

PROCESS STANDARDIZATION STUDIES ON *GHANTHAGA RASAYANAM*

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Received: 16 March 2012, Revised and Accepted: 20 April 2012

## ABSTRACT

*Siddha* system is one of the ancient traditional Indian medicinal systems with Dravidian origin having its entire literature in Tamil language. In *Siddha* system metals and minerals are predominantly used. Alchemy has its origin in *Siddha* system and is connected with the Tantrik culture. *Siddha* means achievement, which was attained by *Siddhars*. '*Siddha* Medicine' means medicine that is perfect. In *Siddha* system *Pasanam* are group of metals and minerals which are considered as toxins and they are around 64 drugs. "*Ghanthagam*" or Sulphur is one among the *Pasanam*. In the present paper Process Standardization Studies on *Ghanthaga rasayanam* is taken up which is prescribed in various diseases such as gastric ulcers, chronic wounds, other skin problems such as leprosy and in Leucoderma, tumors for which modern medicine has no cure. "*Ghanthaga rasayanam*" was prepared, analyzed and characterized employing modern sophisticated techniques such as AAS, FT-IR, HPLC, Zeta Potential and XRF Standards were also determined for the prepared medicines. Lights were also thrown on the various roles of the purifying agents and the purification processes of the selected formulation.

**Keywords:** *Ghanthagam Rasayanam*, *Siddha* Medicine, Purification methods and Chemical studies.

## INTRODUCTION

*Siddha* is a traditional healing holistic medicine of India which emphasizes the maintenance of a relaxed mind and body harmony and insists to keep pace with the laws of nature. In *Siddha* system, besides herbs, metal and mineral drugs were also used as medicines. *Siddhar Bhogar* classified the metals and minerals into four groups in his book "*Bhogar karasara thurai*"

They are-

Metals (*Ulogam*)-12

Toxins (*Pasanam*)-64

Minerals (*Karasaram*)-24

Hydrochemicals (*Uparasam*)-120

In the present paper an attempt is made to understand the significance of various methods adopted in the preparation of *RASAYANAM* which is a Medicated semisolid powder. The herbs are made into fine powder, sugar and ghee are added to it and taken in a semi solid form. With a view to understand the significance of these groups of *Siddha* medicines *Ghanthaga rasayanam* a unique preparation prepared from "*Ghanthagam*" (Sulphur) one of the toxins (*Pasanam*) is selected and studied from standardization point of view employing modern analytical tools and attempts were process made to understand the importance of purification processes involved in the preparation.

Sulphur is a crystalline, non-metal used in the preparations like *Rasayanam*, *mathiri*, *Mezhugu*, *Parpam* and *Chenduram* as major ingredients in *Siddha* therapeutics. It possesses bitter and astringent taste. Traditionally *Sulphur* is known with other names like *Ghandagam*, *Kaarizhai Natham*, *Parai natham*, *Parai Veerayam*, *Atheetha prakasam*, *Beejam*, *Selvi vindhu*, *Sakthi*, *Sakthi peesam*, *Chenduraa thrrathi*, *Theviuram*, *Natham*, *Narram*, *Parai natham*, *Ponnvarni*, *Rasa sronitham*. (Ref.No:2)

Types of *Ghanthagam*:

*Pirappu kandagam*, *Vaipu kandagam*, *Kozhi thalai kandagam*, *VanaKendhi vaipu*, are some of the varieties. *Pirappu kandagam*, is the naturally occurring sulphur from which *Ghanthagam* based formulations were prepared by subjecting it to purification. *Kozhi thalai kandagam*, name itself suggests that it resembles red color of the crown of Cock. (Ref.No:2)

Based on the color Sulphur is of four types:

- White sulphur is used to cure all diseases.
- Parrot nose red color *Ghanthagam* is used for calcinations of nine metals.
- Golden color *Ghanthagam* resembles the color of Gooseberry, easily reacts with Mercury to form *Kajjali*, is used in therapeutics.
- Black *Ghanthagam* similar with the Crow black color is a rare variety used as a rejuvenator. (Ref.No:2)

In nature, both plant and animal origin substances contain Sulphur in a permissible quantity such as leafy vegetables, egg, meat, garlic etc.

The properties of sulphur, makes it unique to act as a precursor in the preparation of *chenduram*.

## Mode of Action

Sulphur is a laxative; it enhances bile juice secretion, alterative, antiseptic and diaphoretic. It is excreted through duct glands like sweat, urine, mammary glands. It enhances secretions of skin, bronchioles and rectum. (Ref.No:2)

## Therapeutic uses of sulphur

Sulphur is mainly used therapeutically to treat eighteen types of Leucoderma, Flatulence, Hepatomegaly, Ascites, Gastric ulcer, Eye diseases, Poisonous bites, chronic Venereal diseases, Rheumatic fever, Diarrhoea and respiratory symptoms and *Kapham* (Sputum accumulation). The oldest *Siddha* text "*Theran porut panbu nool*" says that as a mother nourishes the child, *Ghanthagam* nourishes our body. (Ref.No:2)

## Dose

Medicine purpose: 650 mg--1.9 g medicine;

For Laxative purpose: 4.2 g - 12.6 g (Ref.No:2)

## MATERIALS AND METHODS

*Ghanthaga rasayanam* is a polyherbomineral *Siddha* formulation consisting of sulphur and 13 ingredients [Ingredients are given below table no:1, Ref.no:1,8 &4, 6]. Morphological part of the plants are used as specified in the *Siddha* texts and the formulation prepared according to the formula in *Siddha* Marunthu Seiperu Muraikal, written by Balarama Iyer.

