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PHARMACEUTICAL EVALUATION OF *MUSTA-TRIPHALADI AVALEHA*, AN AYURVEDIC POLYHERBAL FORMULATION

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ABSTRACT
According to the ayurvedic perspective properties and the action of the drug is described with their r*asa panchaka.* And also ancient ayurvedic *aachaaryas* have mentioned various methods to quality control and to standardization the ayurvedic formulations. But in the present scientific era it is a necessity to explain *ayuervedic* fundamentals also in modern scientific manner for the comprehensive acceptance. Hence present study was carried out to ensure the quality standards of the formulation, *Musta-triphaladi Avaleha* with preliminary physico-chemical parameters and pharmaceutical characteristics. This is a modified ayurvedic formulation which is used in the management of Thalassemia Major. *Avaleha* is a herbal drug compound prepared in dosage format of a semi-solid consistency of the drugs meant for licking (lincture) and has been tried clinically as an adjuvant with proven results. The test drug was prepared in the Pharmacy, G.A.U., Jamnagar. Analytical study of test drug was carried out at pharmaceutical laboratory, I.P.G.T. & R.A., Jamnagar. In the pharmaceutical study, it was observed that Loss on drying was 11.094% w/w, pH was 6.0, Water soluble extractive was 76.29% w/w, Alcohol soluble extractive was 83.65% w/w, Ash Value was 0.142 g and Acid insoluble Ash was 0.193% w/w. And also presence of Carbohydrate, Tannins and Phenolic Compounds and Steroid were observed. In HPTLC study 09 peaks at 254 nm and 08 peaks at 366 nm wave lengths were indicated.

KEYWORDS: *Musta-triphaladi Avaleha*, l, Physico-Chemical, Thalassemia Major.

INTRODUCTION
According to the ayurvedic basic principles, properties and action of a drug are described with their *rasa panchaka, i.e. rasa, guna, veerya, vipaka, prabhava*. After analysing the *rasa panchaka* of any drug, it can be explained that how the drug act on *dosha, dhatu & mala* in the body; whether those factors would be aggravated or pacified. But such ayurvedic basic theories like *rasa panchaka, thridosa*,. etc are not been able to understand by the people out of Ayurvedic field. And also in the present scientific era, where everything is looked at with a modern point of view, it is a necessity to explain *ayuervedic* fundamentals also in modern scientific manner for the global acceptance of Ayurveda. Hence present study was carried out to ensure the quality standards of the formulation, *Musta-triphaladi Avaleha* with preliminary physico-chemical parameters and pharmaceutical characteristics. Pharmaceutical analysis is a branch of practical chemistry that involves a series of process for identification, determination, quantification and purification of a substance, separation of the components of a solution or mixture, or determination of structure of chemical compounds.[1] *Musta-triphaladi Avaleha* is a modified ayurvedic formulation which is used in the management of Thalassemia Major.[2] *Avaleha* is a herbal drug compound prepared in dosage format of a semi-solid consistency of the drugs meant for licking (lincture) and has been tried clinically as an adjuvant with proven results.

MATERIAL AND METHODS

INGREDIENTS OF *MUSTA-TRIPHALADI AVALEHA*

Ingredients of *Musta-Triphaladi Avaleha* are as shown in the Table1.

