



RESEARCH ARTICLE

**Pharmacognostical and Phytochemical Standardization of *Vyaaghreeharetakee
Avaleha***

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ABSTRACT

The present work was carried out to standardize the finished product *Vyaaghreeharetakee Avaleha* to confirm its identity, quality, and purity. The two major ingredients of the formulation are *Vyaaghree* (*Solanum xanthocarpum* Schrad. & Wendl.) and *Haretakee* (*Terminalia chebula* Ritz.). The formulation is widely indicated for the management of *Kaasa* (Cough), *Swaasa* (breathlessness) due to *Tamak Shwaasa* (Bronchial Asthma). Due to various types of side-effect of available chemical formulations to suppress the condition, there has been a worldwide demand of herbal formulations. But quality and purity without side effect is a big concern. Thus the finished products of herbal preparation in the form of *Vyaaghreeharetakee Avaleha* was tried to standardize. Pharmacognostical and phyto-chemical observations revealed the specific characters of all active constituents used in the preparation. The phytochemical analysis and High Performance Thin Layer Chromatography has also been performed. The drug combination was also characterized for its physico-chemical properties. The presence of microsphenoidal crystals of calcium oxalate, tracheids with irregular walls and bordered pits, mesocarp cells, simple, oval shaped starch grains, stone cells varying in sizes, shapes and thickness, stone cells with broad lumen in groups, Fragment of border pitted vessels, Epicorp-beaker shaped, oil and tannin content and acicular crystal, alureon grains, 1 to 2 rosette crystals, prismatic crystals, and immature pollen grains were the characteristic features observed in the microscopy of drug combination. Phyto-chemical analysis indicated presence of alkaloids, tannins, saponins, flavonoids, glycosides, and Steroid.

KEYWORDS

Tamak Shwaasa, Bronchial Asthma, *Solanum xanthocarpum*, Pharmacognosy, Phytochemistry, HPTLC

INTRODUCTION

Vyaaghreeharetakee Avaleha is a poly herbal formulation.

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The formulation is indicated for the management of *Kaasa* (Cough), *Swaasa* (breathlessness) and hoarseness of voice and also known for its immunomodulatory action.¹ Bronchial asthma is a serious global health challenge affecting more than 300 million people all over the world.² Herbal preparations are in great demand in the developed as well as developing countries for

primary healthcare because of their wide biological activities, higher safety margins and lesser costs.² It has been reported that there has been an alarming increase in number of diseases and disorders caused by synthetic drugs prompting a switch over to herbal medicine.³ The WHO assembly in number of resolutions has emphasized the need to ensure quality control of medicinal plant products by using modern techniques and applying suitable standards.⁴ Selection of scientific and systematic approach for the biological evaluation of herbal formulations based on their use in the traditional systems of medicine forms the basis for an ideal approach in the development of new drugs from plants. But the most important challenges faced by these formulations arise because of their lack of complete standardization. Detailed research on the chemistry and pharmacology of products of plant origin are much essential and this may eventually lead to the discovery of medicine that can be used in the treatment of several diseases.

In the light of above background, the present

study aimed to standardize the finished product of *Vyaaghreeharetakee Avaleha* using pharmacognostical and phytochemical parameters.

The authenticity, quality and purity of herbal drugs are established by references given in pharmacopoeia.⁵ Thus this experiment is aimed to validate the content of the formulation through comprehensive clinical trial.

MATERIAL AND METHODS

Collection, Identification, and Authentication of Raw Drugs

All the raw drugs except *Kantakaaree* and *Naagakesara* were collected from the pharmacy, Gujarat Ayurved University, Jamnagar.

Drugs collected from the pharmacy were already authenticated from the Pharmacognosy lab, IPGT & RA, Gujarat Ayurved University, Jamnagar.