Table 1: Ingredients

| S. No. | Name of the plant                                      | Part used | Quantity |
|--------|--|-----------|----------|
| 1.     | <i>Semicarpus anacardium</i> L.f                       | Seed      | 35 gms   |
| 2.     | <i>Plumbago zeylanica</i> L                            | Root      | 35 gms   |
| 3.     | <i>Terminalia chebula</i> Retz.                        | Fruit     | 35 gms   |
| 4.     | <i>Terminalia bellirica</i><br>(Gaertn.) Roxb.         | Fruit     | 35 gms   |
| 5.     | <i>Phyllanthus emblica</i> L                           | Fruit     | 35 gms   |
| 6.     | <i>Pipper longum</i> L                                 | Seed      | 35 gms   |
| 8.     | <i>Pipper nigrum</i> L                                 | Seed      | 35 gms   |
| 9.     | <i>Zingiber officinale</i> Roscoe                      | Rhizome   | 35 gms   |
| 10.    | <i>Elettaria cardamomum</i> (L.) Maton                 | Seed      | 35 gms   |
| 11.    | <i>Embelia ribes</i> Burm.f.                           | Seed      | 35 gms   |
| 12.    | <i>Syzygium cumini</i> (L.) Skeels.                    | Seed      | 35 gms   |
| 13.    | <i>Cinnamomum tamala</i><br>(Buch.-Ham.) Nees & Eberm. | Leaf      | 35 gms   |
| 14.    | <i>Crotalaria juncea</i> L                             | Seed      | 35 gms   |
| 15.    | Purified sulphur                                       |           | 150 gms  |
| 16.    | Sugar  |           | 455gms   |
| 17.    | Ghee   |           | 175gms   |
| 18.    | Honey  |           | 140 gms  |

Certain raw drugs in the selected formulation will be purified before adding to the formulation, such as Sulphur, *Semicarpus anacardium* and *Plumbago zeylanica* using the purification methods given in standard text. (Ref.no:5, 7&9)

After purification toxic ingredients as well as other ingredients of the formulation are made into fine powder mixed with sugar and slightly fried with ghee. After cooling honey is added.

#### Dose

1 gm to 2 gm with milk twice a day.

#### Uses

Veneral diseases, Urinary problems, Leucoderma, Diarrhoea, dysentery, Piles, Peptic ulcer, Vatha diseases, swelling, Seminal problems, flatulence, ascites, chronic wounds, increase rejuvenation and act as an antidote to all animal poisons. (Ref.No:1)

#### Adjuvants

Butter or ghee or banana fruit or sugar or honey.

In the conditions of eczema, itching, insect bites and heat boils this is recommended with ghee or water for 40 days. (Ref.No:1)

#### Diet

Avoid tamarind, gingelly oil, mustard, tubers, non-vegetarian and, allergic food materials and sexual activities. (Ref.No:12).

#### Purification Methods

##### Sulphur purification

There are several types of purifying methods prescribed for *ghanthagam* in *Siddha* literature. Common herbal decoctions used for the purification of the Sulphur as per *Siddha* literature are aqueous extract of *Tamarindus indica*, Sour rice water, Sour butter milk, Mushroom juice, cow's milk and rhizome juice of *Musa paradisiaca*. The paste of *Lawsonia inermis* mixed with curd is also used for purification of sulphur. In the present work cow's milk is used for purification. (Ref.No:2)

##### Milk Purification Method

Various stages of purification process are studied. (Fig: 1, 2, 3, 4 and 5). Sufficient quantity of butter taken slightly heated and added 250 gm of sulphur and melted at a temperature of 60-70C. The above melted sulphur is poured into an earthen container containing cow's milk. The Sulphur poured into the milk is allowed to cool. The above process is repeated for thirty times, and fresh milk is used each time. The purified Sulphur after cooling is filtered and washed with the normal water. An amount of 12 gms of Sulphur before, middle and after the purification process were collected for the chemical analysis. ( i.e 1 stage before purification - raw material), 15th stage i.e during the process and 30th stage i.e after purification.



Fig. 1: Butter



Fig. 2: Melting Sulphur



Fig. 3: Pouring melted Sulphur



Fig. 4: Solidified Sulphur



Fig. 5: Solidified Sulphur

#### Purification of *Semicarpus anacardium*:

This drug possesses latex. It is highly poisonous. The black sap of the fruit shell can cause itching after 12 hours of contact and rashes and blistering after 24 hours. In *Siddha* literature few purification methods are mentioned for this drug. Seeds must be boiled with cow

dung solution for three hours. *Semicarpus* seeds are tied in a cotton cloth and suspended in the solution of cow dung before heating. In *Siddha* system this method is called "*tholaenthira murai*". After cooling washed with water and allowed to dry. In the second step seeds are cut and in the third step boiled with coconut water and after drying, fried with cow's ghee. (Ref.No:7&9)



*Semicarpus* seed



Boiled with cow dung solution (*Thozhayenthiram*)



After boiled in coconut water



After purification

#### ***Plumbago zeylanica* Purification**

Boiled the root bark with milk and allowed to dry.

