ORIGINAL ARTICLE

Impact of Bhavana on *Laghu Sutashekhara Rasa* - A promisable formulation in *Ardhavabhedaka* (migraine)

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Abstract

Aim: To evaluate the impact of *Bhavana* on *Laghu Sutashekhara Rasa* (LSR). **Materials and Methods:** The raw drugs are identified and authenticated, and powder microscopy was done in the Pharmacognosy Laboratory, Institute for Post Graduate Teaching and Research in Ayurveda (IPGT), Gujarat Ayurved University (GAU), Jamnagar, Gujarat, India. The study includes organoleptic evaluation and microscopic evaluation. Physico-chemical parameters and high-performance thin layer chromatography (HPTLC) studies carried out at pharmaceutics laboratory, IPGT and RA, GAU, Jamnagar, Gujarat, India. LSR was levigated with *Swarasa* of *Nagavalli* (*Piper betle* Linn.) to prepare *Bhavita Laghu Sutashekhara Rasa* (BLSR). Both the samples were subjected to accelerated stability by following standard guidelines. **Results and Discussion:** The pharmacognostical results of LSR showed oleoresin content of *Sunthi*, dark brown content of *Gairika,* etc. Contents of BLSR showed Trichome of betel leaves loaded by *Gairika,* stomata with epidermal cells of betel leaves, fragments of annular vessels of *Sunthi,* etc. Physico-chemical analysis of LSR revealed loss on drying 1.0376% w/w, water-soluble extract 1.32% w/w, etc. Physico-chemical analysis of BLSR revealed loss on drying 1.8793% w/w, water-soluble extract 4.5% w/w, etc. In HPTLC, almost all spots were merging in both samples showing common characters in both the wavelengths. **Conclusion:** Disturbed walls ruptured cellular particles of ingredients signify that *Bhavana* incorporates additional therapeutic attributes and also increases the potency of drug.

Key words: *Bhavana*, high-performance thin layer chromatography, *Laghu Sutashekhara Rasa,* pharmacognosy, physico-chemical

INTRODUCTION

Laghu Sutashekhara Rasa (LSR) is a herbo-mineral formulation. It is promisable formulation in the management of *Ardhavabhedaka* (Migraine) and acid peptic disorders. It is one such prime formulation which takes corrective action on the *Pitta Dosha*. LSR - a herbo-mineral-powder formulation has been mentioned in *Rasatarngini*.[1] The trial compound has only two contents, which are *Gairika* (Fe_2O_3) and *Sunthi* (*Zingiber officinale* Roxb.). Both contents are easily available. Individual drugs *Gairika* and *Sunthi* were powdered and blended in specified ratio, i.e., 2:1, respectively, to obtain LSR. LSR was levigated with *Swarasa* of *Nagavalli* (*Piper betle* Linn.) to prepare *Bhavita Laghu Sutashekhara Rasa* (BLSR). Proper identification of raw materials at the basic level with the help of microscopic and morphological characteristics and adequate analytical methods are

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essential to ensure the quality and standards of the prepared medicine. Here, an attempt is made to observe the changes in microscopic examination and physico-chemical parameters after levigation process by *Nagavalli Swarasa*. Previously, some worked carried out on the concept of *Bhavana*[2,3] and also on physico-chemical parameters of LSR tablets[4] but till date; there is no data available regarding pharmacognostical evaluation of LSR and especially on the effect of *Nagavalli Swarasa* by *Bhavana* (levigation process). With this background, LSR with levigation process and without levigation process was subjected for pharmacognostical and physico-chemical analysis.

MATERIALS AND METHODS

Plant Material

The raw drug materials were collected from the Pharmacy Department, Institute for Post Graduate Teaching and Research in Ayurveda (IPGT), Gujarat Ayurved University (GAU), Jamnagar, Gujarat, India.

