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# Quality Control Evaluation Of Guduchi Ghana (Dried Aqueous Extract) Prepared From Guduchi (Tinospora Cordifolia Willd.) Swarasa

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**ABSTRACT**

**Introduction:** Ayurvedic pharmaceutics seeks to comprehend diverse pharmaceutical formulations. SwarasaKalpana (juice form of medicine) stands as a paramount dosage form, albeit challenged by issues of shelf life, palatability and storage. A resolution to these challenges lies in the preparation of Ghana (aqueous extract) but no published information exists to date on the analytical profile of Guduchi Ghana prepared from GuduchiSwarasa. **Aim:** To assess the quality control parameters for Guduchi Ghana prepared from GuduchiSwarasa. **Materials and Methods:** Three batches of Guduchi Ghanawere prepared and their characteristics were documented. Organoleptic parameters, physicochemical parameters, qualitative tests for functional groups, quantitative tests for total alkaloids, high-performance thin-layer chromatography (HPTLC) profiles and heavy metal analyses were conducted.**Results:** The average yield percentage and time duration for Guduchi Ghana prepared from GuduchiSwarasa were 5.25 % and 11 hours 36 minutes respectively. Physicochemical parameters revealed pH, Loss on Drying, Total ash,Acid Insoluble Ash, Water-Soluble Extractive and Alcohol-Soluble Extractive were 4.84, 6.07%, 12.48%, 0.64% and 44.45% respectively. HPTLC analysis displayed six peaks under 366 nm. The percentage of berberine and total alkaloid content was 0.11% and 0.50%, respectively. No heavy metals were detected in thesample. **Conclusion:** The present observation can be considered as standard for quality control parameter.

**Keywords:**Swarasa, Ghana,quality control parameter, Tinosporacordifoliawilld.

## I. INTRODUCTION

Ayurvedic pharmaceutics deals with herbal medicine and their preparations, which have been widely used for the thousands of years owing to its natural origin. However, one of the characteristics of oriental herbal medicine preparations is that all the herbal medicines, either presenting as single herbs or as collections of herbs in composite formulae is extracted with water. This may be the main reason why quality control of oriental herbal drugs is more difficult than that of western drug. The World Health Assembly - in resolutions WHA31.33 (1978), WHA40.33 (1987) and WHA42.43 (1989) – has emphasized the need to ensure the quality of medicinal plant products by using modern techniques and suitable standards.

PanchavidhaKashayaKalpana (five fundamental dosage form) being the primary preparations and the most widely used preparation as a starting dosage form as well as a base for many different dosage forms, the modification that can be done for these are a point of immense interest.Among PanchavidhaKashayaKalpana the use of SwarasaKalpana was very much reduced because of its short shelf life, unpleasant palatability, lack of proper protocol for testing and practice of this Kalpana become difficult. These factors gave thoughts to ponder over neo-formulations that can be made from Swarasaof plant.

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Guduchi (Tinosporacordifolia Willd.) is one of the vastly used plant in Ayurvedic system of medicine since ancient time. Ghana of a plant is refers to the aqueous extractable solid substance collected from herbal drug. EspeciallyGuduchiGhana (dried aqueous extract ofTinosporacordifolia) is commonly recommended in conditions like Jwara (fever), Daha (burning sensation) andPitta(Doṣharesponsible for regulating body temperature and metabolic activities) predominant disorders. The analytical techniques have always been mentioned in classical texts to understand the quality of the end product. However, qualitative and quantitative analysis of drugs by using the modern techniques and instruments of the science is also the need of time. Though,GuduchiGhana is widely used in practice, till date no published information available on analytical profile ofGuduchi Ghana prepared from GuduchiSwarasa(GGGS). Considering this, an effort has been made todevelop analytical profile of it.

## II. MATERIAL AND METHODS

### Procurement of raw material and its authentication

The fresh samples of Guduchi stem were procured from Junagadh, Gujarat in the month of March 2022 by adopting Good Collection Practices guidelines. Identification andauthentication of the Guduchi stem was done at Pharmacognocy laboratory of upgraded department of Dyavyaguna, Government Ayurved College Vadodara, Gujarat.

