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Preparation and Physicochemical Evaluation of *Grahanibeelu Leha*

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ABSTRACT

Ahara (Food) is one of the supporting pillars of life which reflects once healthy life. The qualitative food in balanced proportion gives proper nourishment which helps in attainment of growth and development. The lacking in nourishment leads to various nutritional disorders in any stage of life from fetal life to adulthood. Under nourishment is the burdening factor in most of the developing countries irrespective of raised concern which accounts for 16.3% as per global hunger index 2022. In Ayurveda, undernourishment disorders are grouped under *Kuposhanajanya vyadhis* (Undernutritional disorders) which includes *Ksheeralasaka* (Multisystem disease of the breast-fed child), *Phakka* (Walking inability even by 1 year), *Parigarbhika* (Disease caused by consuming milk of pregnant women), *Karshya* (Underweight), *Balashosha* (Undernourished child). Ayurvedic treatment principles such as *Agnideepana* (Carminative), *Srotoshodhana* (Purification of channels) etc helps to rectify the pathology, make body to absorb proper nutrients and helps to overcome undernourishment.

Worldwide herbal drugs occupy the majority source as the part of medicine. *Grahanibeelu (Leucas biflora [Vahl] R. Br)* is one such plant belongs to locality of Udupi used in various conditions such as *Agnimandya* (Weak digestive fire), *Ajeerna* (Indigestion), *Grahani* (Malabsorption syndrome), *Jwara* (Fever) etc. in various *Aushadha kalpanas* (Formulations). There is no source of classical reference of this drug. We have attempted to prepare the Grahanibeelu leha as per Ayurvedic principles of *Aushadha Kalpana* and *Pramana* (Quantity). The present work aims at reporting the physicochemical evaluation of *Grahanibeelu leha* as per the physico-chemical parameters of API. Since the formulation is not standardized in API, here is an attempt to standardize this *Leha* to serve the purpose of further research in the area of Ayurvedic formulation.

Key Words *Grahanibeelu (Leucas biflora [Vahl] R. Br),* physicochemical parameters, *Grahanibeelu leha*, *Karshya* (Underweight)

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INTRODUCTION

Underweight is one among the diet related non communicable disease, became the burdening crisis over the underdeveloped and developing countries worldwide. According to 2022 estimation, nearly half of the deaths among children under 5 years of age are linked to undernutrition[1].

Balyavastha (Childhood) is the stage where the *Dhatus* are in the state of physiological

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immaturity. Deprivement of optimal nourishment required for the age make children undernourished. *Karshya* is one among the *Kuposhanajanya vikara* (Undernutritional disorders). Due to various etiological factors, Vata gets aggravated, affecting the digestive fire leads to *Upashoshana* (Drying up) of the *Rasa Dhatu*, which further exert influence on remaining Dhatus leads to Karshya[2]. Basically, correction of *Agni* from the *Deepana- Pachana dravyas* (Appetizer- Digestant) and *Vatanulomana* are the major contributing factor in the treatment of *Kuposhanajanya vikara*.

Herbal preparations are relied upon by 80% of the global population to address their healthcare needs. Some of these herbs are selective to particular locality, formulated and prescribed in their own way. One such plant is *Grahanibeelu (Leucas biflora [Vahl] R. Br)*, documented in 'Flora of Udupi' textbook not mentioned in any classics used in various disorders such as *Jwara* (Fever), *Aruchi* (Aversion to food), *Agnimandya* (Loss of appetite), *Grahani vikaras* (Irritable bowel disease). *Grahanibeelu leha* (Description of drugs and *Rasapanchaka* explained in table 1 and 2) is indicated for underweight children documented in previous researches[3].

Leha Kalpana (Semi-solid preparations) is one of the *Upakalpana* where any liquid preparation if boiled and reduced over mild fire to a thicker consistency by adding some *Madhura dravyas*[4]. Palatability is the prior choice in selecting medicines to children, where *Leha kalpana* are more advisable.