Pharmacognostical Evaluation

The raw drugs are identified and authenticated, and powder microscopy was done in the Pharmacognosy Laboratory, IPGT and RA, GAU, Jamnagar, Gujarat, India. The study includes organoleptic evaluation and microscopic evaluation.[5]

Microscopic Study

The individual powered drug is first examined under distilled water for the observation of calcium oxalate crystals and other cellular materials, then stained with phloroglucinol and conc. HCl[6] for the lignified characters, then stained with iodine to observe the starch grains. Raw drugs were separately studied under microscope; the diagnostic characters microphotographs are taken using Carl Zeiss Trinocular microscope.[7]

Organoleptic Study

Contents of LSR and BLSR were evaluated for organoleptic characters such as taste, odor, and color.[8]

Physico-chemical Parameters

LSR and BLSR were analyzed at the Pharmaceutical Chemistry Laboratory of IPGT and RA, Gujarat Ayurved University, Jamnagar. The common parameters mentioned for in Ayurvedic pharmacopoeia of India[9] and CCRAS[10] guidelines were considered for pharmaceutical evaluation. The presence of more moisture content in a sample may create preservation problem. Hence, loss on drying was also selected as one of the parameters.

High-Performance Thin Layer Chromatography (HPTLC) Profile[11]

Instrument used was CAMAG make HPTLC with WINCATS 1.4.3 software and Linomat 5 sample applicator. The stationary phase used was HPTLC plates silica gel 60 F254, and mobile phase was toluene:Et. acetate (8:2). The sample was prepared in methanol, and 2 µl sample was applied as 8 mm band for each spot. The plate was visualized under short and long ultraviolet (UV) radiations, and density of the separated spots was recorded using scanner III. The plate was sprayed with vanillin-sulfuric acid reagent and observed in daylight. The R_f values were recorded in 31°C temperature and 48% humidity.

RESULTS AND DISCUSSION

Microscopic Study

Diagnostic characters of LSR showed oleoresin content of *Sunthi*, oil globule of *Sunthi*, silica deposition of *Gairika*, starch grain of *Sunthi*, fibers of *Sunthi*, brown content of *Sunthi*, cork in surface view of *Sunthi*, cork in tangential view of *Sunthi*, dark brown content of *Gairika,* and parenchyma cells with brown content of *Sunthi* [Plate 1 - Figures 1-10]. Diagnostic characters of BLSR showed starch grains of *Sunthi* and other particles of *Gairika. Gairika* particles are loaded on the cellular particles of *Sunthi*, Trichome of betel leaves loaded by *Gairika,* oil globule of betel leaves, stomata with epidermal cells of betel leaves, prismatic crystals of betel leaves, fragments of annular vessels – *Sunthi,* disturbed walled cork cells of *Sunthi* loaded by *Gairika* [Plate 1 - Figures 11-18].

The boarders of cellular constituents of *Sunthi* and *Nagavalli* are damaged due to levigation process, and it also increases the potency of the drug.

Pharmacognostical Evaluation

Organoleptic characters of LSR

On comparison, both the samples are brick red and aromatic odor with light crispy sound. BLSR is found astringent with piercing nature and hard touch in comparison of LSR [Table 1].

Physico-chemical tests

Both the samples LSR and BLSR have same pH value which suggests slide acidic nature of medicine. Ash values are helpful in determining the quality and purity of crude drugs, especially in powder form. The objective of ashing vegetable drugs is to remove all traces of organic matter, which may otherwise interfere in an analytical determination[12]