### Preparation of drug

All the samples of GGGSwere prepared in pharmaceutical laboratory of Upgraded Department of Rasashastra and BhaishajyaKalpana, Vadodara, Gujarat.A total of 3 batches of GGGS were prepared as per the reference of SharangadharaSamhita. Initially 20 kg fresh Guduchi stems were taken and cleaned well with water for 3 times.Then they were converted into small pieces (avg. size of 1 inch) with the help of cutter. After that the pieces of Guduchi stem were made into paste form in mixer by adding doublewater. Then paste was squeeze with the help of cotton cloth and green colored liquid (GuduchiSwarasa) was obtained. GuduchiSwarasa was subjected to the mild flame in s.s. vessel. Then it was stirred intermittently and temperature was measured. When consistency became thicker, temperature was gradually reduced. Heat was stopped when most of the water was evaporated. Ghana was collected in a food grade plastic lined s.s. tray and spread in very thin layer. Then it was subjected to dry in a hot air oven, at 50°C temperature for 3 hours.After complete drying it was scrapped and collected as Guduchi Ghana. After that it was stored into airtight glass container. The average yield percentage and time duration for the preparation were 5.25 % and 11 hours 36 minutes respectively.

### Analytical study

Fresh Guduchi stem and GGGS were analyzed by employing various analytical parameters. Organoleptic parameters, physicochemical parameters.Qualitative tests for various functional groups, quantitative estimations of total alkaloids, HPTLC profile under 366nm ultraviolet lightand tests for presence of certain heavy metals were also carried out.

### Organoleptic parameters

The organoleptic parameters of GGGS are evaluating the qualities of preparation by color, texture, test, odor and appearance through sense organs and it is providing the idea about the quality of formulations without using chemical tests. The final product was made of fine powder form. Results of organoleptic parameters are mentioned in Table 3.

### Physicochemical parameters

Different physicochemical parameters of Guduchi stem and GGGSwere carried out on all the three batches using standard API methods including pH value, loss on drying at 110°C, ash value, acid insoluble ash, specific gravity at 40°C, total solid content, water soluble extractives and alcohol soluble extractives at Quality control Laboratory of Upgraded Department of Rasashastra and BhaishajyaKalpana, Vadodara, Gujarat. Results of Physicochemical parameters are mentioned in Table 4-5.

### Test for various functional groups

The techniques employed to isolate active substance are termed as extraction method. Crude extracts obtained from such processes can be qualitatively tested to ascertain the presence of different types of components. Qualitative tests are used to detect the presence of functional groups and quantitative test for alkaloids used to calculate the percentage of total alkaloids content, which plays very important role in the expression of biological activity. Results of qualitative test for

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functional groups and quantitative test for alkaloids are mentioned in Table 6 .

**HPTLC Profile**

HPTLC is a sophisticated and automated form of TLC. HPTLC is an Invaluable quality assessment tool for the evaluation of botanical materials. It allows for the analysis of a broad number of compounds both efficiently and cost effectively. Additionally, numerous samples can be run in a single analysis thereby dramatically reducing analytical time with HPTLC, the analysis can be viewed using different wavelengths of light thereby providing a more complete picture of the plant than is typically observed with more specific types of analyses. HPTLC was carried out at Vasu Research Centre, Division of Vasu Healthcare PVT. LTD. Vadodara, Gujarat.

**Principle of HPTLC**

Principle remains the same as of TLC i.e. adsorption. One or more compounds are spotted in a thin layer of adsorbent coated on a chromatographic plate. The mobile phase solvent flows through because of capillary action. The component with more affinity towards stationary phase travels faster. Thus the components are separated on a thin layer chromatographic plate based on the affinity of the components towards the stationary phase.

