

An Evaluation of Physicochemical and Phytochemical Analysis of SIDDHA Poly-herbo-mineral Formulation Gandhaga Rasayanam

Dr Zeenath Roshan Ara ^{*1}, Dr. M. Thiruthani ²

1.PG Scholar, Department of PG Nanju Maruthuvam, 2. Head of the Department of Nanju Maruthuvam, Government Siddha Medical College, Palayamkottai, Tirunelveli.

ABSTRACT

Aim and Objective- The aim of the study is to do physicochemical analysis and preliminary phytochemistry for the drug.

Material and Methods- The drug is prepared as per the method mentioned in the classic Siddha literature Siddha Vaithiya Thirattu and in the text Gunapadam Thathujeeva vaghuppu. The drug is subjected to physicochemical analysis such as total ash, loss on drying, total sugar, reducing sugar, fat content, water soluble ash, acid insoluble ash, water soluble extractive, alcohol soluble extractive, microbial load, heavy metal analysis, and preliminary phytochemistry for the drug as per the pharmacopeial laboratory standards of Indian medicine.

Result -The drug is free of microbial contamination, the heavy metal such as lead arsenic, mercury, and cadmium are below detectable limit. Alfa toxin are also below the detectable limit. The total ash value is below 3%. Phytochemical analysis shows positive for the presence of alkaloids, tannin, phenols, steroids, flavonoids, carbohydrate etc.

Conclusion- Thus the preliminary chemical analysis of a Siddha drug will give fingerprints to the clinical studies.

KEYWORDS

Gandhaga Rasayanam, Siddha polyherbal formulation, Standardization, Morphological evaluation, Physicochemical evaluation.

INTRODUCTION

Siddha system is the foremost of all medicinal system and it is practiced in South India, especially in Tamil Nadu and Kerala. It is also called as Dravidian's System of medicine, since it is evolved along with Dravidian's culture. Siddhars' are the founder of the system of medicine possessed siddhic powers (supernatural powers). They have left their imprints in many disciplines like medicine, alchemy, philosophy, yoga, Varma and other external therapies]. They persist preparation of medicines, adjuvant, alchemy, kayakalpam and astrology.

Siddha system is a holistic science which aims at treatment of various infirmities of the body, mind and soul. Siddha treatment consists of preparation of herbs, minerals and metals in the form of decoction, paste, kizhi, powder, chenduram, chunnam, kattu, kazhanghu etc. As per Siddha texts, the medicine has been divided into 32 types of internal medicine and 32 types of external medicine.

Gandhaga Rasayanam, is one of the types of internal medicine. Till date, most of the Siddha formulations are lacking in the quality control parameters and method of it's evaluation. Each traditional system of medicine has their own type of standardization for assuring quality most in human linguistic term and this method of evaluation must be taken into consideration in standardization of Gandhaga Rasayanam – internal medicine. Standardization is an important step for the establishment of a consistent biological activity. A consistent chemical profile or a simple quality assurance program for production and manufacturing of herbal drugs.

However, an attempt was made to standardize the Siddha poly herbo mineral formulation of Gandhaga Rasayanam through scientific method of physicochemical and phytochemical screening.

1. AIM and OBJECTIVE

The aim of the study is to do physicochemical analysis and preliminary phytochemistry for the drug.

2. MATERIAL AND METHODS

A – Drug Selection and Reference

Gandhaga Rasayanam is a classical Siddha herbo mineral formulation mentioned in Siddha Vaithiya Thirattu and in the text Gunapadam Thathujeeva vaghuppu.

B – Ingredients of Gandhaga Rasayanam

Honey, sugar, and ghee are one of the ingredients used for the preparation in the required quantities and below table includes other ingredients used for the preparation of the Gandhaga Rasayanam.