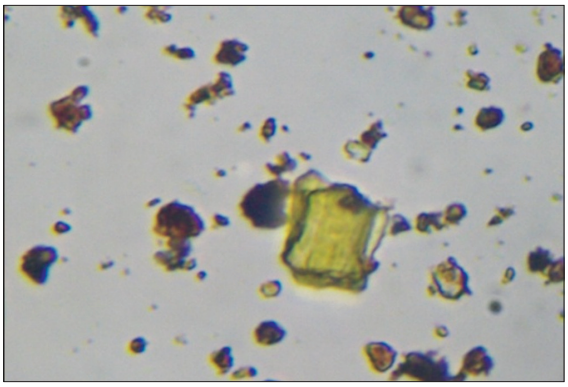

Figure: 1 Oleoresin content – *Sunthi*

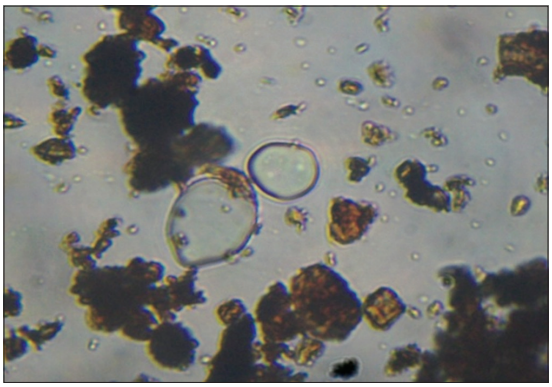

Figure 2: Oil globule – *Sunthi*

Figure 3: Silica deposition – *Gairika*

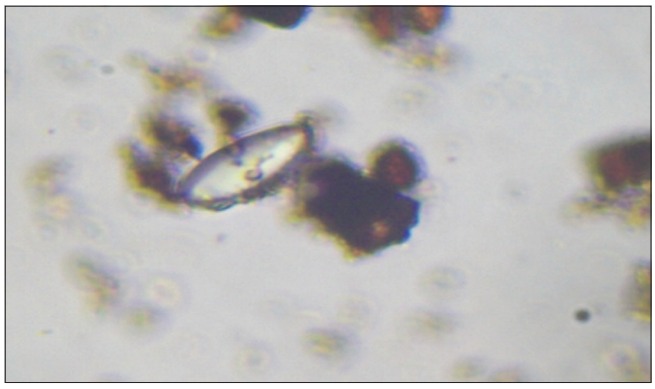

Figure 4: Starch grain – *Sunthi*

Figure 5: Fibers – *Sunthi*

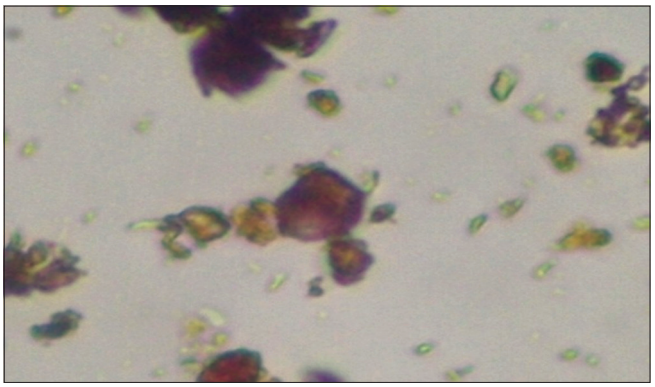

Figure 6: Brown content – *Sunthi*

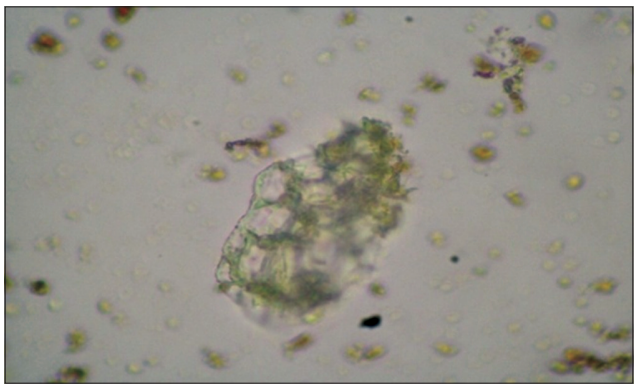

Figure 7: Cork in surface view – *Sunthi*

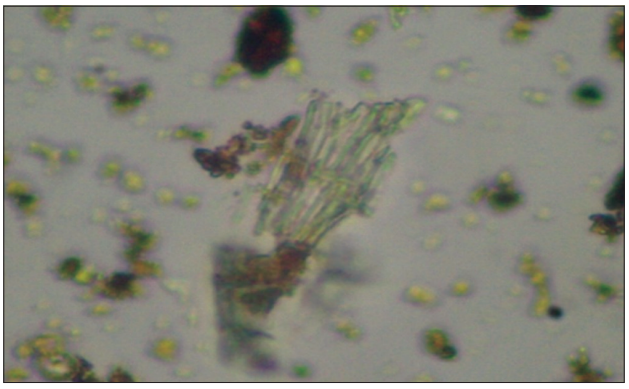

Figure 8: Cork in tensential view – *Sunthi*

Plate 1: Microphotographs of *Laghu Sutashekhara Rasa*

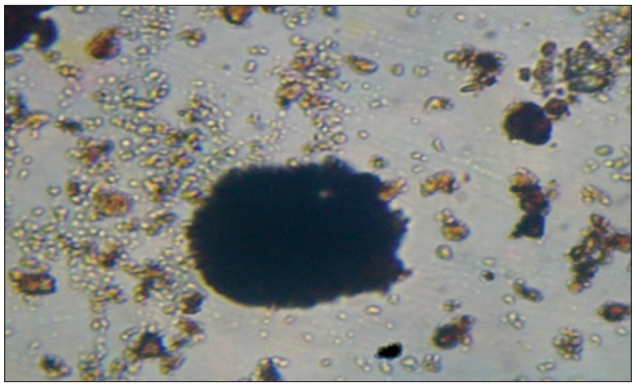

Figure 9: Dark brown content – *Gairika*

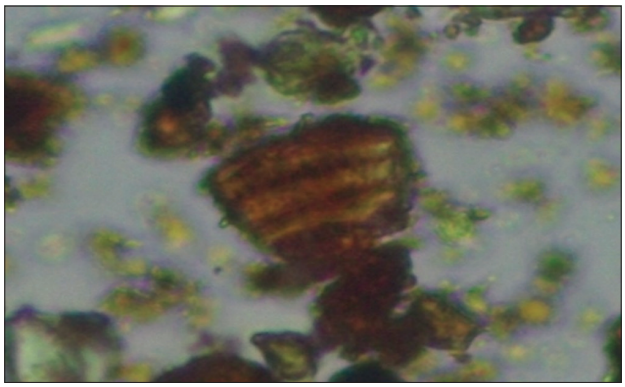