**Instrument specification:**

**Table No. 1.** Instrument specification for HPTLC

| | |
|---|---|
| **Sample application device** | **4 spotter - Linomat 5** |
| **Company** | **CAMAG® Switzerland** |
| **Name of software** | Vision CATS |
| **Scanner** | HPTLC scanner 4 |
| **Photo documentation** | UV cabinet |
| **Run** | Twin through chamber |
| **Object support** | For objects up to 20 x 20 cm |
| **Stage drive** | Stepping motor 3200 steps/rotation, 8 steps = 0.1 mm Band length 0 (spot) - 190 mm in steps of 0.1 mm Speed approx. 10 mm/s. |
| **Dosage syringe drive** | Stepping motor 1600 steps/rotation<br>100 nL = 120 steps with 100 µL syringe<br>100 nL = 24 steps with 500 µL syringe |
| **Sample dosage syringe** | Selectable 100 µL or 500 µL |
| **Memory** | 10 methods, backup min. 10 years |
| **LCD display** | 2 lines of 16 characters ea. |
| **Power connection** | 85–250 V~ 47–63 Hz 30 VA. |
| **Dimensions (W x D x H)** | 360 x 510 x 410 mm |
| **Weight** | 12.5 kg |

Step involved in HPTLC were selection of chromatographic layer, Sample and standard preparation, Layer pre-washing, Layer pre-conditioning, Application of sample and standard, Chromatographic development, Detection of spots, scanning and Documentation of chromatic plate.

**Preparation Of Test Solutions**

Take 0.1gofsampleinatesttube and dilute itwith1mlofhexane.Mixwell. Use the upper hexane solution thus obtained for HPTLC fingerprinting. (Table no. 8)

**Table No. 2.** Chromatographic conditions of HPTLC

| | |
|---|---|
| **Application Mode** | CAMAG Linomat 5–Applicator |
| **Filtering System** | What man Filter paper no.1 |
| **Stationery Phase** | MERCK-TLC/HPTLC Silicagel 60F254on Aluminum sheets |
| **Application(Yaxis) Start position** | 10 mm |

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| | |
|---|---|
| **Development End position** | 80mmfromplatebase |
| **Sample Application Volume** | 5.0µl of each |
| **Distance Between Tracks** | 16 mm |
| **Development Mode** | CAMAGTLCT win Through Chamber |
| **Chamber Saturation Time** | 30 minutes |
| **Mobile Phase(MP)** | Petroleumether: Di-ethylether:Aceticacid (9:1:0.1v/v) |
| **Pre-chromatographic derivatization** | After sample spotting pre-chromatographic derivatization done with 5% Alcoholic KOH (2.0 µl)followedbyheatingtheplatefor10minuteson TLCPlateHeaterPreheatedat100± $5^0$C |
| **Visualization** | @254nm,@366nm (after derivatization) and@540 nm(after derivatization) |
| **Spray reagent** | 5% Sulphuric acid in Methanol |
| **Devatization mode** | CAMAG-Diptankabout1minute |
| **Drying Mode, Temp. & Time** | TLC Plate Heater preheated at100±$5^0$C for 3Minutes |

**Preparation of Sprayre agent [5% Sulphuricacid in Methanolre agent]:**5 ml Sulphuric acid is cautiously mixed with 100 ml Methanol.

**Tests for Heavy metals**

Heavy metal analyses were carried out byICP-OES Inductive Coupled Plasma Optical Emission Spectrometer (Make: Perkin Elmer Model: Optima 3300 RL) at Vasu Research Centre, Division of Vasu Healthcare PVT. LTD.Vadodara. Reagents used in this preparation were deionized water(resistivity > 18.2 M ohm cm), Metal stock solution 100 mg/L (Multi std. CPA Ltd. Bulgeria1000 mg/L Multi std. VHG Lab. USA), Hydrochloric acid (37% GR, Merck), Nitric acid (69%GR, Merck). (Table no. 10)

## III. OBSERVATIONSAND RESULTS

Organoleptic character observed in the fresh Guduchi stem, GuduchiSwarasaandGGGS. The most important analysis is organoleptic character because the palatability of a formulation is highly dependent on these characteristic.

