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Research Article

## Total phenolic and flavonoids contents in the standardized polyherbal formulation “vayusadi guggulu”

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### ABSTRACT

Vayusadi guggulu vati (VGV), a polyherbal formulation is recommended for the management of diseases like obesity, arthritis, hyperlipidaemia and hyper cholesterol. Though Vayusadi guggulu vati is widely used for the treatment of obesity in Ayurvedic System of Indian Medicine, but till date, its quality standard study has not been carried out. In the present article, we evaluated the total phenolic and flavonoid contents in the standardised polyherbal VGV. The total phenolic content was determined by Folin Ciocalteu reagent (FC reagent) method. Aluminum chloride colorimetric method was used for total flavonoids contents determination. The VGV was standardized by physico-chemical parameters like total ash value, acid insoluble ash, loss on drying (LOD), PH, extractive value, phytochemical tests and thin layer chromatography (TLC). Determination of microbial load in different dilutions was also performed. Quality determination of vati (tablet) was also evaluated with help of various tablet parameters. Microbial load study revealed the growth of microbes increases with increases the dilutions. The phenolic and flavonoid contents in VGV were  $190.16 \pm 5.07$  mg/g and  $80.216 \pm 2.07$  mg/g respectively. Physicochemical parameters such as total ash value ( $9.73 \pm 1.45\%$  W/W), acid insoluble ash ( $1.85 \pm 0.40\%$  W/W), LOD ( $4.77 \pm 0.45\%$ W/W), PH 1% (4.6) water soluble extract ( $64.69 \pm 3.42\%$  W/W), alcohol soluble extract ( $50.56 \pm 2.48\%$  W/W) were assessed in preliminary physicochemical scanning. Thin layer chromatography (TLC) fingerprints study revealed that alcoholic and hexane extract of formulation showed 3 spots with different resolution in long wave UV 366 nm. TLC study revealed genuinely, quality and purity of formulation. Physico-chemical and microbial load result revealed that the formulation has a good quality. The inference from the present study may be used as reference standard in the further quality control researches.

**Key words:** Phenolic contents, flavonoid contents, microbial load, vayusadi guggulu vati

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### INTRODUCTION

*Guggulu* is an oleo gum resin obtained from the stem of *Commiphora wightii* (Hook ex. Stocks), belonging to family burseraeae. It is known to have analgesic, hypolipidaemic and anti-inflammatory action<sup>1</sup>. *Guggulu* is the principal ingredient of VGV formulations. It is also the main ingredient of several formulations such as *Rasnadi guggulu*, *Vatari guggulu* and *yogaraja guggulu* etc are traditionally used for musculoskeletal problems, body pain, osteoarthritis, obesity, sciatica and rheumatoid arthritis etc<sup>2</sup>. The preparation of VGV is based on traditional method mentioned in the Ayurvedic Pharmacopoeia of India. It is prepared from 10 ingredients (It is shown in table 1). Quality control studies of the herbal products are very important to justify its acceptability in the modern system of medicine. The change

from batch to batch begins with the collection of the raw materials crude drugs themselves in the absence of any reference standard for identification and authentication. World Health Organization (WHO) has emphasized the need to standardization (ensure quality and purity) of herbal products using modern techniques and applying appropriate standards and parameters. Phenolic and flavonoids compounds are a class of antioxidant agents which act as free radical terminators. Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity<sup>3, 4</sup>. Many published data reported that these compounds are very important for preventing the metabolic disorders<sup>5, 6</sup>. Therefore, the present work is aimed to determine the total phenolic and flavonoids contents and also evaluated the physico-chemical parameters and microbial load in VGV.

Table 1. Composition of VGV

| S. no | Ingredients | Botanical name              | Part used      | Quantity |
|-------|-------------|-----------------------------|----------------|----------|
| 1     | Saunthi     | <i>Zingiber officinale</i>  | Rhizomes       | 1 Part   |
| 2     | Marica      | <i>Piper nigrum</i>         | Flower         | 1 Part   |
| 3     | Pippal      | <i>Piper longum</i>         | Flower         | 1 Part   |
| 4     | Citraka     | <i>Plumbago zeylanica</i>   | Root           | 1 Part   |
| 5     | Musta       | <i>Cyperus rotundus</i>     | Rhizomes       | 1 Part   |
| 6     | Haritaki    | <i>Terminalia chebula</i>   | Plant          | 1 Part   |
| 7     | Bibhitaka   | <i>Terminalia bellerica</i> | Plant          | 1 Part   |
| 8     | Ēmalaki     | <i>Emblica officinalis</i>  | Plant          | 1 Part   |
| 9     | Vidanga     | <i>Embelia ribes</i>        | Flower         | 1 Part   |
| 10    | Guggulu     | <i>Commiphora wightii</i>   | Oleo gum Resin | 9 Part   |

## MATERIALS AND METHODS

### Procurement of VGV

It was procured from local market at the month of January. The tablets were looking good and non-sticky. The colour, shape of vati (tablets) was analysed with naked eye.