Table 2.1 – Ingredients of Gandhaga Rasayanam

S. NO	DRUG	BOTANICAL NAME	FAMILY	PART USED	QUANTITY
1	Sulphur	Sulphur			10 parts (350 g)
2	Ammukura	Withania Somnifera (L.) Dunal	Solanaceae	Root	5 parts (175 g)
3	Parangi Pattai	Smilax Sp	Liliaceae	Root	2 parts (70 g)
4	Kaddukai	Terminalia Chebula Retz.	Combretaceae	Dried fruit	1 part (35 g)
5	Nellikai	Phyllanthus Emblica L.	Euphorbiaceae	Dried fruit	1 part (35 g)
6	Chukku	Zingiber Officinale Roscoe	Zingiberaceae	Rhizome	1 part (35 g)
7	Tippli	Piper Longum L.	Piperaceae	Fruit	1 part (35 g)
8	Valmilaggu	Embelia tsejeriam - cottam (Roem.&Schul.)A.DC.	Primulaceae	Fruit	1 part (35 g)
9	Kodiveli	Plumbago zeylanica L..	Plumbaginaceae	Root	1 part (35 g)
10	Serangottai	Semecarpus anacardium L.f.	Anacardiaceae	Fruit	1 part (35 g)

11	Elakkai	Elettaria cardamomum (L.) Maton	Zingiberaceae	Fruit	1 part (35 g)
12	Santhanam	Santalum album L.,	Santalaceae	Stem	1 part (35 g)
13	Bengal Gram	Cicer arietinum L.,	Fabaceae	Seed	1 part (35 g)
14	Milaggu	Piper nigrum L.,	Piperaceae	Fruit	1 part (35 g)
15	Lavanga pattai	Cinnamomum verum J.Presi	Lauraceae	Stem bark	1 part (35 g)
16	Tantrikai	Terminalia balerica	Combretaceae	Fruit	1 part (35 g)

C - Collection of Raw Materials

The raw drugs which are used (Sulphur, Ammukura, Parangi pattai, Kaddukai, Nellikai, Chukku, Tippili, Valmilaggu, Kodiveli, Serangottai, Elakkai, Santhanam, Bengal gram, Milaggu, Lavanga pattai, Tantrikai) were procured from local store in Thuckalay, Kanyakumari district. Honey was purchased from the outlet of Khadi Kraft, Palayamkottai, Tirunelveli district. Nattu sakkarai was purchased from Government Co-operative Society store, Palayamkottai, Tirunelveli. Ghee was purchased from Aavin, Palayamkottai, Tirunelveli district.

D - Authentication of Raw Materials

All herbal drugs were authenticated by experts at SCRI – Siddha Central Research Institute, Chennai and the voucher specimen number has been given for reference below and mineral drugs Sulphur was authenticated by experts in Animal and Mineral Origin Drug Research Laboratory (AMDRL) of SCRI – Siddha Central Research Institute, Chennai, Tamil Nadu.

Table 2.2 – Authentication of Raw Materials

S.No	DRUG	BOTANICAL NAME	VOUCHER NO
1	Sulphur	Sulphur	SCRI/AMDRL/2019-20/ICP-OES/16
2	Ammukura	Withania Somnifera (L) Dunal	W01031923S
3	Parangi Pattai	Smilax Sp	S01031924
4	Kaddukai	Terminalia Chebula Retz.	T010310925C
5	Nellikai	Phyllanthus Emblica L.	P01031926E

6	Chukku	Zingiber Officinale Roscoe	Z01031927O
7	Tippli	Piper Longum L.	P01031928L
8	Vaividangam	Embelia tsejeriam - cottam (Roem. &Schul) A.DC.	E01031929T
9	Kodiveli	Plumbago zeylanica L..	P01031930Z
10	Serangottai	Semecarpus anacardium L.f.	S01031931A
11	Elakkai	Elettaria cardamomum (L.) Maton	E01031932C
12	Santhanam	Santalum album L.,	S01031933A
13	Bengal Gram	Cicer arietinum L.,	C01031934A
14	Milaggu	Piper nigrum L.,	P01031935N
15	Lavanga pattai	Cinnamomum verum J.Presi	C01031936V
16	Tantrikai	Terminalia balerica	T01031937B

E - Purification of Raw drugs

The purification protocol was performed as per the description in the classical Siddha text.