Data of Table No.3 shows the variation in the color of fresh Guduchistem, Guduchi Swarasa and GGGS.

**Table No. 3:** Organoleptic characteristic of different sample of Guduchi

| Sr. No. | Ingredients | Color | Texture | Test | Odor | Appearance |
|---|---|---|---|---|---|---|
| 1 | FreshGuduchistem | Creamish brown | Soft, Slimy | Bitter | No specific | Solid |
| 2 | GuduchiSwarasa | Green | Soft, Slimy | Bitter | Characteristic | Liquid |
| 3 | GGGS | Creamish brown | Smooth | Bitter | Characteristic | Powder |

Physico chemical parameters are useful in set standards for a crude drug as these parameters are generally constant for a plant.

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**Table No. 4:**Physicochemical parameters of fresh Guduchi stem and GGGS

| Sr. No. | Parameters | Fresh Guduchi stem | GGGS | | |
|---|---|---|---|---|---|
| | | | Batch 1 | Batch 2 | Batch 3 |
| 1 | pH | 5.03 | 4.89 | 4.80 | 4.84 |
| 2 | Loss on drying (% w/w) | 75 | 6.11 | 06 | 6.12 |
| 3 | Ash value (% w/w) | 1.66 | 12.60 | 12.36 | 12.50 |
| 4 | Acid insoluble Ash (% w/w) | 1.07 | 0.56 | 0.40 | 0.98 |
| 5 | Water soluble extractive (% w/w) | 7.12 | 44.65 | 44.60 | 44.10 |
| 6 | Alcohol soluble extractive (% w/w) | 15.00 | 17.50 | 17.89 | 17.44 |

**Table No. 5:** Physicochemical parameters of GuduchiSwarasa

| Sr. No. | Parameters | Results | | |
|---|---|---|---|---|
| | | Batch 1 | Batch 2 | Batch 3 |
| 1 | pH | 6.61 | 6.62 | 6.60 |
| 2 | Specific gravity (%) | 1.006 | 1.004 | 1.006 |
| 3 | Total solid content (%) | 2.88 | 2.92 | 2.90 |

**Table No. 6:** Results of qualitative test for various functional groups

| Sr.No. | Tests for | Fresh Guduchi stem | GGGS |
|---|---|---|---|
| 1. | Alkaloids | +Ve | +Ve |
| 2. | Glycosides | +Ve | +Ve |
| 3. | Flavanoids | +Ve | -Ve |
| 4. | Tannin | +Ve | +Ve |
| 5. | Steroid | +Ve | +Ve |
| 6. | Terpanoids | +Ve | -Ve |
| 7. | Saponin | +Ve | +Ve |
| 8. | Carbohydrate | +Ve | -Ve |
| 9. | Protein | -Ve | -Ve |
| 10. | Starch | -Ve | -Ve |

+Ve =present, -Ve=absent

Table No.6 shows the results of the qualitative test carried out for various functional Groups. Presence of major active constituents of raw drugs into the finish product suggests the extraction of this functional group in the formulation.

**Table No. 7:** Total alkaloid content (%) in fresh Guduchi stem and GGGS

| Name of Sample | Total alkaloid content (%) |
|---|---|
| Fresh Guduchi stem | 0.57 |
| GGGS | 0.50 |

The alkaloids are insoluble sparingly in water but pass readily into solution on treatment with dilute acids with formation of soluble salts. From aqueous solution of their salts the free alkaloids are precipitated by basification (e.g. by alkali carbonates). In organic solvents such as ether or chloroform, alkaloids themselves dissolve freely, but their salts only sparingly. The % of total alkaloids content was 0.57, 0.50in fresh Guduchi stem and GGGS respectively as shown in table No. 7.

