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The Pharmacognostical and Pharmaceutical evaluation of *Sri Bahusala Guda* – An Ayurvedic Formulation

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ABSTRACT

INTRODUCTION: The Standardization of Ayurvedic formulation is essential to assess the quality of drugs. This study involves *Sri Bahusala Guda*, an *Avaleha* Semisolid preparation, which is prepared by a standardized method. The formulation was standardized using modern scientific parameters in finished products. The standardization of *Avaleha* included organoleptic study and physicochemical parameters such as pH, loss of dry, ash value, total sugar and HPTLC.

MATERIALS AND METHODS: Drug and Preparation obtained in GMP certified Pharmacy. Formulation was prepared by traditional method of ayurveda. This present study aimed to standardize the finished product of *Sri Bahusala Guda* (SBG) by comparing it with API standards through a comprehensive analysis, including physio-chemical analysis and phytochemical constituents (identification and quantification of bioactive compounds). **OBSERVATIONS AND RESULTS:** The present study shows organoleptic features of *Sri Bahusala Guda* were within the standard range. The pH value 5.29, water soluble extract value was 100.00, loss of drying was 12.81, and reducing sugar was 4.62 and HPTLC at 620nm after derivation with VSA. **CONCLUSION:** *Sri Bahusala Guda avaleha* is acidic in nature. Loss on drying as humid more. Total Ash value and acid insoluble ash, the results indicate that no adulteration was found in the sample content. HPTLC profile - result showing total 9spots, it may indicate higher quality and purity of the sample. This study will help in understanding the quality, safety and also within the standard parameters.

Key Words Pharmacology, *Sri Bahusala Guda*, Phytochemical analysis

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INTRODUCTION

Pharmacology It can be defined as the study of substances that interact with living system through chemical processes, especially by binding to regulatory molecules and activating or inhibiting normal body processes[1].

Avaleha or *Lehya* is a semi solid preparation of herbal drugs, prepared in *kashaya* by adding sweetening agents like jaggery, sugar or sugarcandy. It is also known as *Modaka, Guda, Khanda, Rasayana* or *Leha*[2]. *Guda paka kalpana* is one among the *Upakalpana* of *Avaleha*, it can

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be either in *Avaleha, Khanda,* or *Modaka* form. Considering its major quantity as *Guda*[3].

Ayurvedic classics have broadly explained many formulations related to different diseases. *Sri Bahusala Guda* is one such formulation explained in *Chakradatta's Arsaroga chikitsa adhyaya*. It consists of *Trivrt, Surnakhanda, Haritaki, Tejovati, Gokshura, Vidunga* etc drugs which is having therapeutic action on *Arsas, Amavata, Gulma, Grahani, Rasayana* and also includes *Pratishyaya*[4].

The present study is aimed to standardize and analyze the physico chemical properties of the final product *Sri Bahusala Guda*.

MATERIALS & METHODS

Drug source & Preparation – All ingredients were collected and the preparation was carried out at the GMP-certified SDM Pharmacy, Udupi. Method of preparation adopted as standard procedure from AFI.

Loss on drying at 105°C[5]

A 10 g sample was placed in tared evaporating dish. It was dried at 105˚C for 5 hours in a hot air oven and then weighed. The drying continued until the difference between two successive weights was no more than 0.01g after cooling in desiccators.

Total Ash[6]

A sample weighing 2g was incinerated in a tared platinum crucible at temperature not exceeding 450˚C until carbon free ash was obtained.

Acid insoluble ash[7]

Add 25 ml of dilute HCl to the crucible containing the total ash and boil the mixture. Collect the insoluble matter on ashless filter paper and wash with hot water until the filtrate is neutral. Transfer the insoluble matter to the crucible, dry it and weigh it. Allow to cool in suitable desiccators for 30 mins and weigh it without delay.

Water soluble ash[8]

Boil the ash for 5 min with 25 ml of water; collect the insoluble matter on filter paper and wash with hot water, and ignite for 15 min at a temperature not exceeding 450˚C.

Alcohol soluble extractive[9]

Weigh 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled Alcohol. Shake occasionally for 6 hours and allow standing for 18 hours. Filter it, taking care not to lose solvent. Pipette out 25ml of the filtrate in a 100 ml beaker. Evaporate to dryness on a water bath, kept in an air oven at 105°C for 6 hours, and allow cooling in desiccators for 30 minutes and weighing.

