


PHYSICOCHEMICAL AND BIOCHEMICAL ANALYSIS OF GANDHAGA CHENDHOORAM
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ABSTRACT

Background: *Gandhga chendhooram* is the one of the valuable herbo-mineral formulation in *Siddha* literature. It used to treat various conditions like PCOS. Physicochemical and biochemical characterization of GC is evaluated scientifically and documented. **Aim:** The present study deals with the safety and efficacy of GC were validated through physicochemical and biochemical analysis. **Materials and Methods:** *Gandhga chendhooram* was analyzed by various methods, like Anti-microbial load, physicochemical parameters and Biochemical analysis. **Result:** In microbial load analysis of GC shows 10^{-4} dilution is 1 and in 10^{-6} dilution is 5. Total fungal load in 10^{-2} dilution is nil and in 10^{-3} dilution is nil. Results of physicochemical analysis shows specific gravity – 0.976, pH – 6.28, LOD- less than 1%, Total ash value- 96.8% which ensure the purity and efficacy of GC. And biochemical analysis results shows presence of magnesium, iron and sulphate which increase the therapeutic effect of GC.

KEYWORDS: *Siddha, GC, Physicochemical, biochemical, Gandhagam, Lingam.*
INTRODUCTION

Siddha system is a traditional system of medicine in India. *Siddhars*, who are the initiator of traditional system. They are experts in to treat the disease and also well known in medicine preparation. In *Siddha* system of medicine *Chendhooram* belongs to 32 types of internal medicine. *Chendhooram* is the one of the higher order drug in *Siddha* medicine; it has long shelf life.^[1]

Gandhaga chendhooram is one of the valuable herbo mineral formulation available in the book of *Anuboga Vaithiya Navaneetham*. GC is used to treat the PCOS; it is the most complaint in young girls in nowadays. GC has good therapeutic effect in PCOS. Safety, purity and efficacy of the GC has established through the standardization method. In this article GC was evaluated scientifically through the physicochemical and biochemical parameters.

MATERIALS AND METHODS
Materials

Herbo mineral formulation of *Gandhaga chendooram* was taken as the compound drug preparation for Polycystic Ovarian Syndrome which taken from the *Siddha* classical literature “*Anuboga Vaithiya Navaneetham*” written by **Hakkim P. Mohamad Abdul Sayabu** Page No-38.^[2]

Collection of the trial drug

The raw drugs *Gandhagam* and *Lingam* were bought from Ramasamy chetty country shop at Parrys, Chennai. Plant material was collected from around Anna Hospital campus, Arumbakkam, Chennai.

Identification and authentication

All raw drugs were identified and authenticated by the Botanists and the experts of *Gunapadam* (Pharmacology) at Government Siddha Medical College, Arumbakkam, and Chennai. The specimen samples of the identified raw drugs were preserved in the laboratory of PG *Gunapadam* for future references.

Ingredients

- Sulphur
- Red Sulphide of Mercury
- *Calotropis gigantea*

Methods

Purification process was done as per classical *Siddha* literature “*Gunapadam Thathu seeva vaguppu*”.^[3] After the purification GC was prepared by as per the procedure of “*Anuboga Vaithiya Navaneetham*”

AVAILABILITY OF MICROBIAL LOAD^[4]

Enumeration of bacteria by plate count – agar plating technique

The plate count technique is one of the most routinely used procedures because of the enumeration of viable cells by this method.

Principle

This method is based on the principle that when material containing bacteria are cultured, every viable bacterium develops into a visible colony on a nutrient agar medium. The number of colonies, therefore are the same as the number of organisms contained in the sample.

PHYSICOCHEMICAL ANALYSIS^[5]

Physico-chemical studies like total ash, water soluble ash, acid insoluble ash, water and alcohol soluble extract, loss on drying at 105°C and pH were done at, Dr. MGR University, Chennai.

Solubility Test

A pinch of sample (GC) was taken in a dry test tube and to it 2 ml of the solvent was added and shaken well for about a minute and the results are observed. The test was done for solvents like distilled water, Ethanol, Petroleum ether, Propylene glycol, Toluene, Benzene, Chloroform, Ethyl alcohol, Xylene, Carbon tetra chloride and the results are observed individually.

pH value

Potentiometrically, pH value is determined by a glass electrode and a suitable pH meter. The pH of the *Gandhaga chendooram* was written in results column.