Figure 10: Parenchyma cells with brown content – *Sunthi*

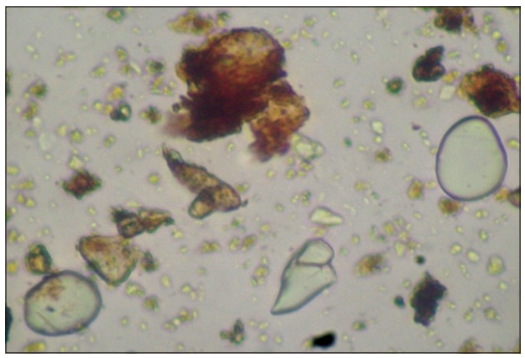

Figure 11: Starch grains of *Sunthi* and another cellular particles of *Gairika*

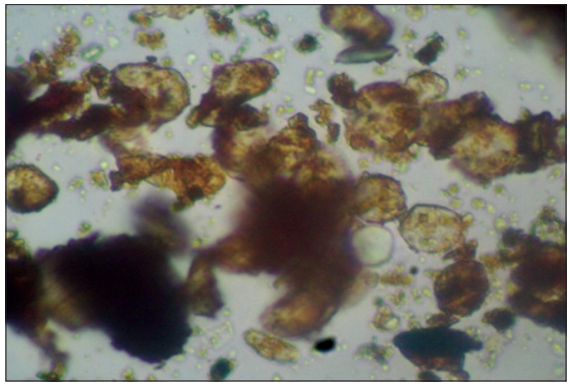

Figure 12: *Gairika* particles are loaded on other particles of *Sunthi*

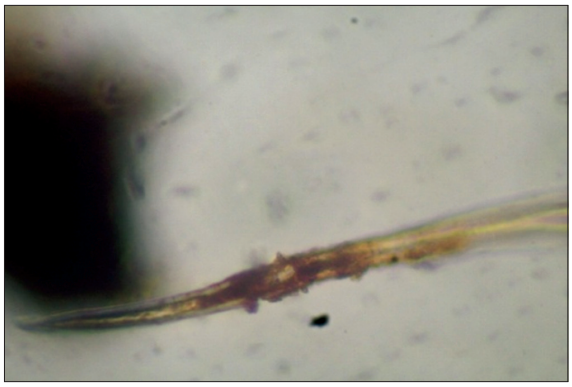

Figure 13: Trichome of betel leaves loaded by *Gairika*

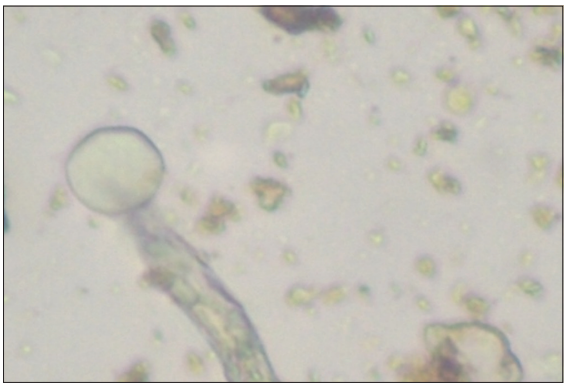

Figure 14: Oil globule of betel leaves

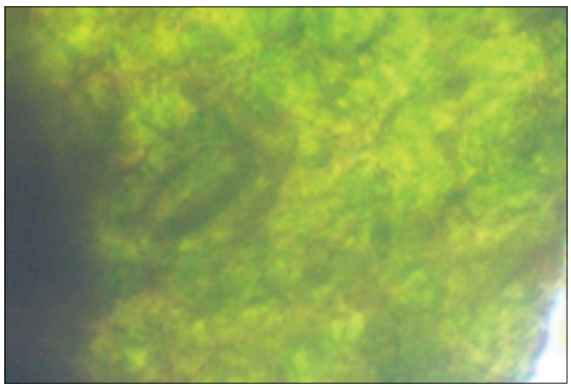

Figure 15: Stomata with epidermal cells of betel leaves

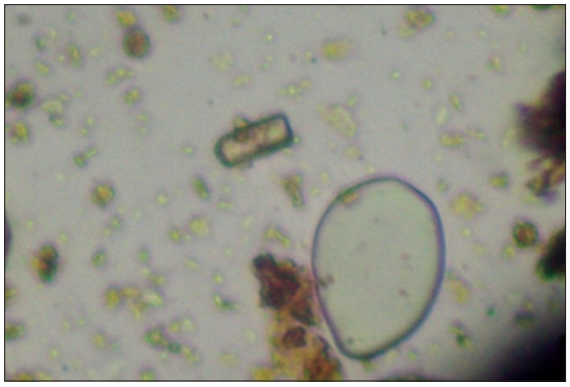

Figure 16: Prismatic crystals of betel leaves

Plate 1: *Continued*....

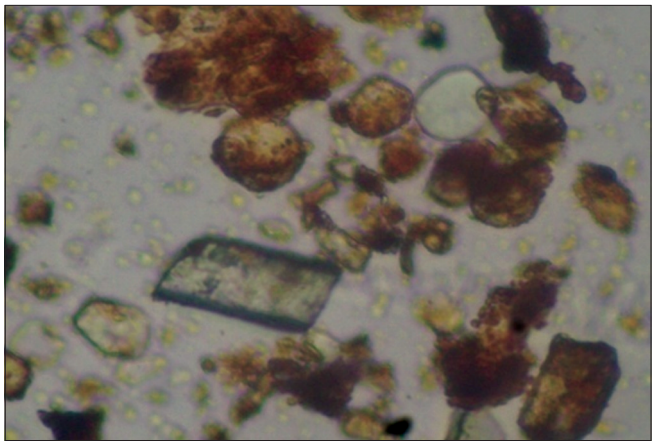

Figure 17: Fragments of annular vessels – *Sunthi*