#### ***Terminalia chebulla* fruit Purification**

Inner kernel is removed from the dry fruit before using in the formulation.

#### ***Terminalia bellerica* Purification**

Inner kernel is removed from the dry fruit before using in the formulation.

#### ***Phyllanthus emblica* Purification**

Inner kernel is removed from the dry fruit before using in the formulation.

#### ***Zingiber officinalis* Purification**

Outer skin of the rhizome of the ginger is peeled and used. (Ref.no:14).

#### **Methodology**

Physico-chemical parameters such as ash values and extractive values (from water- and alcohol) were determined according to Indian Pharmacopoeia (1996) Volume-2, Published by The Controller of Publications, Delhi.

Physical properties and physicochemical standards were determined as per Standard texts. (Ref no: 13). Microbial load also detected as per Indian pharmacopeia.

HPTLC finger printing was determined for Gallic acid present in the formulation. Sample Preparation method -2.504gm of sample was extracted with 50ml of methanol and kept for maceration for 24hrs, filter and remove the solvent under reduced pressure. Dissolve 0.315gm of the extractive in 10ml of methanol and it is taken for TLC analysis. Methanol extracts of *Ghanthaga rasayanam* were spotted on a 10\*10cm precoated silicagel 60 F<sub>254</sub> TLC plate (E.merck) of thickness 0.2mm, 7mm wide band by using automatic TLC applicator Linomat V, 8mm from the bottom. The mobile phase used was Toluene:Ethylacetate:Formic acid(5:5:1 v/v/v). The plates were eluted by ascending mode to 8cm in a CAMAG chamber previously saturated with the mobile phase vapour for 20min. The plates were dried after development, in hot air oven and scanned at 278nm for gallic acid using CAMAG Scanner3with WINCAT software version 1.3.4 by using deuterium lamp. The plates were photographed at 254 nm and 366 nm by using CAMAG Reprostar.

#### **RESULTS AND DISCUSSION**

In the present study a traditional Siddha preparation *Ghanthaga rasayanam* is prepared as per classical methods given in *Siddha Marunthu Seiperu Muraikal*<sup>2</sup> and evaluated chemically which included Heavy metals analysis, FT-IR, Physico-chemical analysis, HPTLC finger printing, Zeta Potential and XRF analysis. Chemical analysis of the final product indicated this medicine is safe and manifest a diverse range of beneficial biological activities.

#### ***Ghanthaga rasayanam* description**

A Brown Colored Coarse Powder



*Ghanthaga rasayanam*

Table 2: Physical properties of *Ghanthaga rasayanam*:

| S. No | Parameters     | <i>Ghanthaga rasayanam</i> |
|-------|----------------|----------------------------|
| 1     | Bulk density   | 0.513gm/ml                 |
| 2     | Tapped density | 0.745gm/ml                 |

Table 3: Physico-chemical parameters of *Ghanthaga rasayanam*

| S. No | Parameters                 | <i>Ghanthaga rasayanam</i>          |
|-------|----------------------------|-------------------------------------|
| 1     | Colour                     | Brown                               |
| 2     | Odour                      | Aromatic                            |
| 3     | Taste                      | Characteristic                      |
| 4     | Solubility                 | Partially Soluble in Water          |
| 6     | pH 1% solution             | 3.63 %w/v                           |
| 7     | Loss on Drying at 105°C    | 5.2932%w/w                          |
| 8     | Total Ash                  | 3.5334%w/w                          |
| 9     | Acid Insoluble Ash         | 0.4990%w/w                          |
| 10    | Alcohol Soluble Extractive | 8.3277%w/w                          |
| 11    | Water Soluble Extractive   | 12.2082%w/w                         |
| 12    | TLC Reports                | Gallic acid - R <sub>f</sub> - 0.24 |
| 13    | Sulphur                    | 28.9867 %                           |

Table 4: Qualitative Analysis of Heavy Metals

| S. No | Sample Name        | Qualitative Analysis of Heavy Metals |         |         |         |
|-------|--------------------|--------------------------------------|---------|---------|---------|
|       |                    | Lead                                 | Arsenic | Mercury | Cadmium |
| 1     | Ghanthagarasayanam | +                                    | +       | +       | +       |