MATERIALS AND METHOD:

Grahanibeelu leha[5]

Table 1 Description of *Grahanibeelu Leha* drugs

INGREDIENTS	BOTANICAL NAME	FAMILY	PARTS USED	QUANTITY
Grahanibeelu	*Leucas biflora* (*Vahl R. Br*)	Lamiaceae	Whole plant	1 part
Draksha	*Vitis vinifera Linn.*	Vitaceae	Fruit	3/4th part
Maricha	*Piper nigrum Linn*	Piperaceace	Fruit	1/12th part
Jeeraka	*Cuminum cyminum*	Umbelliferae	Fruit	1/12th part
Ksheera	Milk			4 parts
Ghrita	Ghee			1/3rd part
Khanda sharkara	Sugar candy			2 parts
Madhu	Honey			1/5th part

Table 2 Description of *Grahanibeelu Leha* drugs *Gana*, Phytochemicals, *Rasapanchaka* and *Karma*

DRUG	GANA	PHYTOCHEMICALS	RASAPANCHAKA	KARMA
Grahanibeelu[6]	*No classical reference.*	*glycosides, saponins, flavonoids and tannins.*	*Pradhana rasa: Tikta, Katu.* *Anurasa: Madhura* *Guna- Laghu, Ruksha, Teekshna.* *Veerya- Ushna* *Vipaka- Katu*	*Kapha- Vata shamaka used in Karshya, Aruchi, Agnimandya, Grahani, Jwara.*
Draksha[7]	*Charaka- Kanthya, Virechanopaga, Kasahara, Shramahara,*	*Catechin, Epicatechin, B-Sitosterol, Ergosterol, Jasmonic acid, Resvertrol*	*Rasa - Madhura* *Guna - Guru, Snigdha, Mrudu* *Virya - Sheeta* *Vipaka - Madhura*	*Vatapitta shamaka Brimhaniya, Vrushya, Chakshushya, Virechanopaga, Swarya*

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	Madhura skandha, Susrutha- Parushakadi Vagbhata- Parushakadi Bhavaprakasha- Amradi phala varga			
Maricha[8]	*Charaka- Deepaniya, Sulaprashamana, Krimigna, Sirovirechanopaga. Susrutha- Pippalyadi, Tryusana Bhavaprakasha: Haritakyadi varga*	*Piper longumine, Piperlonguminine, Piperine, Essential oil, Pipercide, Beta sitosterol.*	*Rasa- Katu Guna- Guru, Teekshna Veerya- Natyushna Vipaka- Madhura*	*Kaphavata shamaka Deepana, Krimighna, Sulahara, Rochana, Chedana, Avrishya*
Jeeraka[9]	*Charaka- Shulaprashamana Susrutha- Pippalyadi Vagbhata- Pippalyadi*	*Cuminin, Cuminaldehyde, Diacyl glycerol, Imperatorin, Apigenin, Oxalic, P-cymene.*	*Rasa - Katu Guna – Laghu, Ruksha Virya - ushna Vipaka - Katu*	*Kapha- Vata shamaka Deepana- Pachana, Grahi, Vrushya, Balya, Garbhashaya shodhaka*

Method of Preparation of *Grahanibeelu leha:*

The fresh parts of the *Grahanibeelu* plant were collected from a natural source, thoroughly washed, and boiled in cow's milk over low heat. After boiling, the mixture was dried using a tray drier to ensure that even the fiber content of the plant was finely powdered. The drug was then fried in cow's ghee. A syrup (Paka) was prepared using the remaining milk and *Khanda Sharkara* (unrefined sugar). The ghee-fried drug was mixed with *Draksha* paste (Grape pulp) and other *Prakshepaka Dravyas* (preservative or enhancing substances). The mixture was gently heated until it reached the desired *Leha* (semi-solid) consistency. Once cooled completely, honey was added to the preparation. The finished *Leha* was then tightly packed in an airtight container to preserve its quality.

Organoleptic parameters of *Grahanibeelu leha*:

Color, Odor, Taste of Grahanibeelu leha were documented.

1. Physical State: Viscous liquid
2. Colour: Dark Brown
3. Odor: Pleasant
4. Taste: Sweet
5. Clarity: Opaque

Physicochemical parameters of *Grahanibeelu leha*: (All physicochemical parameters of *Grahanibeelu leha* are tabulated in table 3)

Determination of pH[10]**:**

Standard buffer solution is prepared by dissolving one tablet of pH 4, 7 and 9.2 in 100 ml of distilled water separately. 1 ml of sample was taken and make up to 10 ml with distilled water, stirred well and filtered. The filtrate was used for

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the experiment and pH meter is the instrument. The pH 4 solution was first introduced and the pH adjusted by using the knob to 4.02 for room temperature 30°C. The pH 7 solution was introduced and the pH meter adjusted to 7 by using the knob. Introduced the pH 9.2 solution and checked the pH reading without adjusting the knob. Then the sample solution was introduced and reading was noted. Repeated the test four times and the average reading were taken as result.