Kantakaaree was collected from the periphery of Jamnagar city while *Nagakeshara* (*Mesua ferrea*

Table 1: Ingredients of *Vyaaghreeharetakee Avaleha*

S.N.	Name	Botanical Name	Part Used	Form
1.	<i>Kantakaaree</i>	<i>Solanum xanthocarpum</i> Schrad. & Wendl.	Fresh whole plant	Plant Decoction
2.	<i>Hareetakee</i>	<i>Terminalia chebula</i> Ritz.	Fruit pericarp	Pulp
3.	<i>Guda</i>	Jaggery	---	---
4.	<i>Shunthee</i>	<i>Zinziber officinarum</i> Roxb.	Dry Rhizome	Powder
5.	<i>Maricha</i>	<i>Piper nigrum</i> Linn.	Fruit	Powder
6.	<i>Pippalee</i>	<i>Piper longum</i> Linn.	Fruit	Powder
7.	<i>Tvak</i>	<i>Cinnamomum zeylanicum</i> Blume.	Bark	Powder
8.	<i>Patra</i>	<i>Cinnamomum tamala</i> Ness.	Leaves	Powder
9.	<i>Eelaa</i>	<i>Elattaria cardamom</i> Maton.	Seed	Powder
10.	<i>Naaga</i>	<i>Mesua ferrea</i> Linn.	Stamens	Powder
11.	<i>Pushpa rasa</i>	Honey

Linn.) was collected from the source place Koppa (12° 33' 0" N, 75° 21' 0" E 12.55, 75.35), Karnataka India. Pharmacognostical evaluation and authentication of both drugs were done in the Pharmacognosy lab, IPGT&RA, Gujarat Ayurved University, and Jamnagar.

Preparation of Drug

Hareetakee fruits were tied in cotton cloth (pottali) and boiled along with *Kantakaare* to prepare the decoction. The well-cooked *Hareetakee* fruits were then taken out of the pottali and the seeds were removed to collect the pulp of the fruit. After collecting the pulp of the fruit, it was grind well to make fine paste of it. Then the *avaleha* was prepared following the principles of *Avaleha Kalpana*. Then fine powders of *prakshepa dravyas* were added after self-cooling and stirred thoroughly to get a homogeneous mixture. Finally *madhu* (honey) was added and mixed. After cooling the prepared material for 24 hours, the drug was packed in airtight plastic containers.⁶

Pharmacognostical Evaluation

One gram of finished product *Vyaaghreeharetakee Avaleha* dissolved in distilled water then filtered through filter paper, the filtrate used study under the corl zeiss microscope with stain (Phloroglucine and concentrated HCl) and without stain to study the characters of the product. The microphotographs were taken attached with the microscope.

Phytochemical Assay of Drug

Vyaaghreeharetakee Avaleha was analyzed by using qualitative and quantitative parameters at Pharmaceutical Chemistry Laboratory, Institute of Post Graduate Teaching & Research in Ayurveda, Gujarat Ayurved University, Jamnagar. All Physico-chemical parameters such as Loss on Drying, Ash value, Water soluble extract, Methanol soluble extract, PH, Reducing sugar, Non- reducing sugar and Total sugar were determined. The water and methanol extract of the sample was analyzed qualitatively for different functional groups.⁷

High Performance Thin Layer Chromatography (HPTLC)

Methanol extract of *Vyaaghreeharetakee Avaleha* was used for High performance thin layer chromatography (HPTLC) study. Methanol extract of *Vyaaghreeharetakee Avaleha* was spotted on pre-coated silica gel GL60254 aluminum plate as 10mm bands by means of a Camag Linomate V sample applicator fitted with a 100 µL Hamilton syringe. Toluene (9ml) and ethyl acetate (1ml) was used for *Vyaaghreeharetakee Avaleha* as a mobile phase. The development time was 30 minutes. After development, Densitometry scanning was performed with a Camag TLC scanner III in reflectance absorbance mode at 254nm and 366 nm under control of Win CATS software (V1.2.1. Camag).^{8,9} Then the plate was sprayed with Anisaldehyde sulphuric acid followed by heating and then visualized in day light.

RESULTS AND DISCUSSION

Organoleptic Parameters

The organoleptic characters of the formulation are tabulated in the table 2.