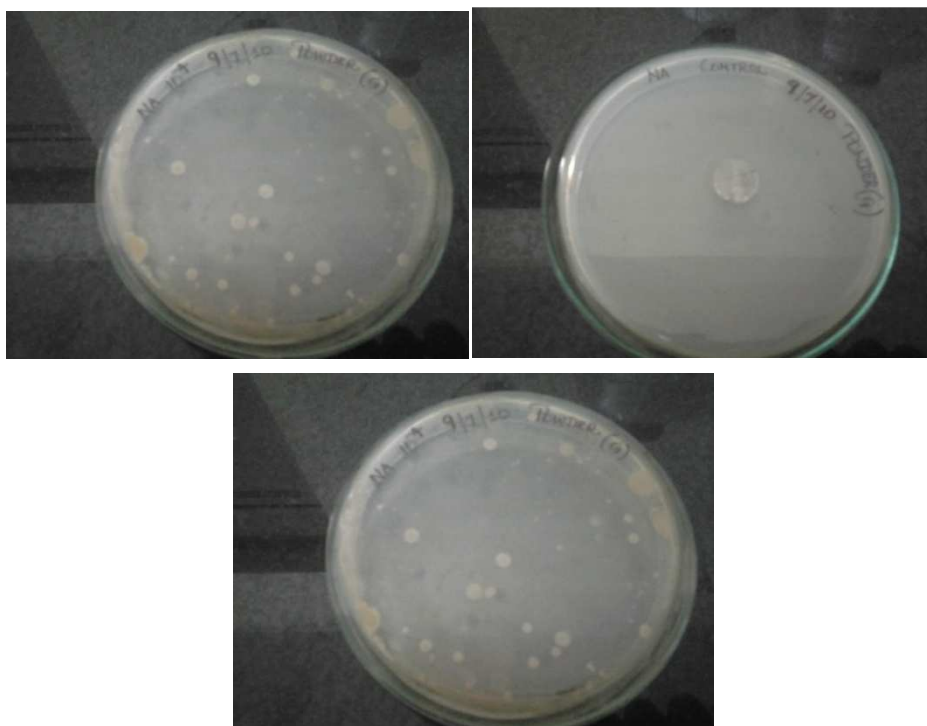
Table 5: Heavy Metal Analysis using Atomic Absorption spectrometer

| S. No | Heavy Metals (PPM) | Ghandhaga rasayanam(PPM) | WHO Permissible limits | Inference    |
|-------|--------------------|--------------------------|------------------------|--------------|
| 1.    | Mercury            | 0.16                     | 1ppm                   | Within limit |
| 2.    | Arsenic            | 0.09                     | 10ppm                  | Within limit |
| 3.    | Lead               | <1                       | 10ppm                  | Within limit |
| 4.    | Cadmium            | <0.1                     | 0.3ppm                 | Within limit |

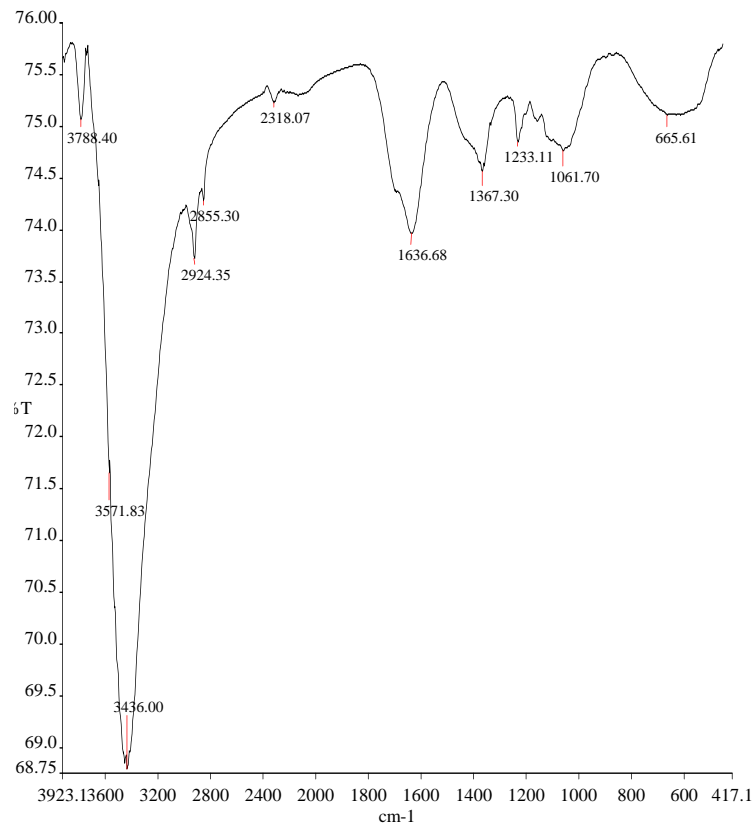
#### Microbial analysis

Table 6: Total Microbial count

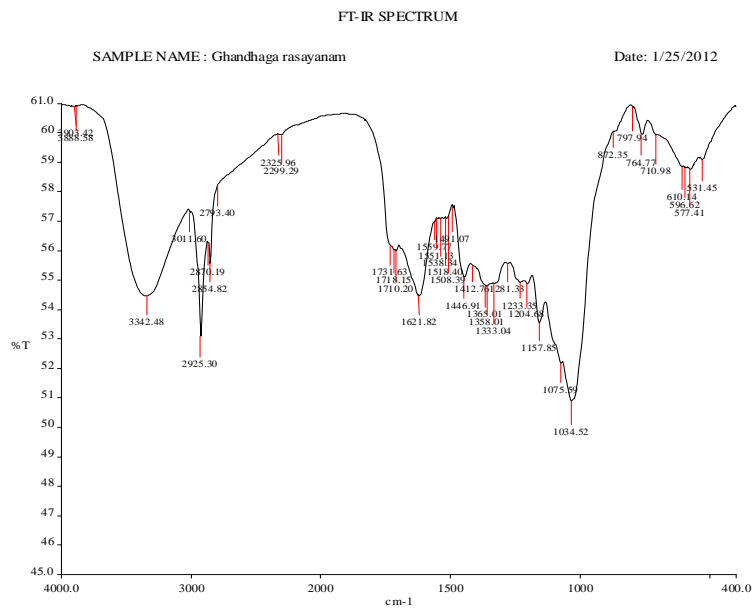
| S. No | Bacterial Name               | Cells in Sample/g    | WHO Limit       | Inference    |
|-------|------------------------------|----------------------|-----------------|--------------|
| 1.    | Total Heterotrophic Bacteria | 13 x 10 <sup>4</sup> | 10 <sup>7</sup> | Within limit |