Loss on drying[11]:

A sample weighing 10 grams was placed in a pre-weighed evaporating dish. It was dried at 105˚C for 5 hours in hot air oven and weighed. The drying was continued until difference between two successive weights was not more than 0.01 after cooling in desiccator. Percentage of moisture was calculated with reference to weight of the sample.

Total Ash[12]:

2 g of sample was incinerated in a tared platinum crucible at temperature not exceeding 450˚C until carbon free ash is obtained. Percentage of ash was calculated with reference to weight of the sample.

Acid insoluble Ash[13]:

To the crucible containing total ash, add 25ml of dilute HCl and boil. Collect the insoluble matter on ash less filter paper and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot plate and ignite to constant weight. Allow the residue to cool in suitable desiccator for 30 mins and weigh without delay. Calculate the content of acid insoluble ash with reference to the air-dried drug.

Total fat[14]:

1 g of the sample mixed with 4gm of silica and was introduced into a thimble and placed it in a soxhlet fitted with a condenser. Taken 90 ml of petroleum ether (B.P. 40 - 60°C) in the 150 ml RB flask and boiled for 6 hours. The extract was taken in a pre-weighed conical flask and petroleum ether was evaporated on a water bath. Removed the traces of petroleum ether in vacuum pump. Taken the weight of fat to constant weight.

Reducing and non-reducing sugar[15]:

The filtrate was prepared by 10 g of sample was taken in a 250 ml volumetric flask and 200 ml of water was added. Slight excess solid basic Lead acetate was added to remove tannins, Shaken and filtered. To remove excess of basic Lead acetate, Sodium oxalate is used, shake and filtered.

Reducing sugar: Take the sugar solution in a 50 ml burette.

Preliminary titration: 10 ml of Fehling's solution was pipette into a 250 ml conical flask, from the burette, 15 ml of the sugar solution was added. The liquid boiled on asbestos-covered gauze and further quantities of the sugar solution was added (One ml at a time) at 10 to 15 second intervals to the boiling liquid until the blue colour is nearly discharged. 3-5 drops of aqueous Methylene blue solution (1%) is added and continued the titration until the indicator is completely decolourised.

Accurate titration: The titration repeated, before heating, almost all of the sugar solution required

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to effect reduction of copper added. Gently boiled for two minutes. 3-5 drops of Methylene blue indicator is added and the titration was completed within a total boiling time of three minutes. At the end point all the blue colour should be discharged and the liquid should be red.

Total Sugar: 20ml of reducing sugar solution was taken and 10ml of Concentrated HCl was added and kept aside overnight.

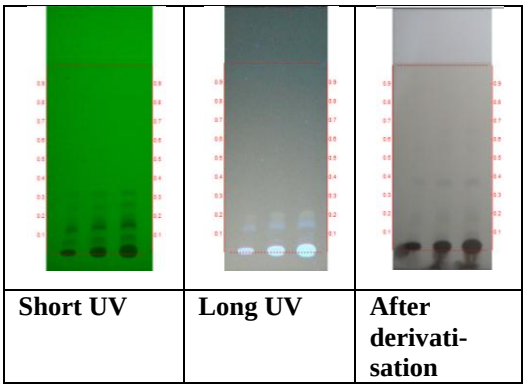
Neutralized with approximately 1ml of Sodium hydroxide solution or with solid sodium carbonate and made up to 100 ml in a volumetric flask. Determined the total sugar content by this titrimetric method. The experiment was repeated twice and the average value was taken.

Table 3 Physicochemical parameters of *Grahanibeelu leha*

Parameters	Result n = 3 (% w/w)
	Grahanibeelu leha
pH	4.90
Loss on drying	6.86±0.00
Total ash	2.14±0.47
Acid insoluble ash	0.61±0.01
Total solids (%)	93.0
Fat content	10.0
Total sugar	81.83
Reducing sugar	16.36
Non reducing sugar	65.47

HPTLC[16]:

1g of *Grahanibeelu leha* was extracted with 20 ml of alcohol kept for cold percolation for 24hours, filtered into a pre weighed chinadish evaporated to dryness. The extract was then dissolved in 10.0ml of alcohol. 3, 6 and 9µl of the above extract were applied on a pre-coated Silica gel F_{254} on Aluminium plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (9.0: 1.0). The developed plates were visualized in short UV, long UV, and then derivatised with Vanillin sulphuric acid and scanned under UV 254nm, 366nm and 620nm (Fig.1 shows Ethanolic extract of *Grahanibeelu leha* under Short UV, Long UV and after derivatisation and fig 2a, 2b and 2c shows the Densitometric scan of *Grahanibeelu leha* at 254nm, 366nm and 620nm respectively). R_f, colour of the spots and densitometric scan were recorded. (R_F values of samples given in table 4).