Table 2.3: Procedure for purification of raw drugs

S.NO	DRUG	PURIFICATION METHOD	REFERENCE
1	Sulphur	Melt sulphur in a spoon with butter. Pour into cow's milk. Repeat this method for a total of 30 times. Wash in water and dry it.	Process from Thathujeevam and Gunapadam
2	Ammukura	Cut the drug into pieces and put in a pot. Add equal volume of cow milk and water. Boil till 3/4th of the mixture has evaporated. Then wash the dug in cold water and dry.	Process from Gunapadam

3	Parangi Pattai	Cut the drug into pieces and put in a pot. Add equal volume of cow milk and water. Boil till 3/4th of the mixture has evaporated. Then wash the dug in cold water and remove the skin and dry.	Process from Gunapadam
4	Kaddukai	Discard the seed and collect the fruit rind alone for use.	Process from Gunapadam
5	Nellikai	Discard the seeds and take only the rind portion alone for the use.	Process from Gunapadam
6	Chukku	Remove the skin and dry.	Process from Gunapadam
7	Tippli	Clean, sorted and dried.	Process from Gunapadam
8	Vaividangam	Clean, sorted and dried.	Process from Gunapadam
9	Kodiveli	Cut into pieces, then soak in chunnambu neer (lime water) for one saamam - 3 hours. Finally washed and dried again. The procedure has to be repeated till the color of lime water becomes white.	S. Kumarasamy Acharya Vaidyaratna P A Krishnaswamy Pillai Vaidyaratna Vachnabushnan, 1929 SA Muthukrishnaswamy Publishers
10	Serangottai	Carefully cut the nuts into piece and soak for 3 days in cow dung solution. Wash with water and then with tender coconut water and dry.	Process from Gunapadam
11	Elakkai	Clean, sorted and dried.	Process from Gunapadam
12	Santhanam	Clean, sorted and dried.	Process from Gunapadam

13	Bengal Gram	Clean, sorted and dried.	Process from Gunapadam
14	Milaggu	Clean, sorted and dried.	Process from Gunapadam
15	Lavanga pattai	Clean, sorted and dried.	Process from Gunapadam
16	Tantrikai	Discard the seeds and take only the rind portion alone for the use.	Process from Gunapadam

F – Preparation of Gandhaga Rasayanam

The ingredients mentioned in the formulations are taken and cleaned. Purification of the required raw drugs are done as per the text properly. After purification, the drugs are made to dry in shade completely. Then the raw drugs are powdered separately and then sieved. Add all the powdered drug together in pestle. Then cow's ghee is added little by little and grinded well, when necessary palm jaggery, honey can be added. The mixture must be in the form of granules (sand like).

G – Dose – 1.3 to 1.9 grams (10-15 kundrimani)

H – Uses

The Gandhaga Rasayanam is useful in the treatment of venereal diseases, dysuria, dermatological diseases, anorectal diseases, ulcer, vatha and swelling.

F- Storage of The Drug

The prepared test drug was stored in a clean, air-tight glass container.

G- Standardization Parameters

The various standardization parameters organoleptic properties, physicochemical investigations, preliminary phytochemical analysis, Heavy metal analysis, microbial load analysis and aflatoxins investigations were studied.

H- Physicochemical analysis⁵

The sample is tested for the following parameters as per the guidelines followed by WHO. Loss on drying, Total ash, Water soluble ash, Acid insoluble ash, Water soluble extractive, Alcohol soluble extractive, Fat content, Reducing sugar, Total sugar, Microbial load Aflatoxins and Heavy metals.

I- Preliminary Phytochemical Tests^{6,7}

Preliminary Phytochemical tests such as test for Proteins (Biuret test), Steroids (Lieberman Burchard Test), Flavonoids (Shinoda test), Triterpenoids (Noller's Test), Phenol, Tannin, Alkaloids (Dragendorff's Test), Glycosides, Cardiac glycoside (Keller-Killani Test), Reducing sugar (Fehling's Test), Saponins were carried out.

RESULT

I ORGANOLEPTIC CHARACTERS

S. No	CHARACTERS	RESULT
1	Colour	Dark Brown
2	Odour	Characteristic
3	Taste	Sweet, Pungent
4	Consistency	Semi- solid

The characteristic odour is due to the presence of sulphur. The sweet taste and semisolid consistency are due to the addition of sugar, ghee and honey.