Table 2: Organoleptic characters of *Vyaaghreeharetakee Avaleha*

Organoleptic parameters	<i>Vyaaghreeharetakee Avaleha</i>
Consistency	Semisolid sticky paste
Color	Brownish Black
Odor	Characteristic
Taste	Sweet, Astringent

Pharmacognostical Evaluation

Pharmacognostical study of the finished product of *Vyaaghreeharetakee Avaleha* was performed and following diagnostic characters were observed. The diagnostic characters observed were fibers, microsphenoidal crystals of calcium oxalate, tracheids with irregular walls and bordered pits (*Solanum xanthocarpum* Schrad. & Wendl.), mesocarp cells (*Terminalia chebula* Ritz.), simple, oval shaped starch grains (*Zinziber*

officinarum Roxb.), stone cells varying in sizes, shapes and thickness (*Piper nigrum* Linn.), stone cells with broad lumen in groups of 2 to 8 of (*Piper longum* Linn.), Fragment of border pitted vessels, Epicorp-beaker shaped (*Piper longum* Linn. and *Piper nigrum* Linn.), oil and tannin content and acicular crystal of (*Cinnamomum zeylanicum* Blume. and *Cinnamomum tamala*

Ness.), alureon grains, 1 to 2 rosette crystals, prismatic crystals (*Elattaria cardamom* Maton.), and immature pollen grains (*Mesua ferrea* Linn.).¹⁰ (Figure 1 a-j).

Qualitative Test for Various Functional Groups

Qualitative test for various functional groups revealed the presence of alkaloid, Tannin and