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**Table No. 8:** Rf values of Guduchi stem and GGGS under 366 nm

| Sample | Under 366 nm | | Sample | Under 366 nm | |
|---|---|---|---|---|---|
| | No. of spot | $R_f$ value | | No. of spot | $R_f$ value |
| Guduchi stem | 6 | 0.22 | GGGS | 6 | 0.18 |
| | | **0.31** | | | 0.22 |
| | | 0.58 | | | **0.31** |
| | | 0.66 | | | 0.51 |
| | | 0.77 | | | 0.58 |
| | | 0.80 | | | 0.80 |

HPTLC study of fresh Guduchi stem and GGGSwere carried out in comparison.At 366 nm 6 prominent spots bearing $R_f$ value 0.22. 0.31, 0.58, 0.66, 0.77, 0.80 in track of fresh Guduchi stem while 6 prominent spots bearing $R_f$ value 0.18. 0.22, 0.31, 0.51, 0.58, 0.80 in track of GGGScould be seen from thetable No. 8.(Figure no.1.)

**Table No. 9:** Percentage content of berberine estimated using HPTLC

| Sr. No. | Sample | Berberine (% w/w) |
|---|---|---|
| 1 | FreshGuduchi stem | 0.074 |
| 2 | GGGS | 0.11 |

In HPTLC peak areas and absorption spectra were recorded and the amount ofberberine was calculated using its calibration curve.

**Table No. 10:**HeavyMetalAnalysisoffinish products

| Sr.No. | HeavyMetal | GGGS | PermissibleLimitsasper API |
|---|---|---|---|
| 1. | Lead | 1.632 ppm | NMT10ppm |
| 2. | Cadmium | 0.019 ppm | NMT0.3ppm |
| 3. | Arsenic | 0.402 ppm | NMT3ppm |
| 4. | Mercury | 0.984 ppm | NMT1ppm |

The heavy metal content in the analysis falls within acceptable limits as per the results of the heavy metal test.

## IV. DISCUSSION

Raw drug was procured in month of March 2022. AcharyaYadavjiTrikamji has mentioned to use ‘Angusthapramana’ (thumb size) of Guduchi stem, accordingly thumb sized or medium size stem diameter(1.6-2.0 cm) was selected for study.It was authenticated and analyzed before processing because good quality products mainly depend on genuine raw materials.

The organoleptic characters, which correspond to the Panchagyanedriya Pariksha (perception by five sense organs) of Ayurveda, were performed at three stages of preparation (for fresh Guduchi stem, GuduchiSwarasa and powdered Ghana), because these parameters can change at different stages (Table no. 3). Data shows the variation in the color of fresh Guduchistem and Guduchi Ghana. Due to absence of Terpanoids color of GGGS was changed to creamish brown. Terpanoids are the most abundant group of compound in Guduchi these compounds are involved in giving plants their colors. Pigments are the responsible for natural spectacular color of plants. Red-yellow betalains, green chlorophylls, red-purple anthocyaninand yellow-orange carotenoids are the most common pigments found in vegetables and fruits. Apart from their coloring properties of Guduchi exhibit potential health-promoting functions. GGGS was bitter in taste because sample having more concentration of berberine (0.11%)

Observations of physicochemical data are tabulated in Table no. 4-5. Here, mean pH of GuduchiSwarasa (6.61) and different batches of GGGS (4.84) showed significant difference, which indicate few chemical changes were occurring on this phase between solvent and solute, which leads the change in pH. Loss on drying value of fresh Guduchi stem was 75.00 % w/w. It is because of high moisture content in green state. Average value of loss on drying was 6.07 % w/w in GGGS. Material absorbs moisture during the storage. In conjunction with a suitable temperature moisture will lead to the activation of enzymes and given

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suitable condition to the proliferation of living organism. Hence, moisture contents may affect the quality of the drug. Although the weight loss in the samples is principally due to water, small amount of other volatile materials will also contribute to the weight loss. Loss on drying values was high in GGGS sample may be due to hygroscopic nature of Ghana. Therefore, it must be stored in damp proof polythene covers or glass containers.The average ash values (% w/w) of freshGuduchi stem and GGGS was 1.66 and 12.48, respectively. The total ash figure is important as it indicates to some extent the amount of care taken in the preparation of the drug. The total ash usually consist mainly carbonates, phosphates, silicates and silica. Lower value of ash value in prepared drug indicates presence of very fewer inorganic materials in it. Also the value of water soluble extract (% w/w) of GGGS (44.45) was more than fresh Guduchi stem (7.12) indicating role of AgniSamsakara(heat transform) in extraction. There is no much difference in alcohol soluble extractive value of freshGuduchi stem andGGGS sample indicating no role of heating process in changing the alcohol soluble extractive values.