Short UV	**Long UV**	**After derivati-sation**

Track 1- *Grahanibeelu leha* – 3µl
Track 2- *Grahanibeelu leha* – 6µl
Track 3- *Grahanibeelu leha* – 9µl

Figure 1 HPTLC photo documentation of ethanolic extract of *Grahanibeelu leha*

Table 4 R_f values of samples

Short UV	Long UV	After derivatisation
0.08 (Green)	-	-
0.14 (Green)	0.14 (F. blue)	-
-	0.20 (F. yellow)	0.19 (Purple)
0.25 (Green)	-	0.24 (Purple)
0.32 (Green)	-	-
-	-	0.37 (Purple)
-	-	0.65 (Purple)

- F - fluorescent

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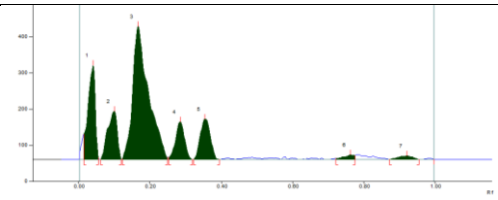
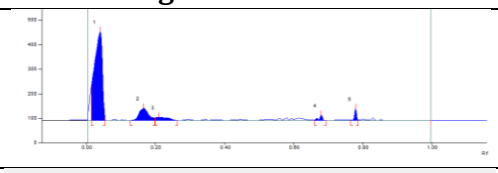


Track 3, ID: Grahanibeelu leha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	69.8 AU	0.04 Rf	259.6 AU	25.95 %	0.05 Rf	4.3 AU	3830.5 AU	16.74 %
2	0.06 Rf	0.2 AU	0.10 Rf	133.6 AU	13.36 %	0.12 Rf	0.2 AU	2571.4 AU	11.24 %
3	0.12 Rf	1.0 AU	0.17 Rf	367.6 AU	36.75 %	0.25 Rf	5.6 AU	11740.6 AU	51.31 %
4	0.25 Rf	5.7 AU	0.29 Rf	104.2 AU	10.42 %	0.32 Rf	0.1 AU	1899.6 AU	8.30 %
5	0.32 Rf	0.3 AU	0.35 Rf	112.1 AU	11.20 %	0.40 Rf	0.0 AU	2281.6 AU	9.97 %
6	0.72 Rf	2.3 AU	0.76 Rf	12.8 AU	1.28 %	0.78 Rf	10.7 AU	272.9 AU	1.19 %
7	0.87 Rf	0.1 AU	0.92 Rf	10.4 AU	1.04 %	0.96 Rf	0.0 AU	284.1 AU	1.24 %

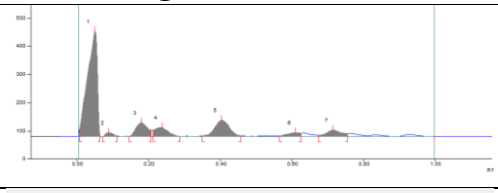
Fig 2a. At 254nm



Track 3, ID: Grahanibeelu leha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	141.0 AU	0.04 Rf	361.1 AU	73.49 %	0.05 Rf	7.2 AU	5912.4 AU	77.98 %
2	0.13 Rf	0.0 AU	0.17 Rf	49.3 AU	10.03 %	0.20 Rf	11.1 AU	981.6 AU	12.95 %
3	0.20 Rf	11.2 AU	0.21 Rf	12.2 AU	2.49 %	0.26 Rf	0.3 AU	337.1 AU	4.45 %
4	0.66 Rf	1.6 AU	0.68 Rf	20.5 AU	4.16 %	0.70 Rf	0.7 AU	132.0 AU	1.74 %
5	0.77 Rf	1.8 AU	0.78 Rf	48.3 AU	9.83 %	0.79 Rf	1.8 AU	219.2 AU	2.89 %

