to 8 cm distance.After development, the plate was photo documented using Camag's TLC visualizer under UV 254 nm and UV 366 nm. Scanned the plate using Camag's scanner 4 at UV 254 nm (D2 lamp/ Absorption mode) finger print profiles of the extract were documented. Then the plate was dipped in vanillin- sulphuric acid reagent followed by heating at 105°C till the development of coloured spots. The plate was then photo documented in white light and scanned at 520 nm (W lamp/ absorption mode). The photographs were captured, the plate was scanned and all the data were integrated. The fingerprint of each track was recorded [9,10].

Microbial Load

Microbial quality assessment, including the isolations and identification of pathogenic bacteria from both commercial and homemade herbal medicines, was conducted in accordance with WHO standards (2007) [11]. The procedure quantified the number of bacteria and fungi capable of aerobic growth in 1 g of the sample. Samples were homogenized by mixing with water. 1 g was transferred to 9 ml of peptone broth. Serial dilutions were prepared to achieve appropriate concentrations. All microbial analyses were performed in triplicate. Viability was assessed using the pour plate method on casein soyabean digest agar for bacterial counts and Sabouraud dextrose agar for fungal identification. Dehydrated media were prepared according to the manufacturer's instructions, seeded, incubated at 37°C for 24 to 48 hours for bacterial screening and at 25° C for 48 to 72 hours for fungal screening. After incubation, the number of colony forming units pergram(CFU/g) was calculated by multiplying the average colony count by the dilution factor. The resulting CFU/g values were compared with WHO standards. Samples exhibiting bacterial growth exceeding 105 CFU/g of herbal medicine were classified unsatisfactory or inadequate according to WHO guidelines for aerobic bacteria.

Identification of bacteria

For bacterial isolation and identification, samples were diluted in water to their appropriate concentrations and homogenized by vigorous mixing. Aliquots of 1 ml were transferred to 9 ml of peptone broth and cultured under recommended conditions. All microbial analysis were performed in triplicate. To investigate

Escherichia coli, Salmonella species, Pseudomonas aeruginosa, Staphylococcus aureus, EMB agar, Mac Conkey agar, Deoxycholate citrate agar, Cetrimide agar, Mannitol salt agar was used, respectively11. After incubation, pathogenic bacterial isolates were preliminarily characterized based on colony morphology, gram staining and biochemical tests, including oxidase, gas, and catalase production.

Test for Aflatoxins [12, 13].

Aflatoxins are a group of naturally occurring toxins produced by Aspergillus flavus and Aspergillus parasiticus. The Afla Test is a quantitative method for detecting aflatoxins B1, B2, G1, G2, M1, and M2. Five grams of *Gandhaga rasayanam*(GRM) and 0.4 g of sodium chloride were mixed with methanol: 2% Tween 20 or phosphate buffer (60:40 v/v). The extract mixture was vortexed at high speed for 3 minutes. The extract was filtered through fluted filter paper. 10 ml of filtered extract was added to the measuring cylinder. To that, 20 mL of purified water was added, and the mixture was vortexed on high for 1 minute. Then the diluted extract was filtered through a prewetted glass microfiber filter (1.5 µm). 10 mL of diluted extract was passed through the Afla Test WB column. Pressure was applied to get 1-2 drops per second. The column was washed with 10mL 2% Tween 20, followed by 10 mL purified water twice. The Afla Test WB columns were eluted by passing 1 mL HPLC-grade methanol (100%) through the column, and pressure was applied to get 1 drop per second. Eluate was collected in a sterile VICAM cuvette. To that, 1.0 mL of Afla Test Developer was added and mixed well, then immediately placed in the fluorometer (VICAM fluorometer-series 4EX). The concentration was read by the fluorometer after 60 seconds.

Test for Pesticide residue [14].

The test sample was 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 40° C until the solvent has almost completely evaporated. To the residue a few millimetres of toluene was added and it was heated again until the acetone was completely removed. Resultant residue was dissolved using toluene and filtered through membrane filter. The values obtained were noted and tabulated.