Loss on Drying

An accurately weighed 2gm of *Gandhaga chendooram* formulation was taken in a tarred glass bottle. The crude drug was heated 105°c for 6 hours in an oven till a constant weight. The percentage moisture content of the sample was calculated with reference to the shade dried material.

Determination of total Ash

Weighed accurately 2g of *Gandhaga chendooram* formulation was added in crucible at a temperature 600°c in a muffle furnace till carbon free ash was obtained. It was calculated with reference to the air dried drug.

Determination of acid insoluble ash

Ash above obtained was boiled 5min with 25ml of 1M hydrochloric acid and filtered using an ash less filter paper. Insoluble matter retained 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 as was calculated with reference to the air dried drug.

Determination of water soluble ash

Total Ash 1g was boiled for 5min with 25ml water and insoluble matter collected on an ash less filter paper was

washed with water and ignited for 15 min at a temperature not exceeding 450°c in a muffle furnace. The amount of soluble ash is determined by drying the filtrate.

BIOCHEMICAL ANALYSIS^[6]

The bio-chemical analysis was done to identify the acid and basic radicals present in the sample.

Preparation of extract

5g of GC was taken in a 250 ml clean beaker and 50 ml of distilled water was added, boiled well and allowed to cool and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water.

Preliminary Basic and Acidic radical studies

Test for basic radicals

1. Test for Potassium

To a pinch of the GC 2 ml of sodium nitrate and 2 ml of cobalt nitrate solution in 30% glacial acetic acid was added and observed for the presence of yellow precipitate.

2. Test for Calcium

To 2 ml of the GC extract 2 ml of 4% ammonium oxide solution was added and observed for the formation of white precipitate.

3. Test for Magnesium

To 2ml of GC extract, drops of sodium hydroxide solution was added and watched for the appearance of white precipitate.

4. Test for Ammonium

To 2ml of GC extract few ml of Nessler's reagent and excess of sodium hydroxide solution are added for the appearance of brown color.

5. Test for Sodium

Hydrochloric acid was added with a pinch of the GC sample and a paste was made and introduced into the blue flame of Bunsen burner and observed for the appearance of intense yellow color.

6. Test for Iron (Ferrous)

The GC extract was treated with Conc. HNO₃ and ammonium thiocyanate and waited for the appearance of blood red color.

7. Test for Zinc

To 2 ml of the GC extract drops of sodium hydroxide solution was added and observed for white precipitate formation.

8. Test for Aluminium

To the 2ml of the GC extract sodium hydroxide was added in drops and noted for any characteristic changes.

9. Test for Lead

To 2 ml of GC extract 2ml of potassium iodide solution was added and noted for yellow colored precipitate.

10. Test for Copper

a. A pinch of GC sample was made into a paste with concentrated HCl in a watch glass and introduced into the non-luminous part of the flame and noted for blue color appearance.

b. To 2 ml of GC extract excess of ammonia solution was added and observed for the appearance of blue colored precipitate.

11. Test for Mercury

To 2ml of the GC extract sodium hydroxide solution was added and noted for yellow precipitate formation.

12. Test for Arsenic

To 2 ml of the GC extract 2ml of Sodium hydroxide solution was added and brown wash red precipitate if appeared was noted.

Test for acid radicals**1. Test for Sulphate**

To 2 ml of the GC extract 5% of barium chloride solution was added and observed for the appearance of white precipitate.

2. Test for Chloride

The GC extract was treated with silver nitrate solution and observed for the appearance of white precipitate.

3. Test for Phosphate

The GC extract was treated with ammonium molybdate and concentrated HNO₃ and observed for the appearance of yellow precipitate.

Table.No.2. Results of Physicochemical analysis.

S.No	Parameter	Result
1.	Specific gravity	0.976
2.	pH	6.28
3.	Particle size	Completely passes through sieve no.120
4.	Loss on drying at 105 degree Celsius	Less than 1%
5.	Total ash value	96.8%
6.	Acid insoluble ash	Less than 1%
7.	Water soluble ash	Less than 1%

DISCUSSION ON PHYSICOCHEMICAL ANALYSIS

- GC is soluble in major solvents and sparingly soluble in water, well soluble in HCl, H₂SO₄. GC proves that its efficiency of solubility increasing the bio availability.
- The trial drug *Gandhaga Chendooram* shows low specific gravity (0.976) compared to water. This indicates its good nature of absorption of drug.
- *Gandhaga Chendooram* shows weak acidic pH (6.28). It is also important factor for drug

4. Test for Carbonate

The GC extract was treated with concentrated HCl and observed for the appearance of effervescence.