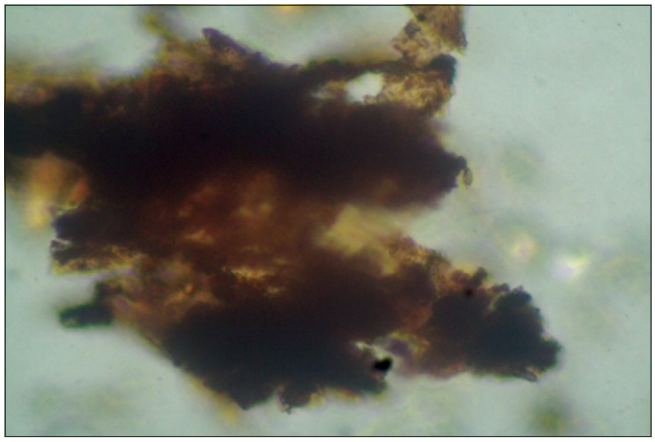

Figure 18: Cork cells of Sunthi loaded by *Gairika*

Plate 1: *Continued....*

and extracting values are useful for determining of crude drugs and it gives an idea about the nature of the chemical constituents present.[13] The less value of moisture content of drugs could prevent content bacterial, fungal, or yeast growth through storage.[14] For present study, the values for LSR and BLSR are ash values are 71.62% w/w and 61.639% w/w, acid insoluble ash value 62.062% w/w and 48.80% w/w, water-soluble extract are 1.32% w/w and 4.5% w/w, and methanol-soluble extract are 1.48% w/w and 2.28% w/w, loss on drying values are 1.0376% w/w and 1.8793% w/w, iron estimation values are 7.3% w/w and 7.8% w/w, respectively [Table 2].