### Determination of phenolic contents

Sample Preparation: 1gm sample was dried & powdered then reflux in 50 ml of methanol for 2 hours. Collected the filtrate after filter it and evaporated the methanol and weight the residue & reconstituted in methanol 10 mg/ml.

Preparation of reagent: 10 % phenolic reagent and sodium carbonate (1M) were prepared with distilled water

Methanol extract: Then taken 0.5 ml of each methanolic extract and added in 5 ml FC reagent and 4 ml of sodium carbonate then taken absorbance at 765 nm after 15 min.

Preparation of standard solution: Standard (Gallic acid), 1mg/ml solution in methanol was prepared. Then made dilution from 25 microgram to 300 microgram with methanol. Taken 0.5 ml standard dilution and added into 5 ml FC reagent & 4 ml of sodium carbonate then taken absorbance at 765 nm after 15 min.

Blank solution: Taken 0.5 ml methanol and 5 ml FC reagent & 4 ml sodium carbonate solution then taken absorbance at 765 nm after 15 minutes<sup>7</sup>. At first, prepared the curve and found out regression equation and calculated the total phenolic content. The standard curve of gallic acid is shown in figure 1.

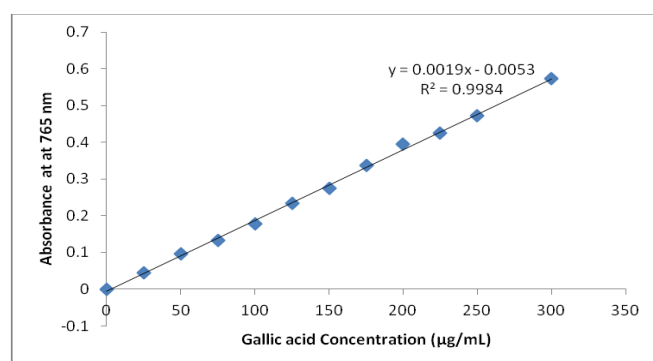


Figure 1. The standard curve of gallic acid

### Determination of flavonoid contents

Sample preparation: Taken 0.5 ml of extract from each solution, added 1.5 ml of methanol, 0.1 ml aluminium chloride and 0.1ml of sodium acetate then added 2.8 ml of distilled water & kept for 30 minute after taking absorbance at 415 nm.

Preparation of standard solution (quercetin): Taken 0.5 ml of standard dilution, added 1.5 ml methanol, 0.1 ml aluminium chloride, 0.1ml sodium acetate then added 2.8 ml distilled water and kept for 30 minute after taking absorbance at 415 nm.

Preparation of blank solution: Taken 2 ml of methanol, added 0.1 ml aluminium chlorides and 0.1ml of sodium acetate, & then added 2.8 ml of distilled water. After taking absorbance of standard dilution as mentioned above, draw a calibration curve and found out the regression equation and calculated the total flavonoid content<sup>7</sup>. The standard curve of quercetin is shown in figure 1.

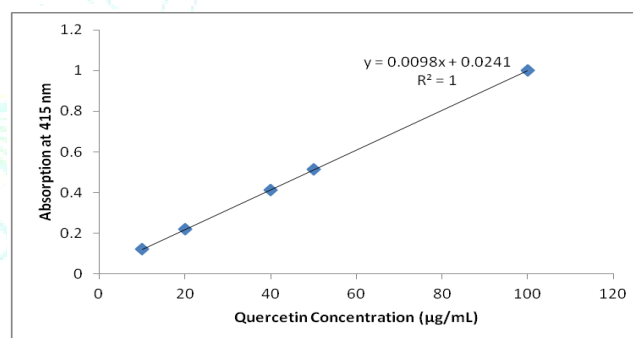


Figure 2. The standard curve of quercetin

## Physico- chemical properties

### Determination of total ash

Incinerated about 2 to 3 g of powdered drug accurately weighed in a tared platinum or silica dish at a temperature not exceeding 450° c until free from carbon, cooled and weighed and finally calculated the percentage of ash with reference to the air-dried drug<sup>8</sup>.