FT-IR Spectrum of Raw Sulphur



FT-IR Spectrum of Ghanthaga rasayanam



Ghandhaga rasayanam 1 \_ 250112.pk

GHANDH-I.ASC 3601 4000.00 400.00 50.89 60.96 4.00 %T 1 0.01

REF 4000 60.96 2000 60.56 600  
 3903.42 60.88 3888.58 60.90 3342.48 54.45 3011.60 57.29 2925.30 53.09  
 2870.19 56.26 2854.82 55.55 2793.40 58.24 2325.96 59.94 2299.29 59.93  
 1731.63 56.16 1718.15 56.00 1710.20 56.00 1621.82 54.47 1559.77 56.97  
 1551.13 57.09 1538.34 57.09 1518.40 57.09 1508.39 57.15 1491.07 57.44  
 1446.91 55.08 1412.76 55.48 1365.01 54.80 1358.01 54.82 1333.04 54.87  
 1281.33 55.55 1233.35 54.92 1204.68 54.88 1157.85 53.53 1075.59 52.16  
 1034.52 50.88 872.35 60.04 797.94 60.87 764.77 59.95 710.98 59.92  
 610.14 58.84 596.62 58.82 577.41 58.76 531.45 59.08

Compare to raw sulphur the *Ghanthaga rasayanam* showed characteristics peaks at various region as given above. This indicated the presence of various groups which might have originated from the purifying herbs indicating that there must be a

complex formation of sulphur with these secondary metabolites of the purifying agents.

Probably toxic sulphur might have become non-toxic due to this interaction.

**XRF Analysis data**

**Elemental form**

**Table: Oxide form**

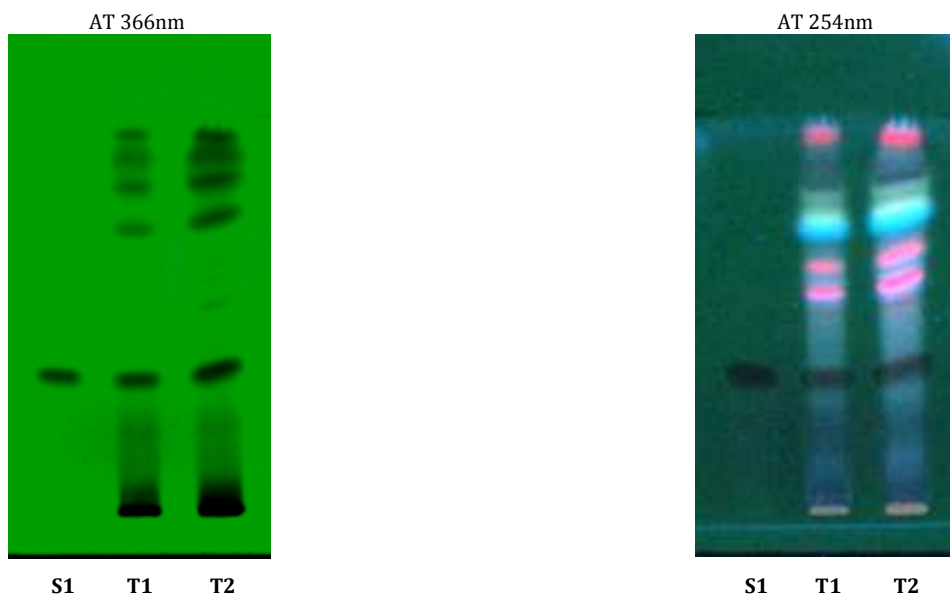
| Formula                        | Z  | Concentration% |
|--------------------------------|----|----------------|
| SO <sub>3</sub>                | 16 | 92.07          |
| K <sub>2</sub> O               | 19 | 3.80           |
| CaO                            | 20 | 1.40           |
| Cl                             | 17 | 0.62           |
| P <sub>2</sub> O <sub>5</sub>  | 15 | 0.59           |
| SiO <sub>2</sub>               | 14 | 0.54           |
| MgO                            | 12 | 0.50           |
| Al <sub>2</sub> O <sub>3</sub> | 13 | 0.17           |
| Na <sub>2</sub> O              | 11 | 0.12           |
| Fe <sub>2</sub> O <sub>3</sub> | 26 | 0.12           |
| MnO                            | 25 | 00.04          |
| MnO <sub>3</sub>               | 42 | 0.01           |
| ZnO                            | 30 | 70ppm          |
| CuO                            | 29 | 69ppm          |
| Rb <sub>2</sub> O              | 37 | 34ppm          |