HPTLC study results

Chromatographic study (HPTLC) was carried out under 254 and 366 nm UV to establish fingerprinting profile. Results showed 4 spots at 254 nm and 6 spots at 366 nm in LSR and 6 spots at 254 nm and 7 spots at 366 nm in BLSR with R_f values were recorded which may be responsible for expression of its pharmacological and clinical actions, i.e., 0.71 for LSR and 0.9 for BLSR [Plate 2].

In HPTLC, almost all spots were merging in both sample showing common characters in both the wavelengths and it

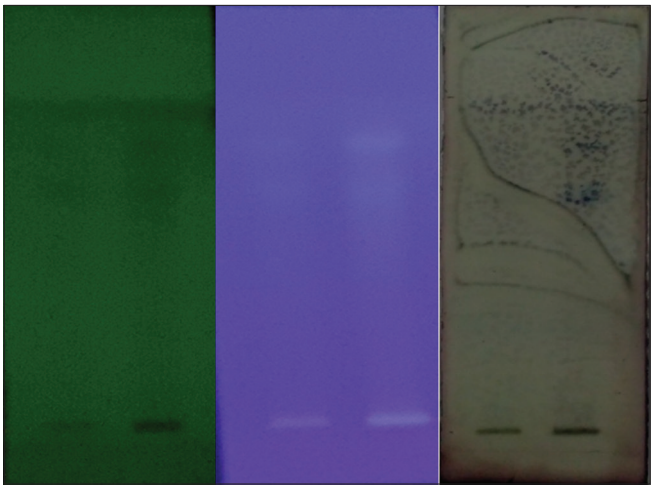

Plate 2: Visualization of *Laghu Sutashekhara Rasa* and *Bhavita Laghu Sutashekhara Rasa* at 254, 366 nm and after visualizing agent

Table 1: Organoleptic characters of samples

Characters	Observed characters of LSR	Observed characters of BLSR
Color	Brick red	Brick red
Odor	Aromatic	Aromatic
Taste	Astringent	Astringent with piercing nature
Touch	Fine powder	Hard
Sound	Light crispy	Light crispy

LSR: *Laghu Sutashekhara Rasa*, BLSR: *Bhavita Laghu Sutashekhara Rasa*

Table 2: Physico-chemical evaluation

Test	LSR	BLSR
Ash value	71.62% w/w	61.639% w/w
Acid insoluble ash value	62.062% w/w	48.80% w/w
Loss on drying	1.0376% w/w	1.8793 w/w
Water-soluble extract	1.32% w/w	4.5% w/w
Alcohol-soluble extract	1.48% w/w	2.28% w/w
pH	6.5	6.5
Iron estimation	7.3% w/w	7.8% w/w

LSR: *Laghu Sutashekhara Rasa*, BLSR: *Bhavita Laghu Sutashekhara Rasa*

reflects the presence of flavonoids components in product [Plates 3 and 4, Tables 3 and 4].

CONCLUSION

Pharmacognostical and pharmaco-chemical evaluation of LSR and BLSR illustrated the specific characters of ingredients which were used in the preparation. All the