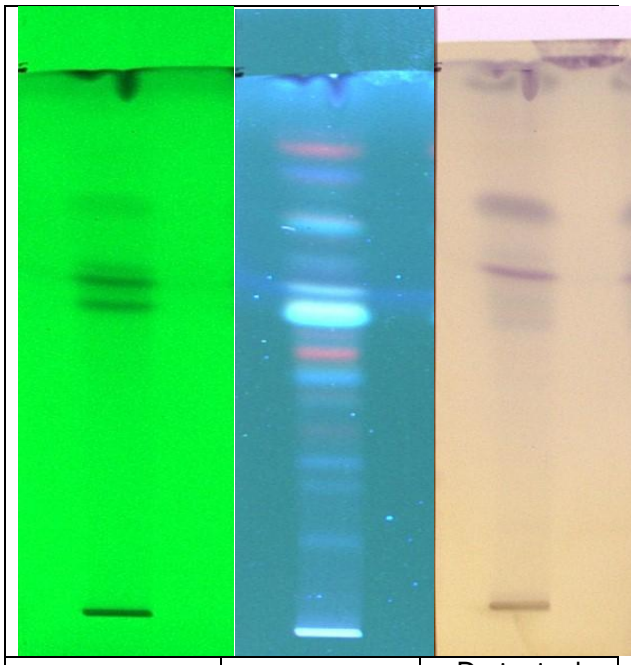
RESULTS

Table 02: Physicochemical parameters

S NO	PARAMETER	I	II	MEAN
1	Loss on drying	2.92%	2.49%	2.70%
2	Total Ash	2.45%	2.34%	2.39%
3	Acid insoluble ash	0.55%	0.45%	0.50%
4	Water soluble extractives	4.87%	5.11%	4.99%
5	Alcohol soluble extractives	9.62%	9.85%	9.74%
6	Fat content	49.44%	49.31%	49.37%
7	pH	5.07	5.07	5.07

Table 03: Phytochemical analysis

S NO	PHYTOCHEMICAL TEST	RESULT
1	Phenols	Present
2	Tannins	Present
3	Saponins	Absent
4	Flavonoids	Absent
5	Protein	Present
6	Cardiac glycosides	Absent
7	Glycosides	Absent
8	Alkaloid	Absent
9	Quinones	Present
10	Steroids	Present
11	Triterpenes	Present
12	Anthraquinones	Absent
13	Coumarins	Absent
14	Acids	Absent
15	Reducing sugar	Absent



UV 254 nm	UV 366 nm	Derivatized plate

Figure 01: TLC Photo documentation

Table 04: Rf Values and Spot Colors

UV 254 nm		UV 366 nm		Derivatized plate	
Rf value	Color	Rf value	Color	Rf value	Color
0,57	Green	0.16	Blue	0.52	Purple
0.61	Green	0.26	Blue	0.57	Purple
0.64	Green	0.31	Blue	0.62	Purple
0.75	Green	0.36	Blue	0.74	Purple
0.94	Green	0.45	Blue	0.97	Purple
		0.50	Red		
		0.57	Aqua		
		0.62	Aqua		
		0.66	Blue		
		0.74	Aqua		
		0.82	Blue		
		0.87	Red		
		0.97	Blue		