Fig 2b. At 366nm



Track 3, ID: Grahanibeelu leha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	24.0 AU	0.05 Rf	371.2 AU	66.52 %	0.06 Rf	2.9 AU	7288.0 AU	59.51 %
2	0.07 Rf	0.0 AU	0.09 Rf	13.6 AU	2.44 %	0.11 Rf	1.7 AU	209.7 AU	1.71 %
3	0.15 Rf	0.5 AU	0.18 Rf	48.4 AU	8.68 %	0.21 Rf	22.3 AU	1125.7 AU	9.19 %
4	0.21 Rf	22.3 AU	0.24 Rf	31.3 AU	5.61 %	0.29 Rf	0.1 AU	852.8 AU	6.96 %
5	0.35 Rf	1.6 AU	0.40 Rf	57.6 AU	10.32 %	0.46 Rf	2.3 AU	1710.4 AU	13.97 %
6	0.57 Rf	2.2 AU	0.61 Rf	13.6 AU	2.43 %	0.63 Rf	11.8 AU	361.6 AU	2.95 %
7	0.68 Rf	4.2 AU	0.71 Rf	22.3 AU	4.00 %	0.76 Rf	10.2 AU	699.4 AU	5.71 %

Fig 2c. At 620nm

Figure 2 Densitometric scan of *Grahanibeelu leha*

DISCUSSION

Karshya is one among *Apatarpanajanya vyadhi* (Catabolism). The treatment modality to be adopted is *Agnideepna* and Bulk promoting agents which are light and nourishing property. *Grahanibeelu* is the main ingredient processing *Tikta, Katu rasa* as *Pradhana* and *Madhura* as *Anurasa*. *Tikta rasa* is *Deepaniya* and correcting the *Agni* at *Dhatu* level. *Draksha is Madhura* and *Anulomaka*. *Maricha* and *Jeeraka* are the *Prakshepaka dravyas* (Synergizer) in this preparation, possessing carminative and digestive properties. *Khanda sharkara* are the *Madhura pradhana Dravya*. *Ksheera and Ghrita* are *Nityasevaneeya, Satmya Ahara Dravya* (Compatible food) which have high nutritive value promotes *Balya*. Overall view of *Grahanibeelu leha* possess is *Deepana, Vata-Khaphahara, Brumhana*.

For the preparation of *Grahanibeelu Leha*, the ingredients are quantified based on *Anuktamana* (Unknown quantity) of *Avaleha* by *Acharya Sharangadhara*. In preparation of the *Leha* drying is adopted, after boiling the drug in milk, to prepare the proper fine powder easily and to prevent wastage. *Avaleha Kalpana* is the most palatable when compared to the basic forms, hence it is easy for administration in children.

Assessment of Physico-chemical parameters is the part of drug Standardization done as per API. The following results pH of *Avaleha* solution is acidic in nature so hydrogen ions are present in the preparation. The loss on drying was 6.86% representing the moisture content. To determining the authenticity and purity of this *Leha*, Total Ash and Acid insoluble ash is done results in 2.14% and 0.61 % respectively. Total fat was 10.0%, showing the amount of oil content in the preparation. Total sugar was 81.83%, Reducing sugar was 16.36%, reflecting the presence of sugar acting as a reducing agent. Non reducing sugar was 65.47% comparing to reducing sugar it producing non reacting agent more in the preparation. HPTLC for

Grahanibeelu Leha reveals two spots of Rf values 0.12, 0.30 in short UV 254nm. In long wave UV 366nm five spots at 0.12, 0.22, 0.46, 0.58, 0.78 Rf values observed. After derivatisation with VSA UV 620nm two spots at 0.12, 0.35 Rf values observed, it shows more therapeutically active ingredients.

CONCLUSION

Traditional practices are boosted with Ayurvedic principles, to through light over the many herbal preparations. *Grahanibeelu leha* is one such product useful in *Agnimanya*, *Ajeerna*, *Grahani* etc. Correction of *Agni* at *Jatharagni* (Digestive fire) and *Dhatwagni* (Digestive fire related to body dhatus) level is needed in *Karshya* before *Brumhana* (Bulk promoting) *chikitsa*.

Standardization of the new product has better hope in determining the quality, purity and assuring safety. The standardization study of *Grahanibeelu Leha* reveals several key findings. Its acidic nature is evident, while its moisture content, as reflected by the loss on drying, falls within normal limits. The total ash and acid-insoluble ash values, representing inorganic residues and non-soluble minerals, are minimal, underscoring the Leha's purity. The fat content is within acceptable levels, and the total sugar content along with both reducing and non-reducing sugars is on the higher side, which enhances the Leha's palatability, stability, and nutritional value. The HPTLC profile shows nine distinct spots, pointing to a diverse range of bioactive compounds that suggest a broad spectrum of therapeutic potential.

The study concludes that standardizing *Grahanibeelu Leha* will enhance understanding of the drug's quality and ensure safety for therapeutic benefits.

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