

Preliminary Pharmacognostical and Analytical study on “*Triphaladi Avaleha*” - A Poly Herbal *Ayurvedic* Formulation



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Abstract

There are ample of collections for effective management of diseases in octapertite *Ayurveda*. The geometrical rise of demand for indigenous medicines, maintaining quality standards is the need of hour in present era. Absence of reference standards for compound formulations is a hindrance on the way towards standardization. The present study was aimed for experimenting preliminary Pharmacognostical and analytical profile of *Triphaladi Avaleha*. Study included preparation of *Triphaladi Avaleha* using pre authenticated raw drugs following all SOPs. Later *Triphaladi Avaleha* was subjected to Pharmacognostical and physicochemical analysis as per standard protocols. The final observations were systematically observed and recorded. Organoleptic features of *Triphaladi Avaleha* were within the standard range as per mention in standard texts. pH of 5% aqueous solution of *Avaleha* observed acidic in nature. Water soluble extractive was 82% w/w while solubility in alcohol was 91.8% w/w. Qualitatively the metabolites like, carbohydrate, steroid, saponin glycoside, flavonoid, alkaloid, tannins & phenolic compounds were found positive. Total sugar was 89.57%. Artificial Invert Sugar test for Honey was negative. Chromatographic evaluation gave a preliminary fingerprint of the formulation with appropriate solvent system in which 7 spots in long UV, 2 in short UV while after spraying 4 spots were observed. This may shows the presence of certain definite constituents in *Triphaladi Avaleha*. The quality of *Triphaladi Avaleha* can be ascertained by a cascade of Pharmacognostical and physicochemical screening for the findings in accordance with the observations of the present study. It was found that the formulation meets the required standards of an *Avaleha* at a preliminary level. The results of this study may be used as the reference standard in further research undertakings of its kind.

Key words: *Triphaladi Avaleha*, Pharmacognosy, Analytical Study

Introduction:

The geometrical rise of demand for indigenous medicines, maintenance of quality standards is the need of hour in present era. Chemical analysis ensures chemical constituents and standards of medicine, responsible for the efficacy. The quality control and standardization facets of herbal drugs

stay as a difficult task even in 21st century. Proper identification and surety of purity through pharmacognosy and analytical measures is unavoidable ladder required for the quality assurance and standardization of the herbal formulation whether it is single drug or polyherbal formulation. Absence of reference standards for compound

formulations is a hindrance on the way towards standardization. The present study was aimed at setting a standard pharmacognostical and analytical profile of *Triphaladi Avaleha* which is a polyherbal formulation of Ayurveda. In present study, the selected *Triphaladi Avaleha* contained *Amalaki*, *Haritaki*, *Vibhitaki*, *Katuki*, *Kakmachi*, *Kutaja*, *Haridra*, *Vidanga*, *Guduchi*, *Shweta Punarnava*, *Sharapunkha*, *Chaturjata*, *Sharkara* and *Madhu*. It is an experience based medicine after reviewing the classical texts of Ayurveda which is used in the management of Thalassemia.

The Thalassemia syndromes are most common single gene disorder in the world and represent a major health burden worldwide. It is a heterogeneous disorder recessively inherited resulting from various mutations of the genes which code for globin chains of haemoglobin (Hb) leading to reduced or absent synthesis of globin chains [1]. As per WHO estimate 4.5% of the world's populations are carriers of haemoglobinopathies [2]. The ingredients of *Triphaladi Avaleha* were selected from *Lohashodhana Gana*, *Lohamarana Gana*, *Lohasevanajanya Vikara Chikitsa*, *Yakrita* and *Pleeha Vikara Chikitsa* and *Pandu Chikitsa* [3, 4, 5]. Details of ingredients of *Triphaladi Avaleha* are mentioned in Table 1.

Materials and Methods

Procurement of drugs

Sharapunkha was collected from the periphery of Jamnagar. *Haridra* was bought from local market of Jamnagar and *Kakamachi* was bought from Ahmedabad. Other remaining drugs were provided by the Pharmacy, I.P.G.T.&R.A., GAU. Then all drugs were identified and authenticated in pharmacognosy department, I.P.G.T.&R.A., GAU. Finally, all the drugs were powdered, packed in air tight containers and taken for further study.