Water soluble extractive[10]

Weigh 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled water shake occasionally for 6 hours and allow standing for 18 hours. Pipette out 25ml of the filtrate in a 100 ml beaker. Evaporate to dryness on a water bath. Keep it in oven at 105°C for 6 hours. Cool in desiccators and weigh.

Total solids[11]

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Weighed 50 g of the sample to an evaporating china dish, that has been dried to a constant weight and evaporate to dryness on a water bath, then dry at 105^0C for 3 hr. After cooling for 30 min, weigh it.

Determination of pH[12]

Preparation of buffer solutions:

Standard buffer solution: Dissolved one tablet each of pH 4, 7 and 9.2 in 100 ml of distilled water.

Determination of pH: 1 ml of the sample was taken and diluted with 10 ml of distilled water. The mixture was stirred well, filtered, and used for the experiment. The pH meter was switched on and allowed to warm up for 30 minutes. The pH 4 solution was then introduced and adjusted to 4.02 at room temperature (30°C) using the control knob. The pH 7 solution was added next and adjusted to 7 using the knob. Finally, the pH 9.2 solution was introduced, and the reading was checked without any adjustment.

Total fat[13]

Weighed 5 g of the sample and placed it in a thimble, which was then inserted into a Soxhlet apparatus fitted with a condenser. Added 90 ml of petroleum ether to a 150 ml round-bottom flask and boiled for 6 hours. The extract was transferred to a pre-weighed conical flask, and the petroleum ether was evaporated using a water bath. Any remaining traces of petroleum ether were removed with a vacuum pump.

Reducing and non reducing sugar[14]

10 g of the sample was placed in a 250 ml volumetric flask, and 200 ml of water was added. To remove tannins, a slight excess of solid basic lead acetate was added. The solution was then made up to the mark with water without disturbing it, and filtered. To remove any excess basic lead acetate, solid sodium oxalate was added, shaken, and the solution was filtered again.

***Reducing Sugar*:** The sugar solution was prepared and filled in a 50 ml burette.

***Preliminary Titration*:** 10 ml of Fehling's solution was pipette into a 250 ml conical flask, and 15 ml of the sugar solution was added from the burette. The mixture was boiled on asbestos-covered gauze, and the sugar solution was added in 10-15 second intervals to the boiling liquid until the blue colour nearly disappeared. 3-5 drops of methylene blue solution were then added, and the boiling continued until the solution was completely decolorized.

Accurate Titration: The entire amount of sugar solution required to reduce the copper was added during the titration. The mixture was gently boiled for two minutes. 3-5 drops of methylene blue were added, and the boiling continued for a total of three minutes. At the endpoint, the blue color should be completely discharged, and the solution should turn red.

Total Sugar: 20 ml of the reducing sugar solution was mixed with 10 ml of concentrated hydrochloric acid and left overnight. The solution was then neutralized with approximately 1 M sodium hydroxide solution or solid sodium carbonate, and the volume was adjusted to 100 ml in a volumetric flask.

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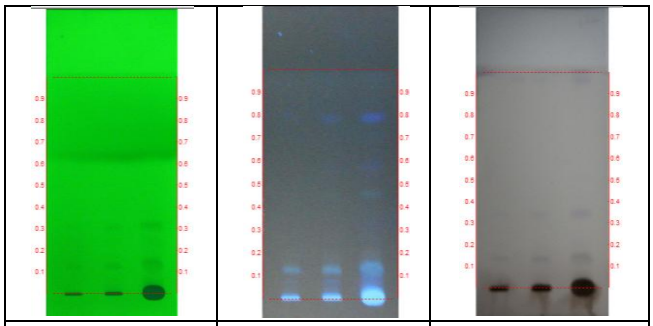
HPTLC (*Sri Bahusala Guda*)[15]