**Table: Elemental form**

| Formula | Z  | Concentration% |
|---------|----|----------------|
| O       | 8  | 57.23          |
| S       | 16 | 36.87          |
| K       | 19 | 3.15           |
| Ca      | 20 | 1.0            |
| Cl      | 17 | 0.62           |
| Mg      | 12 | 0.30           |
| P       | 15 | 0.26           |
| Si      | 14 | 0.25           |
| Na      | 11 | 0.09           |
| Al      | 13 | 0.09           |
| Fe      | 26 | 0.08           |
| Mn      | 25 | 0.03           |
| Mo      | 42 | 77ppm          |
| Zn      | 30 | 56ppm          |
| Cu      | 29 | 55ppm          |
| Rb      | 37 | 31ppm          |

**HPTLC fingerprinting profile of *Gandhaga Rasayanam***

**Photo documentation under UV chamber**



TLC Details: S1 - 5µl of Standard (Gallic acid); T1 -5µl of test Sample ; T2 -10µl of test Sample

**Identity Test**

Standard Preparation:

Dissolve 25.03mg of gallic acid in 25ml of methanol.

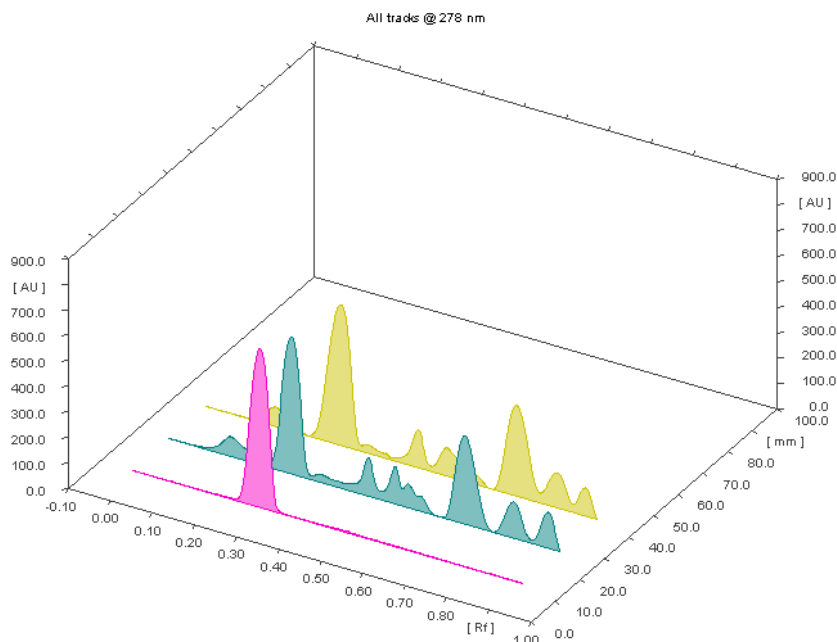
Stationary Phase: Silica Gel 60 F<sub>254</sub>

Mobile Phase: Toluene: Ethyl acetate: Formic acid (5:5:1)

Wave length: 278nm

Evaluation: A band (RF 0.28) corresponding to gallic acid is visible in both reference and test solution tracks.

**3D Display @ 278nm**



**PEAK DISPLAY (5µl of Standard Gallic acid)**

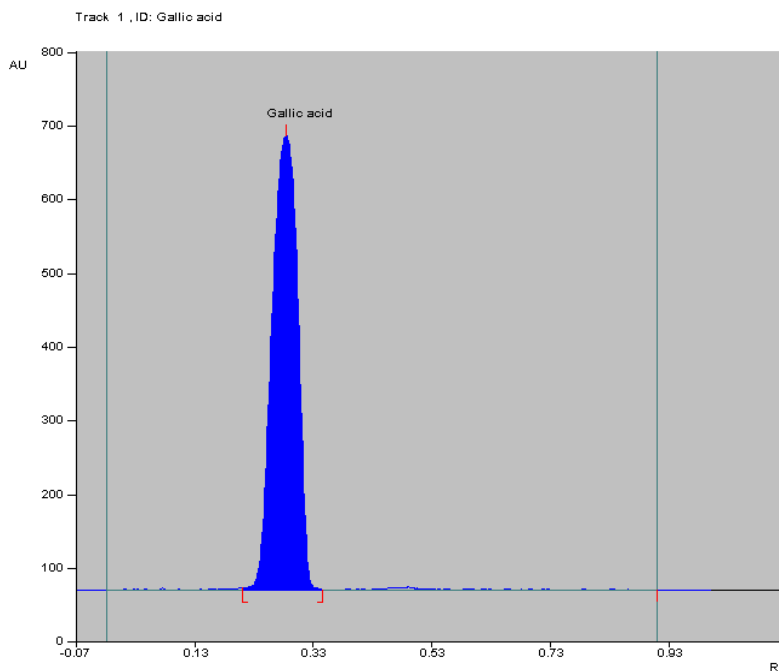


Fig. 1:

| Peak | Start Rf | Start Height | Max Rf | Max Height | Height % | End Rf | End Height | Area    | Area % | Assigned substance |
|------|----------|--------------|--------|------------|----------|--------|------------|---------|--------|--------------------|
| 1    | 0.21     | 2.7          | 0.28   | 616.1      | 100.00   | 0.34   | 0.6        | 20672.6 | 100.00 | Gallic acid        |



PEAK DISPLAY (5µl of Sample)