Table 1: Ingredients of *Musta-Triphaladi Avaleha*

No.	Drug Name	Latin Name	Part Used
1	Musta	*Cyprus rotundus* Nust.	Dry Rhizome
2	Amalaki	*Emblica officinalis* Gaertn.	Dry Fruit
3	Haritaki	*Terminalia chebula* Retz.	Dry Fruit
4	Vibhitaki	*Terminalia bellerica* Roxb.	Dry Fruit
5	Katuki	*Picrorhiza kurroa* Royle ex Benth.	Dry Root
6	Kakmachi	*Solanum nigrum* Linn.	Dry Whole plant
7	Kutaja	*Holarrhena antidysenterica* Wall.	Dry Bark
8	Haridra	*Curcuma longa* Linn.	Dry Rhizome
9	Vidanga	*Embelia robusta* Burm	Dry Fruit
10	Guduchi	*Tinospora cordifolia* Willd.	Dry Stem
11	Shweta Punarnava	*Trianthema portulacastrum* Linn.	Dry Root
12	Sharapunkha	*Tephrosia purpurea* Linn.	Dry Root
13	Apamarga	*Achyranthus aspera* Linn.	Dry Whole plant
14	Kadali	*Musa paradisiacal* Linn,	Dry Rhizome powder
15	Shatavari	*Aspergus recemosus* Willd.	Dry Root
16	Shigru	*Moringa oleifera* Lam.	Dry Root bark
17	Vasa	*Adhatoda vasica* Nees	Dry Leaves
18	Daruharidra	*Berberis aristata* DC	Dry Root
19	Sariva	*Hemidesmus indicus* R.Br.	Dry Root
20	Manjishtha	*Rubia cordifolia* Linn.	Dry Root
21	Madhu	Honey	----
22	Sharkara	*Saccharum officinarum* Linn	Crystal
23	Chaturjata		
a.	Twak	*Cinnamomum zeylanicum* Blume	Dry Bark
b.	Ela	*Elettaria cardamomum* Maton	Dry Seed
c.	Tamalapatra	*Cinnamomum tamala* Nees & Eberm	Dry Leaf
d.	Nagakesara	*Mesua ferrea* Linn	Dry Pushpakalika
24	Trikatu		
a.	Shunthi	*Zingiber officinale* Rosc.	Dry Rhizome
b.	Maricha	*Piper nigrum* Linn.	Dry Fruit
c.	Pippali	*Piper longum* Linn.	Dry Fruit

PREPERATION OF THE DRUG

The test drug was prepared in the Pharmacy, Gujarat Ayurved University, Jamnagar. The Finished product of test drug was used for the Pharmaceutical and physico-chemical Parameters study at the Pharmaceutical chemistry laboratory.

ANALYTICAL STUDY

Analytical study of *Musta-Triphaladi Avaleha* was carried out to evaluate following parameters for *Avaleha* Standardization:

1. Loss on drying.[3]
2. Ash value.[4]
3. Acid insoluble Ash.[5]
4. pH Value.[6]
5. Water soluble extractive.[7]
6. Alcohol soluble extractive.[8]
7. Qualitative Test

a. Test for carbohydrate.[9]
b. Test for steroid.[10]
c. Test for proteins.[11]
d. Test for cardiac glycoside.[12]
e. Test for saponin glycoside.[13]
f. Test for flavonoid.[14]
g. Test for alkaloid.[15]
h. Test for tannins and phenolic compounds.[16]
i. Test for amino acid.[17]

8. Sugar content (Total, Reducing & Non-reducing).[18]
9. Thin Layer Chromatography.[19]

1. Loss on drying (LOD)

2 gram of drug sample was taken in a pre weighed dried and clean petridish. It was dried in an oven at 110ºC until reaching a constant weight. The petridish was taken out, self cooled and weighed immediately. The weight loss i.e. loss on drying was calculated and expressed as % w/w.

2. Ash value

2 gram accurately weighed sample was taken in a pre weighed dried and clean crucible. It was incinerated in a muffle furnace up to 650°C, The crucible was taken out, self cooled and weighed immediately. Then ash value was calculated and expressed in grams.

3. Acid insoluble ash value
Ash obtained by the test of ash value were dissolved with 6N 40ml HCl and poured in to a clean and dried 100 ml becker and heat it. Then filtered with Wattman no.1 ashless filter paper. Washing was given by distilled water throughout the filter paper in order to make it free from chloride. After filtering the all distilled water, the filter paper was folded in to a small piece and kept in a pre weighed dried and clean crucible. It was incinerated in a muffle furnace up to 650°C, The crucible was taken out, self cooled and weighed immediately. Then acid insoluble ash value was calculated and expressed in grams.

4. P^H Value
5 gram of test drug sample was weighted and taken in a clean and dried conical flask. Then added 50 ml water and stirred well for few minutes; kept this solution for some time and then filtered it through filter paper and the filtered solution was taken in a beaker. pH value of the sample was evaluate by using the standardized pH papers according to the change of the color of the paper.

5. Water Soluble Extractive
2.5 gram of the sample was weighed accurately. 50 ml of distilled water was added to it and kept covered overnight. Next day, it was filtered. 20 ml of the filtrate was accurately measured with a pipette and transferred to the already weighed clean and dry evaporating dish. The evaporating dish was placed on a water bath for evaporation of the water. After evaporation of the water it was dried in an oven, allowed cooling and weighed immediately. From the weight of the residue obtained, the percentage of water soluble extractive was calculated and expressed as % w/w.