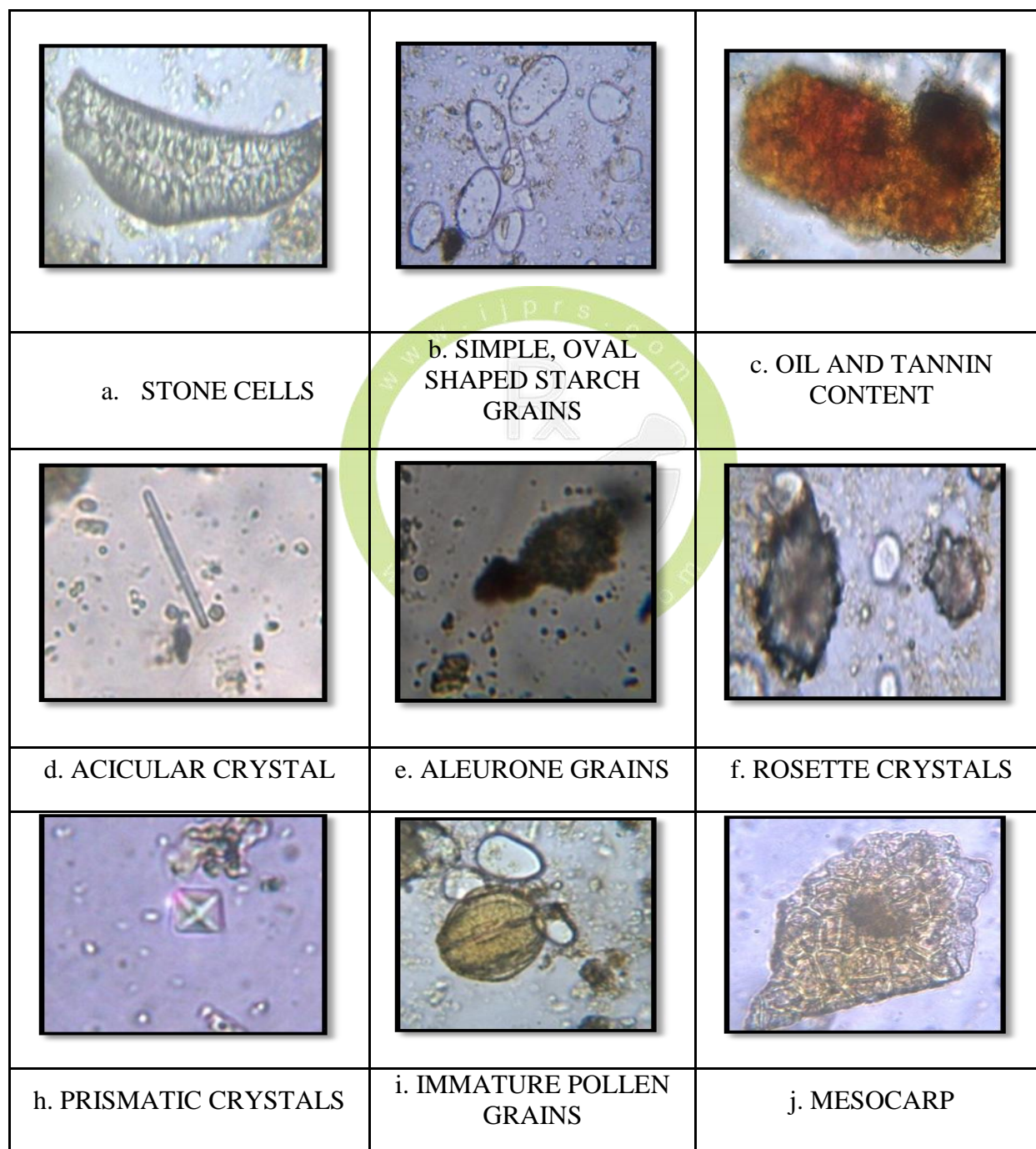


Figure: 1 Microphotographs of finished product *Vyaghreeharetakee Avaleha*

phenolic, glycosides, saponin, flavanoids, protein, steroids and carbohydrates in both the formulations.

Table 3: Qualitative Analysis of *Vyaghreeharetakee Avaleha*

S. N.	Functional group	Test	<i>Vyaghreeharetakee Avaleha</i>
1.	Alkaloid	Dragendroff's & Wagner's test	+
2.	Tannin and phenolic	Lead acetate	+
3.	Glycosides	Keller-Killiani test	+
4.	Saponin Glycosides	Lead acetate solution	+
5.	Flavanoids	Lead acetate solution	+ =
6.	Protein	Lead acetate solution	+
7.	Steroid	Liebermann's Burchard	+
8.	Carbohydrates	Molish's test	+

Physico-Chemical Parameters

Vyaaghreeharetakee was evaluated for various physico-chemical parameters. The results are shown in the table 4.

Table 4: Physic-Chemical Evaluation of *Vyaghreeharetakee Avaleha*

S.N.	Test	<i>Vyaghreeharetakee Avaleha</i>
1.	Loss on Drying at 110 °C	11.20 % w/w
2.	Ash value	4.54 % w/w
3.	Water soluble extract	84.50 % w/w
4.	Methanol soluble extract	88.83 % w/w
5.	PH	5.02
6.	a. Reducing sugar	40.48 % w/w
	b. Non-reducing sugar	40.76 % w/w
	c. Total sugar	81.24 % w/w

High Performance Thin Layer Chromatography (HPTLC) Study

In High performance thin layer chromatography (HPTLC) study, visual observation under UV light showed few spots, but on analyzing under densitometer much more was observed. Chromatogram shows 6 prominent spots at R_f 0.02, 0.24, 0.48, 0.57, 0.81, 0.92 in short wave UV 254 nm in and 5 prominent spots at R_f 0.02, 0.20, 0.48, 0.56, 0.65 in long wave UV 366nm. Details are noted in the table and figure. Then the plate was sprayed with Anisaldehyde sulphuric acid followed by heating and then visualized in day light showed 3 prominent spots at R_f 0.18, 0.36, 0.55 in *Vyaaghreeharetakee Avaleha* and 3 prominent spots at R_f 0.18, 0.35, 0.58 in *Vyaaghreeharetakee Avaleha*. Details are noted in the table 5 and figure 2.