### Determination of acid-insoluble ash

Boiled the ash obtained in for 5 minutes with 25 ml of dilute hydrochloric acid; collected the insoluble matter in a Gooch crucible or on an ash less filter paper, washed with hot water and ignite to constant weight and lastly calculated the percentage of acid-insoluble ash with reference to the air-dried drug<sup>8</sup>.

### Determination of alcohol-soluble extractive

Macerated 5 g of the air-dried drug, coarsely powdered, with 100 ml of alcohol of the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing standing for eighteen hours. Filtered rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105°, to constant weight and weighed and calculated the percentage of alcohol-soluble extractive with reference to the air-dried drug<sup>8</sup>.

### Determination of water-soluble extractive

Macerated 5 g of the air-dried drug, coarsely powdered, with 100 ml of water of the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing standing for eighteen hours. Filtered rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dried at 105°, to constant weight and weighed and calculated the percentage of water-soluble extractive with reference to the air-dried drug<sup>8</sup>.

### LOD (Loss on drying)

Procedure set forth here determined the amount of volatile matter (i.e., water drying off from the drug). For substances appearing to contain water as the only volatile constituent, the procedure given below, is appropriately used. Placed about 10 g of drug (without preliminary drying) after accurately weighing (accurately weighed to within 0.01 g) it in a tared evaporating dish. For example, for unground or unpowered drug, prepare about 10 g of the sample by cutting shredding so that the parts are about 3 mm in thickness<sup>8</sup>.

### PH (1%)

Weighed 1 gm sample and dissolved in 100 ml distilled water to made 1% solution. [8]

### TLC

Extracted 5 g of formulation powder with 75 ml n-hexane under reflux on a water bath for 30 min, filtered and

concentrated to 10 ml and carried out the thin-layer chromatography. Applied 10 ml on TLC plate and developed the plate to a distance of 8 cm using toluen: acetone (9: 1) as mobile phase. After development allowed the plate to dry in air and examine under ultraviolet light (366 nm)<sup>8</sup>.

### Phytochemical test for flavonoids

Shinoda's test:

Taken about 2 ml of ethanolic extract, three pieces of magnesium chips was then added to the filtrate followed by few drops of conc. HCl. A pink, orange, or red to purple colouration indicated the presence of flavonoids<sup>9</sup>.

Alkaline reagent's test

Taken about 2 ml of ethanolic extract in a test tube then added few drops of 2% NaOH solution, it produced dense yellow colour. After addition of few drops of dilute HCL, the solution disappeared the colour indicated the presence of flavonoids<sup>9</sup>.

### Determination of foaming index and swelling index

Swelling index and foaming index of the formulation were measured as per method described in WHO guideline<sup>9</sup>.

### Determination of microbial counts explained as per WHO

The microbial load was determined as per procedure of Dutt et al. 2020<sup>7, 9</sup>. The medium was autoclaved at 151lbs per square inch pressure at 121°C. The growth of microbial in petri-plates.