Pharmacognostical study

The final product *Avaleha* was also observed pharmacognostically and macro & microscopically characters were recorded and photographs were also taken, after proper mounting and staining with different reagents like, Phloroglucinol and HCl for

identification of Lignified elements and Iodine for detection of starch grains in pharmacognosy Lab. I.P.G.T. & R.A., G.A.U., Jamnagar [6].

Analytical Study

The sample of *Triphaladi Avaleha* was subjected to organoleptic and physico-chemical studies in order to develop analytical profile.

Organoleptic characteristics like, *Rupa* (colour), *Rasa* (taste), *Gandha* (odour) and *Sparsha* (consistency) were recorded for the sample.

Physicochemical parameters

Physicochemical study includes the parameters such as Loss on drying, Ash value, Extractive values in water and methanol, pH according to the various textual references [7, 8, 9, 10].

Qualitative Study

In qualitative analysis various chemical tests were performed to find out the presence or absence of chemical constituents of various functional groups like Saponins, Alkaloids, Glycosides etc. and artificial invert sugar test of honey [6, 11]

Quantitative estimation

The final product was analyzed for its sugar content, as per the standard protocol [12].

Chromatographic fingerprint

Preparation of sample solution

10 gm of sample was extracted with 5 ml of Methanol. The material was refluxed for half an hour. The extract was filtered and volume was made up to 10 ml to get solution concentration [13].

Experimental Conditions:

Sample: Methanolic ext.

mobile phase: Toluene : ethyl acetate (7:3 v/v)

Stationary phase: Precoated silica gel GF254 plates

Saturation time : 30min

Spraying Reagent: 10% FeCl₃ aqueous solution

Chromatographic conditions:

Application mode: CAMAG Linomat V Hamilton Syringe

Development chamber: CAMAG Twin trough chamber (20 x 10 cm)

Scanner: CAMAG Scanner III
Scanning mode: Linear at 254 nm and 366 nm
Detection: Deuterium lamp, Mercury lamp
Photo documentation: CAMAG reprostar
Data system: WINCATS software (Ver. 3.17)
Drying device: Oven
U.V. Spectrum Scanning range: 200 nm to 700 nm

Results and observation:

Preparation of *Triphaladi Avaleha*

SOP of *Triphaladi Avaleha* is followed as per AFI Standards. First of all, *Yavakuta* (coarse powder) of all the 11 constituents (each 1200 g) of *Triphaladi Avaleha* (Table 1) was prepared. This was boiled with 16 times of water under low heat and reduced to one-eighth and filtered. Eighteen kilograms of *Sharkara* (sugar) was added to the decoction and again boiled till *Avaleha* is not formed. At this stage, fine powders of *Prakshhepa Dravyas of Chaturjata* (each 300 gm) were added. At the end, six kilograms *Madhu* was added after cooling of the *Avaleha*. The formulation was prepared in Pharmacy, I.P.G.T. & R.A., GAU.

Organoleptic properties

Triphaladi Avaleha had dark brown colour, typical smell, *Madhura*, *Tikta* taste and semisolid consistency. (Table 2)

Pharmacognostical study

The final product of *Avaleha* was analyzed pharmacognostically and revealed the presence of characters like prismatic crystals, rosette crystals, stone cells and sclereids, spiral vessel, cork cells transversely cut and in surface view, transversely cut fibers, unicellular simple trichome, pitted vessel, patches of fibers, reticulated vessel, parenchyma cells with yellowish content, striated thick walled lignified parenchyma, pollen grains, oil globules, brownish content and simple and compound starch grains were found [14] [Photo no. 1-14].

Physico-chemical parameters:

The results are mentioned in Table 3.

Qualitative tests for various primary as well as secondary metabolites and for artificial invert sugar were done and the results were enumerated in Table 4.

Quantitative assessment of sugar was done for *Avaleha* and results are mention in Table no. 5

Thin Layer Chromatography (TLC):

The R_f values from the chromatograms of *Triphaladi Avaleha* under long U.V., short U.V. and in the day light after spraying with 10% $FeCl_3$ aqueous solution are presented in Table 6.