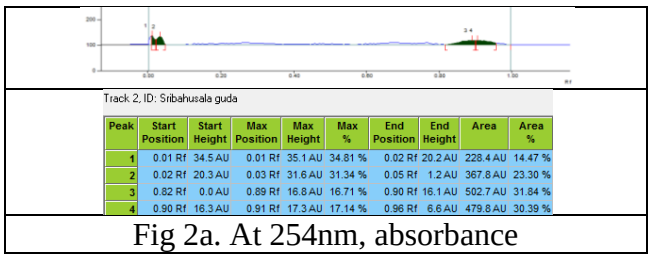
Dissolve 1 g of the sample in 20 ml of n-butanol and evaporate the soluble portion to dryness. The residue was then dissolved in 10 ml of ethanol. Using a Linomat 5 TLC applicator, 3, 6, and 9 µl of the sample were applied onto pre-coated silica gel F254 aluminium plates, with a band width of 7 mm. The plate was developed in a solvent mixture of toluene: ethylacetate (9.0:1.0). After development, the plate was visualized under short and long UV light, and then scanned at 254 nm and 366 nm. It was subsequently derivative with vanillin-sulphuric acid reagent and scanned at 620 nm. The R_f values, spot colours, and densitometry scans were recorded and shows in Figure 1&2.



Short UV	Long UV	After derivatisation (VSA)

Track 1 – Sri Bahusala guda – 3µl
Track 2 – Sri Bahusala guda – 6µl
Track 3 – Sri Bahusala guda – 9µl
Solvent system – Toluene: Ethyl acetate (9.0: 1.0)

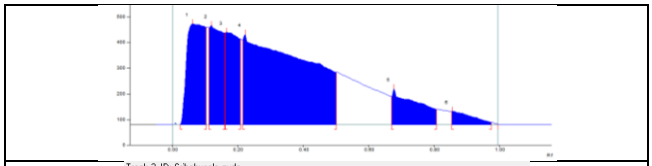
Figure 1 HPTLC photo documentation of ethanolic fraction of *Sri Bahusala Guda*



Track 2, ID: Sribahusala guda

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	34.5 AU	0.01 Rf	35.1 AU	34.81 %	0.02 Rf	20.2 AU	228.4 AU	14.47 %
2	0.02 Rf	20.3 AU	0.03 Rf	31.6 AU	31.34 %	0.05 Rf	1.2 AU	367.8 AU	23.30 %
3	0.82 Rf	0.0 AU	0.89 Rf	16.8 AU	16.71 %	0.90 Rf	16.1 AU	502.7 AU	31.84 %
4	0.90 Rf	16.3 AU	0.91 Rf	17.3 AU	17.14 %	0.96 Rf	6.6 AU	479.8 AU	30.39 %

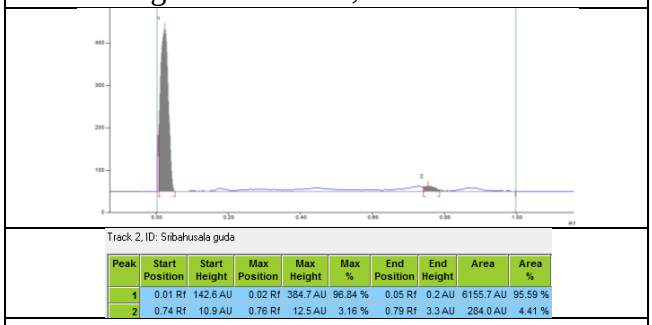
Fig 2a. At 254nm, absorbance



Track 2, ID: Sribahusala guda

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	0.6 AU	0.06 Rf	393.1 AU	23.39 %	0.10 Rf	79.3 AU	15318.1 AU	16.13 %
2	0.11 Rf	376.1 AU	0.12 Rf	385.7 AU	22.95 %	0.16 Rf	56.9 AU	11078.9 AU	11.67 %
3	0.16 Rf	357.1 AU	0.17 Rf	358.5 AU	21.33 %	0.21 Rf	33.1 AU	10481.8 AU	11.04 %
4	0.22 Rf	327.9 AU	0.22 Rf	351.4 AU	20.91 %	0.50 Rf	03.6 AU	47918.8 AU	50.46 %
5	0.67 Rf	109.0 AU	0.68 Rf	139.6 AU	8.31 %	0.81 Rf	60.3 AU	7756.1 AU	8.17 %
6	0.85 Rf	51.8 AU	0.86 Rf	52.2 AU	3.11 %	0.98 Rf	9.6 AU	2413.9 AU	2.54 %