Table 5: HPTLC study of *Vyaaghreeharetakee avaleha*

Extract	Solvent system	Wave lengths	No. of spots	Rf value	Observation under UV light
Methanol extract	Toluene (8ml): Ethyl acetate (2ml)	366nm	5	0.02, 0.20 0.48, 0.56 0.65	Light yellow, Light brown Grayish brown, Light brown brown
		254 nm	6	0.02, 0.24 0.48, 0.57 0.81, 0.92	Light yellow, Gray Grayish brown, Light brown Brown, brown

Table 6: HPTLC of *Vyaaghreeharetakee avaleha* (Methanol extract) After Spraying Anisaldehyde sulphuric acid

Sl.No.	Spray	No. of spots	HRf value	Observation
1	Anisaldehyde sulphuric acid	3	0.18 0.35 0.58	Light brown brown brown

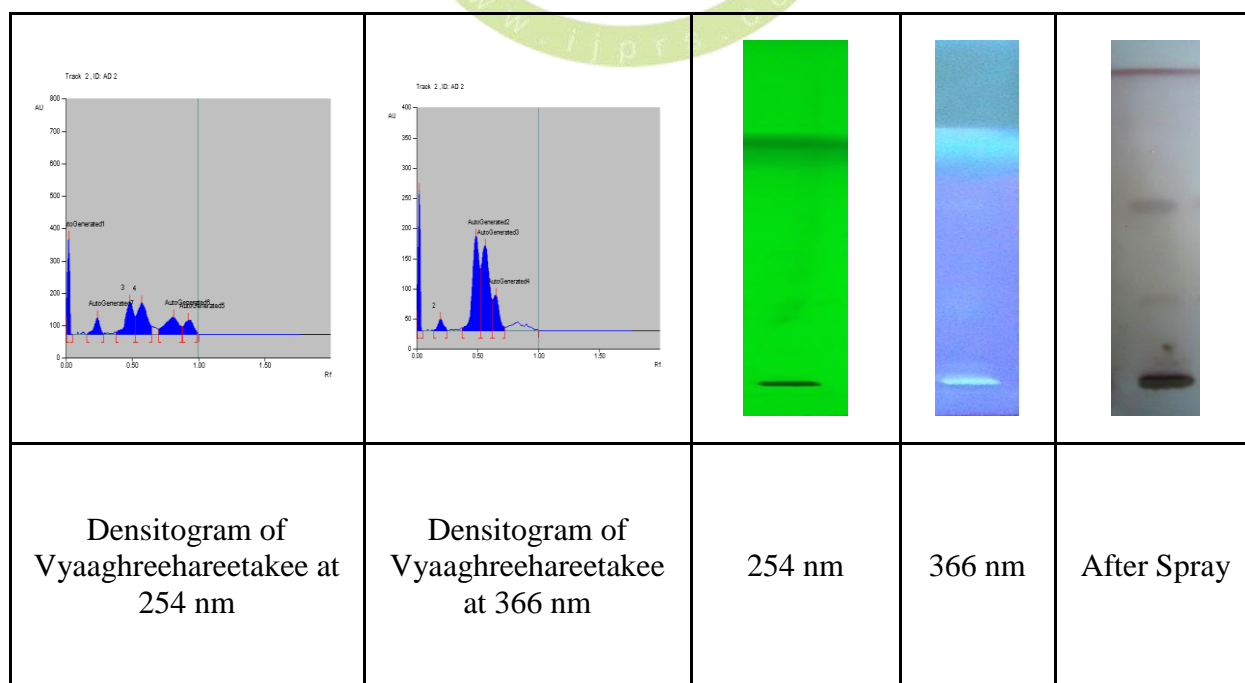


Figure 2: HPTLC of *Vyaaghreeharetakee* (Methanol Extract)

CONCLUSION

Pharmacognostical and Phyto-Chemical evaluation of *Vyaaghreeharetakee Avaleha* illustrated the specific characters of all ingredients which we used in the preparation. For the first time a new pharmaceutical preparation *Vyaaghreeharetakee Avaleha* was tried which is economical in terms of time and machinery usage. On the basis of our observations and experimental results, this study may be used as reference standard in the further quality control researches. Further studies may be carried out on *Vyaaghreeharetakee Avaleha* based on identification and separation of active ingredients with the help of various Biomarkers.

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