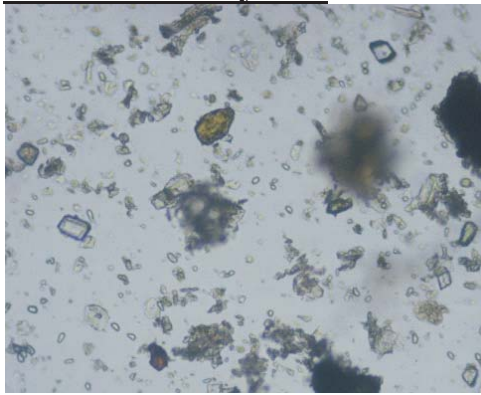
Discussion

The present formulation consisted of seventeen plant ingredients, which were proved to be genuine, by assessing the organoleptic characters and powder microscopy features. Physicochemical parameters were applied for assessing the prepared formulation. Loss on drying indicates the moisture content of the product because higher value of moisture tends to degradation of the components. Ash value shows inorganic compounds in the drug which is found less in amount in the *Avaleha* which also indicates good quality drugs. The pH of *Avaleha* was found to be 4.4, showing the acidic nature of the drug. Higher percentage of extractive values shows the solubility of compounds with respective solvents, which is a good sign for its efficacy because more solubility tends to good activity. Qualitatively the metabolites like, carbohydrate, steroid, saponin glycoside, flavonoid, alkaloid, tannins & phenolic compounds were found positive which may be the responsible active constituent for its activity. Total sugar was 89.57%. Artificial Invert Sugar test for Honey was negative. TLC showed two spots, when the plate was scanned at 254 nm and seven with 366 nm, while four after spraying with $FeCl_3$ solution. The results of R_f values were observed common for 3 values for both the detection wavelengths as well as after derivatisation, which showed that the components were sensitive to both wavelengths and conditions.

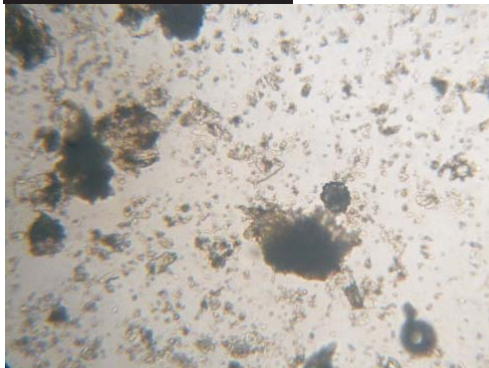
Conclusion

The present work deals with the macroscopic, microscopic, physicochemical and phytochemical evaluation of the *Triphaladi Avaleha*. Observed phytochemical data for *Avaleha* reveals presence of alkaloids, glycosides, carbohydrates,