Fig 2b. At 366nm, fluorescence



Track 2, ID: Sribahusala guda

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	142.6 AU	0.02 Rf	384.7 AU	96.84 %	0.05 Rf	0.2 AU	6155.7 AU	95.59 %
2	0.74 Rf	10.9 AU	0.76 Rf	12.5 AU	3.16 %	0.79 Rf	3.3 AU	284.0 AU	4.41 %

Fig 2b. At 620nm (after derivatisation with VSA)

Figure 2 Densitometric scan of *Sri Bahusala Guda*

HPTLC Remarks

The given sample of *Sri Bahusala Guda* has been standardized as per API specification. HPTLC photodocumentation. Rf values, Densitometric scan are given in respective table no 2 and figures are shown in fig 1&2.

DISCUSSION

Standardization of herbal medicine is crucial for analysis drug identification, quality of final products and detecting adulterations. Physicochemical parameters were compared with API values and present study vales are shown in table 1, revealing the following results:

The pH was measured to analyse the acidity or alkalinity of the sample. This helps to understanding the pharmacological basis of drug absorption and metabolism. In this sample pH is 5.29% (shown in table 1), it is acidic in nature[16].

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Value of Loss on drying formulation at 105^0, it indicates there is presence of moisture and the value obtained as 12.81% (shown in table 1) which was acceptable.

Total Ash represents inorganic contents in formulation whereas acid insoluble ash indicates insoluble inorganic content. Higher inorganic content indicates adulteration of ingredients. Total Ash value and acid insoluble ash of sample is 2.49% and 0% respectively (shown in table 1). The results indicate that no adulteration was found in the sample content[17].

Water and Alcohol soluble extractive value indicate the therapeutic efficacy of the sample. High concentration of compounds, which may contributes to the therapeutic efficacy. The water soluble extractive value of sample is 24.39% (shown in table no 1)and the alcohol soluble extractive value is100%. These results suggest that the ample has high therapeutic efficacy. A water soluble extractive value greater than 70% and an alcohol soluble extractive value greater than 20% indicates a high concentration of active compounds[18].

The reducing sugar value indicates the presence of glucose, fructose and maltose, while the non reducing sugar value suggests the presence of sucrose and lactose. These values are crucial foe assessing the therapeutic properties, shelf life, quality control, and detecting adulteration in the sample. The total sugar content was 23.10%, with reducing sugar was 4.62% and non reducing sugars 18.48% (values shown in table no 1). These results indicate that the sample has therapeutic efficacy and to prevent spoilage within acceptable limits[19].

HPTLC is used to identify and quantify the various phytoconstituents present in the herbal preparation. The number of spots corresponds to the number of phytoconstituents in the sample, while R_f values reflect the migration of these constituents, aiding in the identification of the sample. The R_f value range is 0.00 – 1.00. At UV 254nm, aromatic compounds and flavinoids are detected, while UV366nm identifies alkaloids and glycosides. Visible light is used to detect coloured compounds[20].

In HPTLC, a smaller number of spots (5-10) mentioned in fig 1&2 generally indicate a simple composition, suggesting higher quality and purity, which can help in standardizing the herbal formulation. Conversely, a large number of spots (15-20) may suggest a more complex mixture of phytoconstituents, possibly indicating impurities in the sample.

For this sample, two spots of R_f values 0.12 and 0.30 were observed under short UV (254nm). In long wave UV (366nm), five spots with R_f values of 0.12, 0.22, 0.46, 0.58 and 0.78were detected. After derivatization with VSA, two spots with R_f values of 0.12 and 0.35 were observed under UV 620nm. In total, 9spots were detected in the HPTLC profile, which suggests a high quality and purity of the sample, with minimal or no impurities. (values in table no 2 and figures in Fig 1&2)

CONCLUSION

The present study demonstrates that the preparation dissolves completely in water. *Sri Bahusala Guda avaleha* is acidic in nature. The loss of drying indicates higher humidity content. The HPTLC profile revealed 9 spots, indicating the presence of variety of therapeutically active ingredients, which reflects its high quality and purity. The alcohol and water soluble extractive value further suggest that the sample has significant therapeutic efficacy. As *Sri Bahusala Guda* contains a higher concentration of active ingredients, this study validates its therapeutic potential, supporting its use through well established analytical, experimental and clinical research.

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