Qualitative tests were done to detect the presence of functional groups and presence of major active constituents of raw drugs into the finished product suggests the extraction of these moieties in the formulation. The methanolic extract of the sample was used for analysis [Table no.6]. The study reveals the absence of Flavonoids and Terpanoidsin GGGS. Because both are very heat sensitive so due to heating process Flavonoids and Terpanoids were destroyed.' It can be anticipated that, presence of alkaloids as functional group might be responsible in the expression of biological activity of the formulation. Total alkaloid contents are shown in Table no.7.

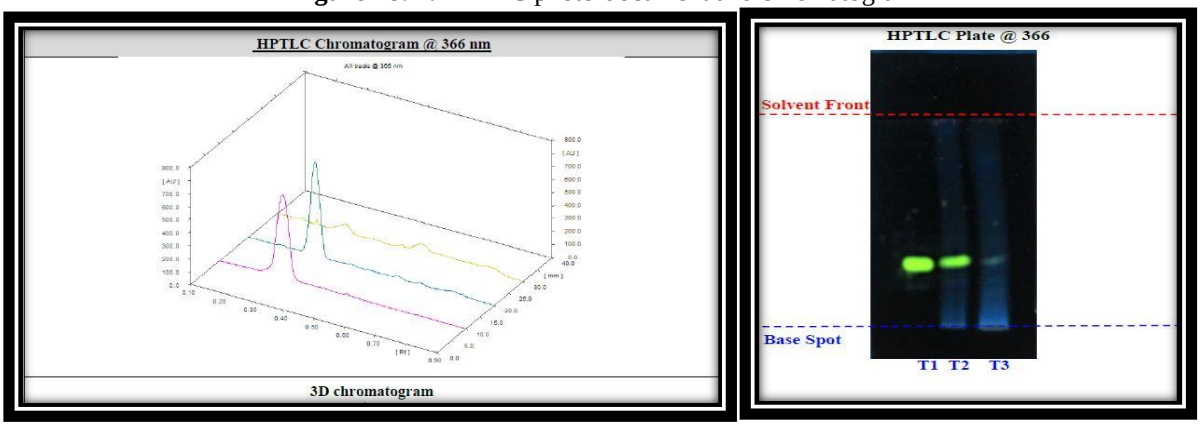
Chromatographic study (HPTLC) was carried out under 366 nm UV to establish the fingerprinting profile. It showed phyto-components with Rf values 0.18, 0.22, 0.31, 0.51, 0.58 and 0.80 in GGGS sample, which may be responsible for expression of its pharmacological and clinical actions.

The heavy metal content of GGGS was carried out to finally fulfill and establish the quality standard at finished product level. No heavy metals were detected [Tables no. 9].

## V. CONCLUSION

The investigation demonstrates adherence to adequate quality control protocols in the development of a novel Ayurvedic preparation. The analysis encompassed fundamental analytical elements of the sample formulation, including examination of phytochemical parameters and HPTLC fingerprinting. These standards serve to establish a comprehensive set of diagnostic criteria, ensuring the identity and authenticity of the formulation. The HPTLC profile generated in this study represents a refined and standardized tool for verifying the genuineness and authenticity of Guduchi Ghanaprepared from GuduchiSwarasa.The present observation can be considered as standard for further studies.

**Figure no. 1:** HPTLC photo-document and chromatogram




**Track T1:** Berberine Standard **Track T2:** Fresh *Guduchi* stem **Track T3:** GGGS

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