1. Prismatic Crystals



2. Rosette Crystals



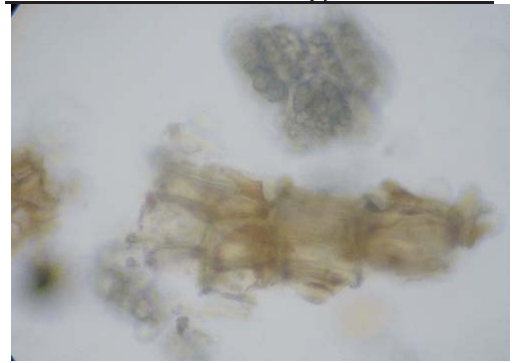
3. Stone Cells & Sclereids



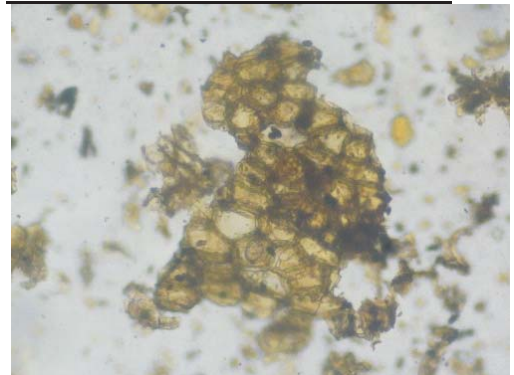
4. Spiral vessel



5. Cork Cells in tangential view



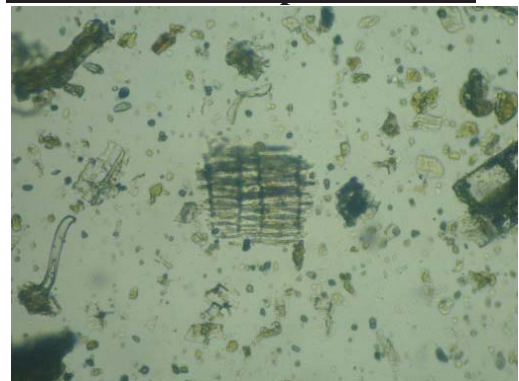
6. Cork Cells in surface view



7. Fibres



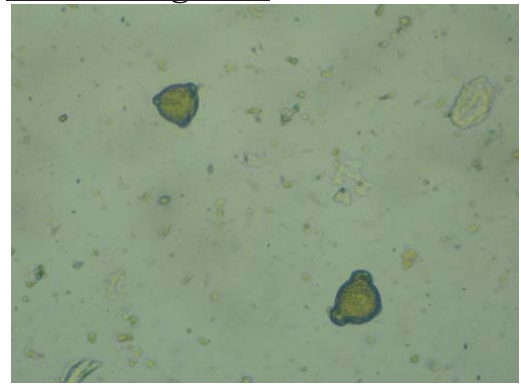
8. Unicellular simple trichome



9. Pitted vessel



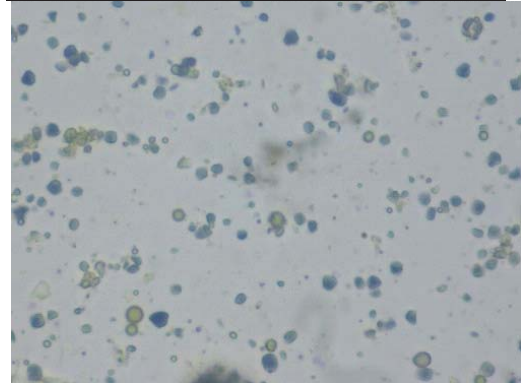
12. Pollen grains



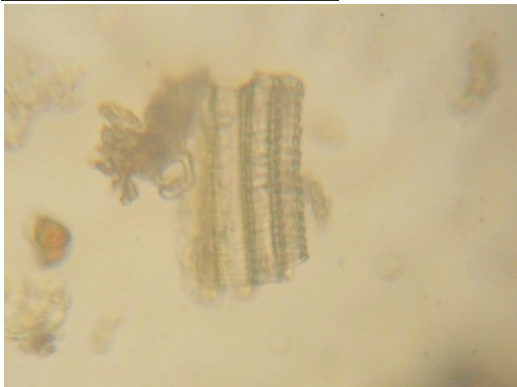
10. Group of vessels



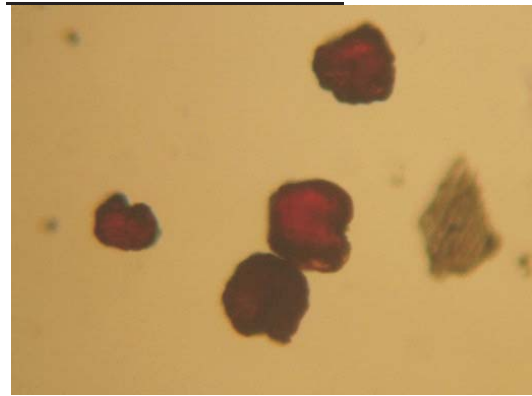
13 Oil globules & Starch grains



11. Reticulated vessel



14. Brownish content



tannins & flavonoids qualitatively and 89.57 % of total sugar. TLC profile shows 2 spots at 254 nm, 7 spots at 366 nm and 4 spots after spraying. All the above Physicochemical & phytochemical data can be useful as diagnostic tool for generated identical profile also all these parameters can act as diagnostic tool for identification and authentication and play an important role in quality control and for further research.