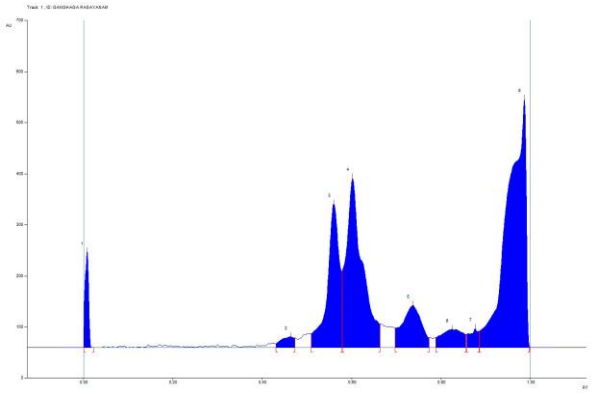


Figure 02: HPTLC Fingerprint profiling at UV 254 nm

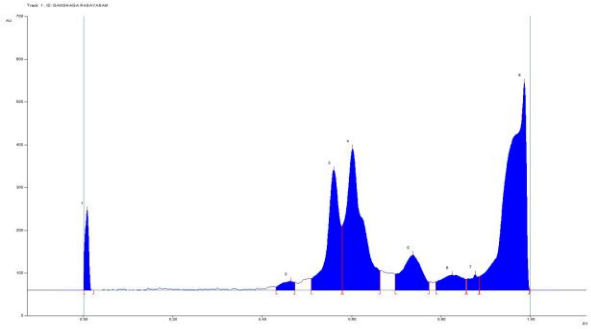


Figure 03: Peak table of GRM at UV 254 nm

Table 05: HPTLC Fingerprint profiling at UV 520nm

Track 1, ID: GANGHAGA RASAYANAM

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.01 Rf	186.7 AU	12.77 %	0.02 Rf	0.0 AU	1419.7 AU	3.23 %
2	0.43 Rf	7.2 AU	0.46 Rf	21.2 AU	1.45 %	0.47 Rf	18.5 AU	583.0 AU	1.33 %
3	0.51 Rf	27.7 AU	0.56 Rf	281.2 AU	19.23 %	0.58 Rf	48.9 AU	6991.7 AU	15.91 %
4	0.58 Rf	150.8 AU	0.60 Rf	331.6 AU	22.67 %	0.66 Rf	46.1 AU	11046.0 AU	25.14 %
5	0.70 Rf	37.9 AU	0.74 Rf	83.0 AU	5.67 %	0.77 Rf	19.1 AU	3305.1 AU	7.52 %
6	0.79 Rf	19.3 AU	0.82 Rf	35.5 AU	2.43 %	0.86 Rf	25.8 AU	1629.9 AU	3.71 %
7	0.86 Rf	26.2 AU	0.88 Rf	37.1 AU	2.54 %	0.88 Rf	32.1 AU	685.2 AU	1.56 %
8	0.89 Rf	32.5 AU	0.99 Rf	486.1 AU	33.24 %	1.00 Rf	3.8 AU	18282.9 AU	41.61 %

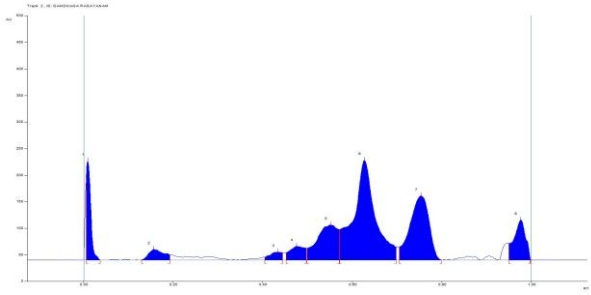


Figure 04: Peak table of GRM at UV 520 nm

Table 06: HPTLC Peak Data

Track 2, ID: GANGHAGA RASAYANAM

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	152.1 AU	0.01 Rf	186.8 AU	26.70 %	0.04 Rf	0.1 AU	1804.5 AU	8.37 %
2	0.13 Rf	0.6 AU	0.16 Rf	20.6 AU	2.95 %	0.19 Rf	10.0 AU	651.3 AU	3.02 %
3	0.41 Rf	5.4 AU	0.44 Rf	15.1 AU	2.16 %	0.44 Rf	13.7 AU	391.2 AU	1.81 %
4	0.45 Rf	13.9 AU	0.48 Rf	25.9 AU	3.71 %	0.50 Rf	22.6 AU	809.0 AU	3.75 %
5	0.50 Rf	22.2 AU	0.55 Rf	66.3 AU	9.48 %	0.57 Rf	57.7 AU	2855.1 AU	13.24 %
6	0.57 Rf	57.7 AU	0.63 Rf	188.2 AU	26.91 %	0.70 Rf	24.7 AU	8455.7 AU	39.21 %
7	0.70 Rf	24.4 AU	0.75 Rf	120.7 AU	17.25 %	0.80 Rf	0.0 AU	4782.2 AU	22.18 %
8	0.95 Rf	31.8 AU	0.98 Rf	75.8 AU	10.83 %	1.00 Rf	3.9 AU	1816.3 AU	8.42 %

Figure

Table 07: Microbial Load

SNO	PARAMETERS	RESULTS
1	Total bacterial Count (TBC)	4 x 10^5 cfu/g
2	Total yeast and mould count	Less than 10 cfu/g
3	Escherichia coli	Absent
4	Salmonella sp.	Absent
5	Staphylococcus aureus	Absent
6	Pseudomonas aeruginosa	Absent

Table 08: Test for aflatoxins

S NO	PARAMETERS	RESULTS
1	Total Aflatoxin B1+B2+G1+G2	BQL (Below Quantification Limit)

Table 09: Pesticide Residue

S NO	SUBSTANCE	RESULTS	PERMISSIBLE LIMIT PRESCRIBED IN API (IN PPB)
1	Alachlor	2.231	20
2	Aldrin and dieldrin	5.467	50
3	Chlordane (Sum of cis-trans- and oxychlordane)	Not detected	50
4	Chlorfenvinphos	Not detected	500
5	Chlorpyrifos	3.317	200
6	Cypermethrin	205.915	1000

	(and isomers)		
7	DDT	Not detected	1000
8	Deltamethrin	5.186	500
9	Diazinon	Not detected	500
10	Ethion	120.134	2000
11	Fenitrothion	2.334	500
12	Fenvalerate	270.372	1500
13	Hexachloroben zene	Not detected	100
14	Malathion	14.127	1000
15	Permethrin	445.088	1000
16	Piperonyl butoxide	63.299	4000