II a. PHYTOCHEMICAL TEST

S. NO	PHYTOCHEMICALS	RESULT
1	Carbohydrate	+
2	Protein	-
3	Alkaloids	+
4	Flavonoids	+
5	Glycosides	-
6	Terpenoid	+
7	Steroid	+
8	Phenol	+
9	Tannin	+
10	Saponin	-

The above table shows the presence of all secondary metabolites. Their presence indicates the antioxidant, antimicrobial, anti-allergic, antifungal, anti-inflammatory property of the drug. its clinical use is thus validated.

b. BIOCHEMICAL ANALYSIS

Results for Basic Radicals

S. NO	PARAMETERS	OBSERVATION	RESULT
1.	Test for Potassium	No formation of Yellow color precipitate	Negative
2.	Test for Calcium	Formation of white color precipitate	Positive
3.	Test for Magnesium	No formation of white color precipitate	Negative

4.	Test for Ammonium	No appearance of brown color	Negative
5.	Test for Sodium	No appearance of intense yellow color	Negative
6.	Test for Ferrous Iron	Appearance of blood red color	Positive
7.	Test for Zinc	No formation of white color precipitate	Negative
8.	Test for Aluminum	No characteristics changes	Negative
9.	Test for Lead	No formation of yellow color precipitate	Negative
10.	Test for Copper	No formation of blue color precipitate	Negative
11.	Test for Mercury	No formation of yellow color precipitate	Negative
12.	Test for Arsenic	No formation of brownish red color precipitate	Negative

Results for Acid Radicals

S. NO	PARAMETERS	OBSERVATION	RESULT
13.	Test for Sulphate	Formation of white color precipitate	Positive
14.	Test for Chloride	No formation of white color precipitate	Negative
15.	Test for Phosphate	No formation of yellow color precipitate	Negative
16.	Test for Carbonate	No formation of effervescence	Negative
17.	Test for Nitrate	No characteristics changes	Negative

S.NO	EXPERIMENT	OBSERVATION	RESULT
18.	Test for Starch	Formation of blue color	Positive
19.	Test for Albumin	No yellow precipitate is formed	Negative
20.	Test for Tannic Acid	Blue black precipitate is formed	Positive

21.	Test for Unsaturated Compounds	Potassium pomegranate gets decolorized	Positive
22.	Test for Reducing Sugar	Color change occurs. (green color)	Positive
23.	Test for Amino Acid	No violet color is formed.	Negative

Interpretation of Biochemical Analysis

The sample contains calcium, ferrous iron, sulphate, starch, tannic acid, reducing sugar, and unsaturated compounds. Absence of heavy metals such as mercury, arsenic, lead, copper, zinc, and aluminum.

III PHYSICAL EVALUATION

S.NO	PARAMETERS	RESULTS
1	Description	Brown coloured powder
2	pH (1% w/v solution)	5.14
3	Total Solids	20.4 % w/w
4	Total Ash	2.99 % w/w
5	Acid Insoluble ash	0.398 % w/w
6	Water soluble ash (NS)	1.85 % w/w
7	Loss on Drying at 105°C	6.38 % w/w
8	Total Fat	13.2 % w/w
9	Water Soluble Extractive (WSE)	53.2 % w/w
10	Alcohol Soluble Extractive (ASE)	30.2 % w/w
11	Carbohydrates	29.6 % w/w
12	Total acidity (NS)	0.8ml of .1M NaOH
13	Total Sugar	26.6 % w/w
14	Reducing Sugar	6.7 % w/w

Loss on drying indicates the moisture content. The total ash content is the measure of inorganic constituents present in the drug. High ash content explains its unsuitable nature to be used as drug.