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TABLE 1 :- Ingredients of Triphaladi Avaleha

No	Drug Name	Family	Latin Name	Part Used	Quantity
1	<i>Amalaki</i>	Euphorbiaceae	<i>Emblia officinalis</i> Gaertn.	Fruit	1 part
2	<i>Haritaki</i>	Combretaceae	<i>Terminalia chebula</i> Retz.	Fruit	1 part
3	<i>Vibhitaki</i>	Combretaceae	<i>Terminalia bellerica</i> Roxb.	Fruit	1 part
4	<i>Katuki</i>	Scrophulariaceae	<i>Picrorhiza kurroa</i> Royle ex Benth.	Root	1 part
5	<i>Kakmachi</i>	Solanaceae	<i>Solanum nigrum</i> Linn.	Whole plant	1 part
6	<i>Kutaja</i>	Apocynaceae	<i>Holarrhena antidysenterica</i> Wall.	Bark	1 part
7	<i>Haridra</i>	Zingiberaceae	<i>Curcuma longa</i> Linn.	Rhizome	1 part
8	<i>Vidanga</i>	Zingiberaceae	<i>Embelia robusta</i> Burm.	Fruit	1 part
9	<i>Guduchi</i>	Menispermaceae	<i>Tinospora cordifolia</i> Willd.	Stem	1 part
10	<i>Shweta Punarnava</i>	Ficoidaceae	<i>Trianthema portulacastrum</i> Linn.	Root	1 part
11	<i>Sharapunkha</i>	Leguminosae	<i>Tephrosia purpurea</i> Linn.	Root	1 part
12	<i>Madhu</i>	-	Honey	Nector	q.s
13	<i>Sharkara</i>	-	<i>Saccharum officinarum</i> Linn.	Crystal	q.s
14	<i>Chaturjata</i>				<i>Prakshepa</i>
A	<i>Twak</i>	Lauraceae	<i>Cinnamomum zeylanicum</i> Blume	Bark	q.s
B	<i>Ela</i>	Zingiberaceae	<i>Elettaria cardamomum</i> Maton	Fruit	q.s
C	<i>Tamalapatra</i>	Lauraceae	<i>Cinnamomum tamala</i> Nees & Eberm	Leaf	q.s
D	<i>Nagakeshara</i>	Guttiferae	<i>Ochrocarpus longifolius</i> Benth. & Hook.	Flower bud	q.s

TABLE 2:- Organoleptic Characters of *Triphaladi Avaleha*

Characters	Results
Rupa (Colour)	Dark brown
Rasa (Taste)	Madhura, Tikta
Gandha (Odour)	characteristic
Sparsha (Consistency)	Semisolid

TABLE 4:- Qualitative Tests of *Triphaladi Avaleha*

No.	Functional groups/ Tests	Results
a	Carbohydrate	+ve
b	Steroid	+ve
d	Saponin glycoside	+ve
e	Flavonoid	+ve
f	Alkaloid	+ve
g	tannins & phenolic compounds	+ve
h	Artificial Invert Sugar test for Honey	-ve

TABLE 3:- Physico-chemical parameters of *Triphaladi Avaleha*

No.	Parameters	<i>Triphaladi Avaleha</i>
1.	Loss on drying at 105°C	9.135% w/w
2.	Total ash	0.796% w/w
3.	pH of 5% aqueous solution	4.44
4.	Water soluble extractive	82% w/w
5.	Alcohol soluble extractive	91.8% w/w

TABLE 5:- Quantitative assessment of *Triphaladi Avaleha*

7.	Sugar content	
a.	Total sugar	89.57%
b.	Reducing sugar	58.37%
c.	Non-reducing sugar	31.20%

TABLE 6:- Results of TLC

DRUG	366 nm		254nm		After spraying 10% FeCl ₃ aqueous solution	
	No. of spots	R _f	No. of spots	R _f	No. of spots	R _f
Triphaladi Avaleha	7	0.08 0.16 0.29 0.39 0.44 0.81 0.95	2	0.08 0.81	4	0.08 0.32 0.81 0.94