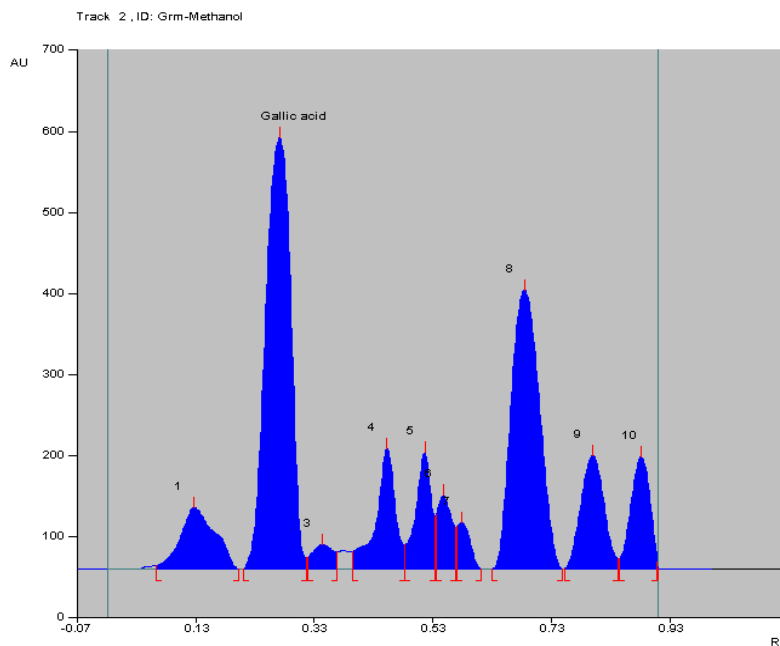


Fig. 2:

| Peak | Start Rf | Start Height | Max Rf | Max Height | Height % | End Rf | End Height | Area    | Area % | Assigned substance |
|------|----------|--------------|--------|------------|----------|--------|------------|---------|--------|--------------------|
| 1    | 0.06     | 4.3          | 0.12   | 75.9       | 4.46     | 0.20   | 0.3        | 3438.9  | 6.72   | unknown *          |
| 2    | 0.21     | 0.6          | 0.27   | 531.6      | 31.26    | 0.31   | 13.7       | 17306.0 | 33.83  | Gallic acid        |
| 3    | 0.31     | 14.2         | 0.34   | 30.5       | 1.79     | 0.36   | 21.3       | 827.2   | 1.62   | unknown *          |
| 4    | 0.39     | 21.8         | 0.45   | 148.8      | 8.75     | 0.48   | 30.2       | 3743.6  | 7.32   | unknown *          |
| 5    | 0.48     | 30.4         | 0.51   | 143.6      | 8.45     | 0.53   | 64.6       | 2907.4  | 5.68   | unknown *          |
| 6    | 0.53     | 66.3         | 0.54   | 90.6       | 5.33     | 0.56   | 52.0       | 1703.4  | 3.33   | unknown *          |
| 7    | 0.57     | 52.2         | 0.57   | 57.3       | 3.37     | 0.61   | 0.2        | 971.6   | 1.90   | unknown *          |
| 8    | 0.63     | 0.0          | 0.68   | 344.1      | 20.24    | 0.74   | 0.1        | 12617.9 | 24.67  | unknown *          |
| 9    | 0.75     | 0.3          | 0.80   | 140.2      | 8.24     | 0.84   | 12.3       | 4209.0  | 8.23   | unknown *          |
| 10   | 0.84     | 12.5         | 0.88   | 138.0      | 8.12     | 0.90   | 9.1        | 3426.3  | 6.70   | unknown *          |

Peak Display (10µl of Sample)

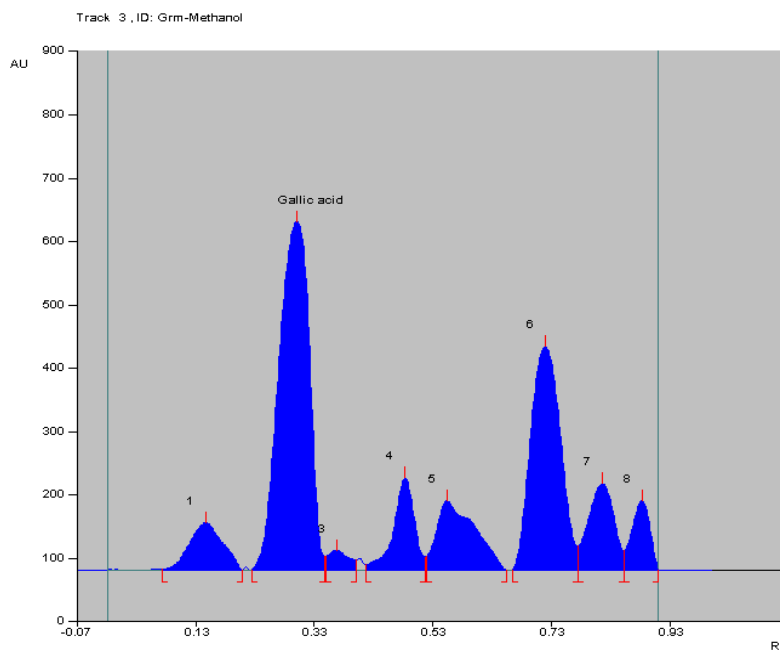


Fig. 3:

| Peak | Start Rf | Start Height | Max Rf | Max Height | Height % | End Rf | End Height | Area    | Area % | Assigned substance |
|------|----------|--------------|--------|------------|----------|--------|------------|---------|--------|--------------------|
| 1    | 0.07     | 1.0          | 0.14   | 75.9       | 5.01     | 0.20   | 0.1        | 3286.1  | 5.80   | unknown *          |
| 2    | 0.22     | 0.3          | 0.30   | 550.4      | 36.37    | 0.34   | 21.7       | 21946.8 | 38.75  | Gallic acid        |
| 3    | 0.34     | 21.8         | 0.36   | 31.6       | 2.09     | 0.40   | 16.0       | 872.4   | 1.54   | unknown *          |
| 4    | 0.41     | 8.7          | 0.48   | 146.0      | 9.65     | 0.51   | 20.9       | 3990.9  | 7.05   | unknown *          |
| 5    | 0.51     | 21.5         | 0.55   | 110.4      | 7.29     | 0.65   | 0.0        | 5515.3  | 9.74   | unknown *          |
| 6    | 0.66     | 0.6          | 0.71   | 353.2      | 23.34    | 0.77   | 38.4       | 13843.2 | 24.45  | unknown *          |
| 7    | 0.77     | 38.7         | 0.81   | 136.7      | 9.03     | 0.85   | 31.1       | 4597.1  | 8.12   | unknown *          |
| 8    | 0.85     | 31.2         | 0.88   | 109.3      | 7.22     | 0.91   | 0.0        | 2578.0  | 4.55   | unknown *          |

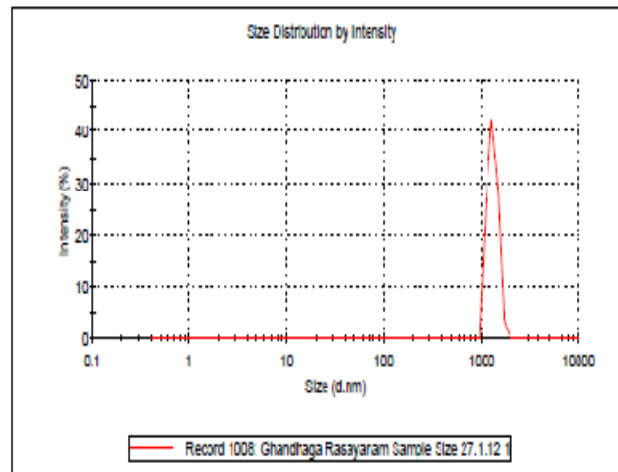
### Zeta Sizer analysis data

Particle size (394.5 nm) determined using zeta sizer indicated the presence of particle in nano range and zeta potential as -17.2mV. These data suggested moderate drug delivery and stability of the selected formulation.

### Zeta size

| Z-Average (d.nm): | Size (d.nm):  | % Intensity | Width (d.nm): |
|-------------------|---------------|-------------|---------------|
| 3373              | Peak 1: 1311  | 100.0       | 159.0         |
| Pd: 0.329         | Peak 2: 0.000 | 0.0         | 0.000         |
| Intercep: 0.657   | Peak 3: 0.000 | 0.0         | 0.000         |

Result quality : Refer to quality report

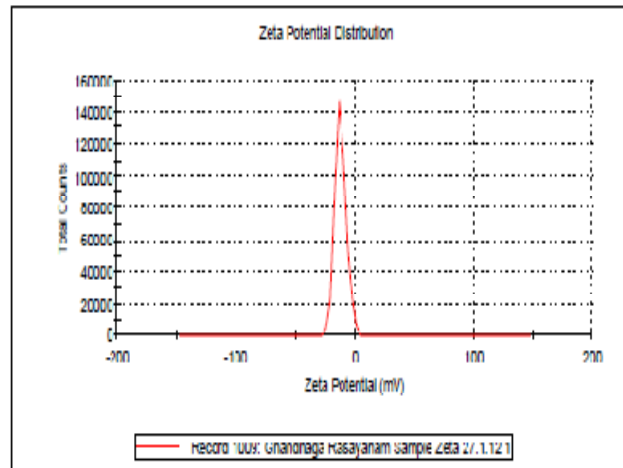


### Zeta potential

#### Results

| Zeta Potential (mV):        | Mean (mV)     | Area (%) | Width (mV) |
|-----------------------------|---------------|----------|------------|
| -11.5                       | Peak 1: -11.5 | 100.0    | 4.51       |
| Zeta Deviation (mV): 1.81   | Peak 2: 0.00  | 0.0      | 0.00       |
| Conductivity (mS/cm): 0.378 | Peak 3: 0.00  | 0.0      | 0.00       |

Result quality : Good



## CONCLUSION

To conclude in the present study delineates information pertaining to the quality control parameters of the selected polyherbo mineral formulation. Ghanthaga rasayanam and the importance of the purification methods and purifying agents in removing the toxicity of the raw materials used in the preparation.

## ACKNOWLEDGEMENT

We place on record our deep sense of gratitude to our Honorable Vice chancellor, SASTRA University, Thanjavur, Tamilnadu for permitting us to carry out this work.

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