IV TEST FOR PESTICIDE RESIDUE

S.NO	TEST	OBSERVED RESULT
Organochlorine Pesticides		
1	Alpha BHC	Not Detected
2	Beta BHC	Not Detected
3	Gamma BHC	Not Detected
4	Delta BHC	Not Detected
5	DDT	Not Detected
6	Endosulphan	Not Detected
Organo Phosphorous		
7	Malathion	Not Detected
8	Dichlorovos	Not Detected
9	Chlorpyriphos	Not Detected
Organocarbamates		
10	Carbofuran	Not Detected
Pyrethroids		
11	Cypermethrin	Not Detected

Pesticides residue in Gandhaga Rasayanam are below the detectable limit.

V TEST FOR ALFATOXINS

S.NO	TEST	RESULT	TEST METHOD	LOD (μ G/KG)
1	AflatoxinB1	BDL*	Lab – SOP – 02 /HPTLC	5
2	AflatoxinB2	BDL*	Lab – SOP – 02 /HPTLC	2
3	AflatoxinG1	BDL*	Lab – SOP – 02 /HPTLC	5
4	AflatoxinG2	BDL*	Lab – SOP – 02 /HPTLC	5

VI TEST FOR BACTERIAL AND FUNGAL COUNT

TEST	OBSERVED RESULT	SPECIFICATION AS PER AYUSH
Total bacterial count	NMT 10 ² CFU / g	Less than 10 CFU / g
Total fungal count	NMT 10 ⁰ CFU / g	Less than 10 CFU / g

E. Coli	Absent / g	Absent / g
Salmonella	Absent / g	Absent / g
Pseudomonas aeruginosa	Absent / g	Absent / g
Staphylococcus aureus	Absent / g	Absent / g

The bacterial and fungal load are within the prescribed limits. The above results suggest that the prepared drug Gandhaga Rasayanam is of standard quality.

VII RESULT OF HEAVY METAL ANALYSIS USING AAS

HEAVY METAL	OBSERVED RESULT	SPECIFICATION AS PER AYUSH/ WHO
Lead	BDL (D. L – 0.04 ppm)	10 ppm
Cadmium	BDL (D. L – 0.04 ppm)	0.3 ppm
Arsenic	BDL (D. L – 0.04 ppm)	3.0 ppm
Mercury	BDL (D. L – 0.01 ppm)	1.0 ppm

The heavy metals such as lead, cadmium, arsenic and mercury are found below detectable limit.

CONCLUSION

The first and foremost challenge to the alternative system of medicine for its globalization is to ensure uniformity and quality of drugs. Various methods and parameters for the assessment of Gandhaga Rasayanam dosage forms are mentioned in different guidelines. Therefore, this study is taken as a standard for quality control purpose to achieve optimum efficacy and safety of Gandhaga Rasayanam. Thus the preliminary chemical analysis of a Siddha drug will give fingerprints to the clinical studies.

ACKNOWLEDGEMENT

The author sincerely thanks the family and HOD and Faculties of Nanju Maruthuvam, Government Siddha Medical College, Tirunelveli for their support and continuous motivation.

REFERENCE

1. WHO Guidelines for assessing quality of Herbal Medicine with reference to contamination and residues – ISBN 97892415941594498 in WHO 2007.
2. Seema Rani, Khaleequrrahman, Mohd. Younis, Sadiya Noorul Basar – Physio Chemical and Microbiological Standardization of sufoofesailan – A Unani Polyherbal formulation. World Journal of Pharmacy and Pharmaceutical Science – Vol 4 Issue 06, 1554-1563.
3. General Guidelines for Drug Development of Ayurvedic Formulations – CCRAS – Central Council for Research in Ayurvedic Sciences – Ministry of AYUSH – Government of India – Vol 1 – 2018 – ISBN No 978-93-83864-23-2.

4. General Guidelines for Safety and Toxicity Evaluation of Ayurvedic Formulations - CCRAS – Central Council for Research in Ayurvedic Sciences – Ministry of AYUSH – Government of India – Vol 2 – 2018 – ISBN No – 978-93-83864-24-9.
5. 13. Anonymous, (Quality control methods for medicinal plant materials, WHO: Geneva; 1998).
6. JB (Phytochemical methods, Chapman and Hill, London 1973).
7. Harbone Trease G. E and W. C. Evans, (pharmacognosy, Brailliar Tiridel and Macmillian Publishers, London Ed-11).