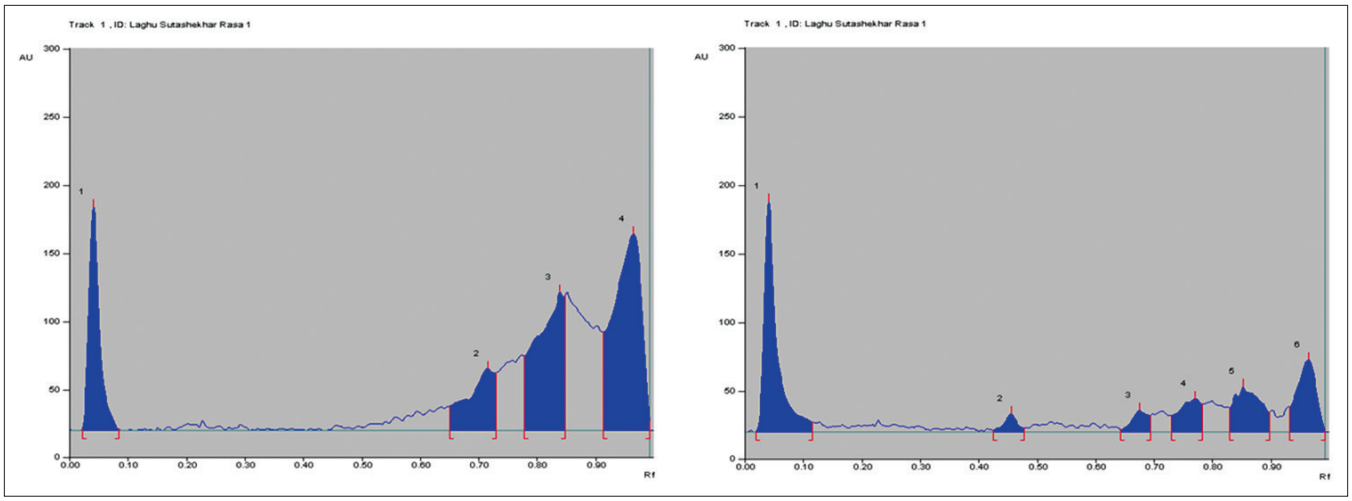

Plate 3: Densitogram of *Laghu Sutashekhara Rasa* at 254 and 366 nm

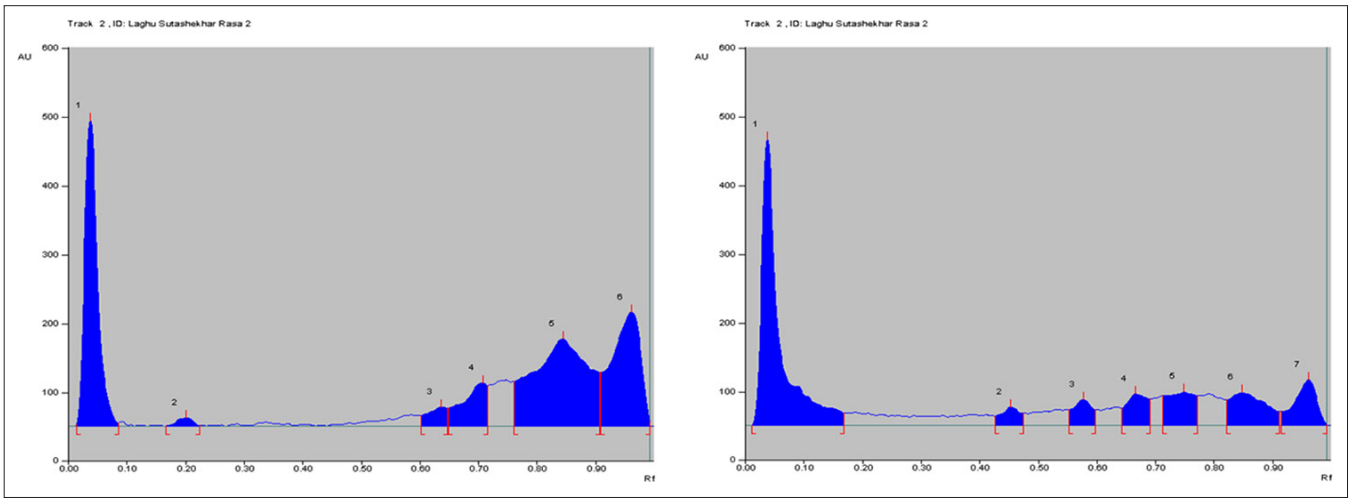

Plate 4: Densitogram of *Bhavita Laghu Sutashekhara Rasa* at 254 and 366 nm

Table 3: HPTLC of LSR

Wavelength (nm)	Number of spots	Maximum R_f values
254	4	0.04, 0.72, 0.84, 0.96
366	6	0.04, 0.46, 0.67, 0.77, 0.85, 0.96

HPTLC: High-performance thin layer chromatography, LSR: *Laghu Sutashekhara Rasa*

Table 4: HPTLC of BLSR

Wavelength (nm)	Number of spots	Maximum R_f values
254	4	0.04, 0.20, 0.64, 0.71, 0.84, 0.96
366	7	0.04, 0.45, 0.58, 0.67, 0.75, 0.85, 0.96

HPTLC: High-performance thin layer chromatography, BLSR: *Bhavita Laghu Sutashekhara Rasa*

pharmaceutical parameters analyzed within the permissible range. Disturbed walls ruptured cellular particles of ingredients signify that *Bhavana* incorporates additional therapeutic attributes and also increases the potency of the drug. As no published data available on consequence of *Bhavana* of LSR; the current observations can be considered as standard for future studies.

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