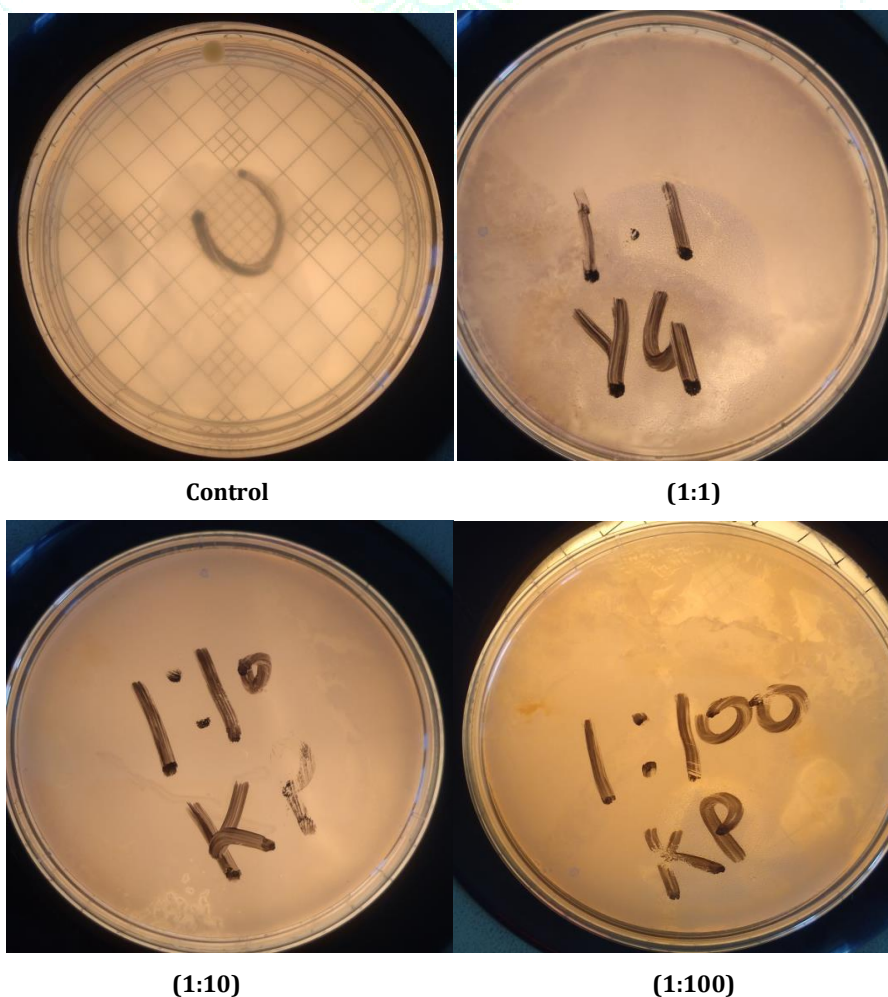


Figure 3. The growth of microbials in petri plates in different dilution.

### Tablet parameters

All tablet parameters such as diameter and thickness, weight variation, hardness, friability and disintegration were evaluated as per described in Indian Pharmacopoeias (IP)<sup>10</sup>.

## RESULTS

### Organoleptic properties

The organoleptic properties of VGV were shown in table 3.

**Table 3. Result of description of VGV**

| S. No | Organoleptic parameters | Observation |
|-------|-------------------------|-------------|
| 1     | Colour                  | Black       |
| 2     | Odour                   | Pleasant    |
| 3     | Taste                   | Bitter      |

### Total phenolic and flavonoids contents

The total phenolic and flavonoid content were found to be  $190.16 \pm 5.07$  mg/g and  $80.216 \pm 2.07$  mg/g dry extract respectively.

### Physico-chemical parameters

Sample was found to be in prescribed range (pharmacopoeial limits) for total ash ( $9.73 \pm 1.45$  % W/W, acid insoluble ash ( $1.85 \pm 0.40$  % W/W), alcohol extractive ( $50.56 \pm 2.48$  % W/W), water extractive ( $64.69 \pm 3.42$  % W/W), LOD ( $4.77 \pm 0.45$  %W/W) and PH (4.6). It is shown in table 4. The swelling index and foaming index of formulation were found to be **zero**. Thus, the sample did not contain saponin and mucilage. The extract for TLC was prepared in n-hexane and alcohol extract. A mixture of toluene: acetone (9:1) was taken as a mobile phase. N-hexane and alcoholic extract showed 4 spot at longer wavelength of 366 nm (properly separated, Figure 4). These standards exhibit a set of diagnostic, identity and authenticity of ayurvedic formulation. TLC profile generated in this study revising a standard tool for the authenticity of Ayurvedic extracts formulation and genuineness of the final product.

**Table 4. Compared with Pharmacopoeial standard**

| S. No | Parameters                | Observed Value   | Pharmacopoeia limit |
|-------|---------------------------|------------------|---------------------|
| 1     | LOD (%W/W)                | $4.77 \pm 0.45$  | NMT 15 percent      |
| 2     | Total Ash (%W/W)          | $9.73 \pm 1.45$  | NMT 11 percent      |
| 3     | Acid insoluble ash (%W/W) | $1.85 \pm 0.40$  | NMT 3 percent       |
| 4     | Alcohol extractive (%W/W) | $50.56 \pm 2.48$ | NLT 21 percent      |
| 5     | Water extractive (%W/W)   | $64.69 \pm 3.42$ | NLT 24 percent      |
| 6     | PH (1%)                   | 4.6              | 4.57- 4.69          |
| 7     | Shinoda's test            | +                | +                   |
| 8     | Alkaline reagent's test   | +                | +                   |

**Alcohol extract**



**n-Hexane extract**



Toluene: Acetone (9:1)

Toluene: Acetone (9:1)

**Figure 4.** TLC profile of VGV extract

### Results of tablet testing parameters

The thickness of tablets was  $0.5 \pm 0.02$  mm. Weight variation of sample was found to be  $-3.1$  to  $+5.1$  % W/W. The hardness and friability of sample were  $2.6 \pm 0.5$  Kg/cm<sup>2</sup> and  $0.562 \pm 0.05$  % W/W. The average disintegration time of sample was found to be  $29 \pm 5.04$  min.<sup>[11]</sup> The results are shown in table 5.