5. Test for Fluoride & Oxalate

To 2ml of GC extract 2ml of dil. acetic acid and 2ml calcium chloride solution were added and heated and watched for cloudy appearance.

6. Test for Nitrate

To 1 gm of the GC, copper turnings was added and again concentrated H₂SO₄ was added, heated and the test tube was tilted vertically down and viewed for any characteristic changes.

RESULT AND INTERPRETATIONS**Table.No.1.Availability Microbial load in *Gandhaga Chendooram*.**

S.NO	Microbes	DILUTION	RESULT
1	Bacteria	10 ⁻⁴	1
2	Bacteria	10 ⁻⁶	5
3	Fungi	10 ⁻²	-
4	Fungi	10 ⁻³	-

Interpretations

- Total bacterial load in 10⁻⁴ dilution is 1 and in 10⁻⁶ dilution is 5.
- Total fungal load in 10⁻² dilution is nil and in 10⁻³ dilution is nil.

Low moisture content suggests better stability against degradation of drug. Here, the contamination of GC is within the WHO guidelines norms. Hence, the drug is collected, prepared, stored and packed and decontaminated prior to formulation.

S.No	Parameter	Result
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absorption. This enhances the bioavailability of the drug.

- Since the GC has low loss on drying (LOD), the moisture content is less than 1% which is suitable for medicine preparation.
- Ash values are helpful in determining the quality and purity of the drugs. The total ash value of *Gandhaga Chendooram* is 96.8%, which determines the presence of inorganic content.
- The Acid insoluble ash of *Gandhaga Chendooram* is less than 1% which ensures the purity of trail drug

- The Water soluble ash of *Gandhaga Chendooram* is less than 1% which increases the facilitation of diffusion and osmosis.

Bio Chemical analysis

Table. No.3: Results of Basic Radical Studies.

S.No	Parameter	Result
1	Test For Magnesium	Positive
2	Test for Iron (Ferrous)	Positive
Test for acid radicles		
1	Test for Sulphate	Positive

Discussion on Biochemical analysis

The Biochemical analysis for basic radicals of GC shows the presence of Magnesium, Iron.

Magnesium

Magnesium activates over 300 enzyme reactions in the body. Which helps in the hormone creation and also it prevent the excess creation of stress hormone cortisol because of excess Cortisol level leads to alteration of progesterone, estrogen, testosterone, FSH and LH will be too, this is the vital when it comes to healing hormonal issue like PCOS. It helps to maintain nerve and muscle function, supports a healthy immune system. Mg regulates the blood glucose levels because it enhances insulin secretion and aid in the production of energy and protein and facilitates sugar metabolism.

Iron

Iron is used to maintain healthy cells, skin, hair and nails. Iron is an essential element for blood production and also production of enzymes, hormones, amino acids and neurotransmitter. Health benefits of iron also include the elimination of unexplained or chronic fatigue, which may occur in both men and women. Its deficiency is a natural cause of fatigue since it is an important component of hemoglobin.^[7]

The biochemical analysis for acidic radicals of GC shows the presence of Sulphate.

Sulphate

Sulphate containing proteins work in indirect ways to maintain the hormone level. So it is useful in menstrual irregularities.^[8]

CONCLUSION

In microbial load analysis of GC shows 10^{-4} dilution is 1 and in 10^{-6} dilution is 5. Total fungal load in 10^{-2} dilution is nil and in 10^{-3} dilution is nil. Availability of microbial load result indicates bacterial and fungal count present within the limits as per the WHO guidelines, which ensure the shelf life of the GC.

Results of physicochemical analysis show Specific gravity – 0.976, pH – 6.28, LOD- less than 1%, Total ash value- 96.8% which ensure the purity and efficacy of GC. Weak acidic pH value shows good absorption in

acidic medium (stomach). Presence of acid basic radicles such as Magnesium, Iron, and Sulphate helps to increase the therapeutic effect of GC. *Gandhaga chenooram* is validating by scientific method to prove the efficacy through the physicochemical and biochemical parameters

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