DISCUSSION

The loss on drying value of 2.70% suggests good stability and reduced microbial susceptibility. The total ash and acid-insoluble ash values are within the permissible range for Herbo mineral formulations. The acid-insoluble ash value of 0.50% indicates minimal silica, sand-like impurities and low inorganic impurities. The pH value is 5.07, weakly acidic in nature that may aid in maintaining stability and facilitate digestion and absorption. The water-extractive value of4.99% indicates good water solubility and the alcohol soluble extractive of 9.74%. Higher alcohol extractive suggests the presence of moderately polar bioactive substances. Fat content of 49.37% indicates lipid rich composition of the drug that could significantly contribute to drug absorption and bioavailability. Preliminary phytochemical analysis of *Gandhaga rasayanam* revealed phenolic compounds, tannins, proteins, quinones, steroids, triterpenes. Therefore, the drug GRM may exhibit anti-oxidant, antimicrobial, anti-inflammatory and immunomodulatory activity and restorative properties. HPTLC analysis showed 5 spots at 254 nm-peaks at Rf 0.57, 0.61, 0.64, 0.75, 0.94 indicating the presence of phenolics and quinones, 13 at 366 nm-peaks at Rf 0.16, 0.26, 0.31, 0.36, 0.45 indicating the presence of aromatic compounds, terpenoids, After derivatization at 520 nm, major spots at Rf 0.52, 0.57, 0.62, 0.74, 0.97 with vanillin- sulphuric acid reagent confirms terpenoids, steroidal compounds, and other complex organic compounds. Total bacterial and fungal counts were below permissible limits suggesting stability of the drug. The total aflatoxin level was also below the WHO reference limit [6]. (WHO Limits: 0.5µg/kg to 15µg/kg (ppb)). Pesticide residues were also within normal limits as prescribed by API.

CONCLUSION

This study presents a comprehensive evaluation of *Gandhaga rasayanam*using microbial, physicochemical, phytochemical and chromatographic analysis. Microbial load, pesticide residue and aflatoxin level assessments confirmed the formulation's safety, while the physicochemical parameters indicated acceptable quality and stability parameters. Phytochemical screening and HPTLC profiling revealed the presence of bioactive components. These findings provide scientific support for traditional claims and contribute to the standardization of the formulation. Overall, the results highlight the potentiality for safe therapeutic use and establish a baseline for further pharmacological and clinical investigations.

CONFLICT OF INTEREST

Nil

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Self

AUTHORCONTRIBUTION

Dr. Pushpa collected, analyzed the data and prepared the manuscript. Dr. Shankar supervised the project, interpreted the data and reviewed the final version.

REFERENCES

1. Ministry of AYUSH. Siddha System of Medicine. New Delhi: Ministry of AYUSH, Government of India; 2016.
2. Siddha Maruthuvanga Churukkam. Chennai: Department of Indian Medicine and Homeopathy; 2005.
3. Koppula Suresh, M. Elango. Traditional and medicinal uses of herbo-mineral formulations in Siddha system. *J Pharm Sci Res.* 2018;10(5):1120-1124.
4. Joseph Thas J. Siddha medicine—background and principles and the application for skin diseases. *Clin Dermatol.* 2008;26(1):62–78. Doi: 10.1016/j.clindermatol.2007.11.010.
5. Muhammad Abdullah Sahib P. *Anuboga Vaidya Navaneetham.* Part 6. Tamil Nadu: Arulmigu Palani Dhandayuthapani Swamy Thirukovil Siddha Medical Manuscripts Publication Committee; 1978.
6. Editor, *Aanaivari Ananthan, Sarakku suthi seimuraigal*, Directorate of Indian medicine and Homeopathy, Chennai. 2008
7. Thiyagarajan IR, *Gunapadam: Thathu- Jeeva vaguppu* (Part II). Chennai: Indian Medicine and Homeopathy Department; 2009.
8. Lohar DR. Protocol for testing of Ayurvedic, Siddha and Unani medicines. Ghaziabad: Government of India, Department of AYUSH, Ministry of Health & Family Welfare, Pharmacopoeial Laboratory for Indian Medicines; 2008; p.21, 40-47.
9. CAMAG. *HPTLC method development and documentation*. Muttenz: CAMAG; 2015.
10. Harborne JB. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd ed. London: Chapman & Hall; 1998.

11. World Health Organization. *WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues*. Geneva: World Health Organization; 2007.
12. Indian Pharmacopoeia. *Indian Pharmacopoeia*. Vol. 1. New Delhi: Government of India, Ministry of Health and Family Welfare; 2018.
13. AOAC International. *Official Methods of Analysis of AOAC International*. 20th ed. Gaithersburg: AOAC International; 2016.
14. World Health Organization, Quality Control Methods for Medicinal Plant Materials, Firstedition, Geneva, World Health Organization, 1998. ISSN: 9241545100