**Table 5. Result of tablet parameters**

| S. No | Parameters       | Observed value                   |
|-------|------------------|----------------------------------|
| 1     | Hardness         | $2.6 \pm 0.5$ Kg/cm <sup>2</sup> |
| 2     | Thickness        | $0.5 \pm 0.02$ mm                |
| 3     | Average wt       | $539 \pm 5.07$ mg                |
| 4     | Weight variation | $-3.1$ to $+5.1$ % W/W           |
| 5     | Friability       | $0.562 \pm 0.05$ % W/W           |
| 6     | Disintegration   | $29 \pm 5.04$ min                |

### Microbial load determination

VGV sample showed very less development microbial growth in 1:1(10 CFU), 1:10 (100 CFU) and 1:100 (220 CFU) dilutions which were under limits. From the results of microbial load reveal that the sample formulation has antimicrobial action, that is why it inhibits the growth of microbes.

## DISCUSSION

VGV is a traditional ayurvedic preparation prescribed for wide range of disorders. In this work an attempt has been made to determine total phenolic and flavonoid contents in standardized VGV. The standardization of VGV was done by different parameters such as ash value, PH, extractive value,



LOD, TLC fingerprinting, phytochemical tests and microbial load. The development of reliable quality protocols for ayurvedic formulations using modern techniques of analysis is extremely important.

The physical parameters such as ash value, extractive value, LOD and PH were found under pharmacopeial limits. The results from physico-chemical parameters indicated that the marketed preparation had good quality, strength and purity. Phenolic compounds are very important plant constituents because of their radical scavenging ability due to their hydroxyl groups<sup>12, 6, 13</sup>. Phenolic compounds are a class of antioxidant agents which act as free radical terminators. Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action. Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity<sup>3, 4</sup>. Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants through their scavenging power are useful for the management of those diseases. It has been recognized that phenolic and flavonoids show antioxidant activity and their effects on human nutrition and health are considerable<sup>14</sup>. The mechanisms of action of flavonoids are through scavenging or chelating process<sup>15, 16</sup>. TLC profile generated in this study revising a standard tool for the authenticity of Ayurvedic extracts formulation and genuineness of the final product. Many pathogens microbes such as *Spirochete*, *Escherichia coli*, *Shigella*, *Salmonella*, *Enterobacter*, *Klebsiella*, *Citrobacter*, *Novoviruses*, enteric hepatitis viruses, gastroenteritis viruses, enteroviruses and parasitic worms are present in water<sup>17, 18</sup>. In addition, different kinds of molds such as *Aspergillus* spp. *Penicillium* spp. are also present in water that are usually allergic and toxigenic<sup>19</sup>. These fungi are not only accountable for the adverse effects on health but also cause taste and odour problems in drinking water<sup>20</sup>. The thickness of tablets was performed on 20 tablets from each formulation. Digital Vernier caliper was used for the study, which permits accurate measurements and provides information of the variation between tablets. Weight variation was carried out to ensure that, each of tablets contains the proper amount of drug. The test was carried out by weighing the 20 tablets individually using analytical balance, then calculating the average weight, and comparing the individual tablet weights to the average<sup>10</sup>. The resistance of tablets to capping, abrasion or breakage under conditions of storage, transportation and handling before usage depends on its hardness. Tablet hardness is defined as the load required crushing or fracture a tablet placed on its edge. Sometimes it is also termed as tablet crushing strength. The hardness test was performed using Monsanto type (Make: Singhla) hardness tester. The instrument measures the force required to break the tablet when the force generated by anvils to the tablet. The tablet was placed between two anvils; force applied to the anvils, and the crushing strength that just causes the tablet to break was recorded. The crushing strength test was performed on 20 tablets from each formulation.

## CONCLUSIONS

The result of the present study showed that the extract of VGV has remarkable phenolic compounds and also contain flavonoid compounds. Due to presence of these compounds, it may show the greatest antioxidant activity and its related activities. VGV also showed good quality, purity and less

development of microbial contamination. The outcomes from various physico-chemicals and testing of tablet parameters may be differentiating qualities from many other vati formulations of guggulu.

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