6. Alcohol Soluble Extractive
2 gram test drug sample was weighed accurately. 50 ml methanol was added to it and kept covered overnight. And same procedure was followed as water soluble extract method. From the weight of the residue obtained, the percentage of alcohol soluble extractive was calculated and expressed as % w/w.

7. Qualitative Test
Preperation of test solution
5g of *Musta-Triphaladi Avaleha* was taken in to the clean and dried conical flask and added 50 ml of methanol. After 2 hours solution was filterd by using a filter paper and that solution was used for qualitative tests.

Details of chemical tests carried out for detection of organic chemical constituents are as follows.
(a) Test for carbohydrate
Molish's test method was followed.

(b) Test for Tannins and Phenolic Compounds
To 2-3 ml of alcoholic extract, few drops of Lead acetate solution were added and observed the color of precipitation.

(c) Test for Steroid
Salkowski reaction test method was followed.

(d) Test for Saponin Glycoside
Form test method was followed.

(e) Test for Flavonoids
Lead acetate solution was added to small quantity of residue and observed the color of precipitate.

(f) Test for Alkaloids
Evaporated alcoholic extracts and to residue, dilute HCl was added. After shaking well it was filtered. With filtrate **Dragendroff's test** method was followed.

(g) Test for proteins
Biuret Test (General Test) method was followed.

(h) Test for amino acids
Ninhydrin test (General Test) method was followed.

8. Sugar content (Total, Reducing & Non-reducing)
This includes estimation of total, reducing and non-reducing sugars. The followed method was slightly modified according to the practice of the laboratory.

Preparation of solution for analysis
2 gm of drug sample was taken in a glass beaker and 25 ml distilled water was added and stirred to make solution. For the proper dissolution it was kept in water bath and filtered with cotton. Hot water was added over the cotton and made final volume up to 75ml. Then the clarifying agent, 10% lead acetate solution, was added drop by drop and warmed for about 3-5 minutes to get the precipitate. Solution was filtered through filter paper. To the filtrate sodium oxalate was added till precipitation was formed to dissolve excessive lead acetate and to get a clear solution. This solution was filtered through the filter paper and washing was given by distilled water and the volume was made up to 250 ml. This straw color clear solution contained both reducing and non reducing sugar.

25 ml of above solution measured with a pipette and added in to 100ml beaker. 6N 5ml HCl was added and keep the solution in water bath under the temperature between 67^0 C – 71^0C for 3 minutes. After cooling one pinch of powder phenopthaline was added to this solution. Then concentrated NaOH solution was added to this solution drop by drop till it become dark pink color. Made final volume of this in to 100 ml by adding distil water. 25 ml each of Fehling's A and Fehling's B solutions were added in to 250 ml cornical flasks marked as T and R. To the 'T' flask 10 ml of pink solution was added and to the 'R' flask 10 ml of straw color solution

was added. Both flasks boiled for three minutes and filtered through filter bed made with glass wool, cotton and Whattman no. 1 filter paper. Repeated washing was given by hot distilled water till clear colorless filtrate was obtained. Precipitate of cuprous oxide (residue) was then taken with acid ferric solution to dissolve the precipitate completely in it. This solution was titrated against 0.1N $KMnO_4$ using ortho phenanthrolin as the indicator. At the end point, the brownish solution changes to greenish blue. From the amount of $KMnO_4$ solution required, the amount of copper % was calculated. Then percentage of sugar content was determined by using 'Revised Hammond table for calag dextrose'.

Determination of reducing sugar:

$$\% \text{ of Cu} = \frac{\text{Factor } (0.00636) \times \text{burette reading of 'R'} \times V^* \times SS^* \times N \text{ of } KMnO_4 \times 100}{W\,S^* \times V^* \text{ taken} \times N \text{ of } KMnO_4}$$

Determination of total sugar

$$\% \text{ of Cu} = \frac{\text{Factor } (0.00636) \times \text{burette reading of 'T'} \times V^* TS^* \times V^* SS^* \times N \text{ of } KMnO_4 \times 100}{W\,S^* \times V^* \text{ taken from } SS^* \text{ to make TS} \times V^* \text{ taken from } TS^* \times N \text{ of } KMnO_4}$$

- V = volume
- SS = stock solution
- TS = test solution
- W S = weight of sample

Determination of non-reducing sugar

The non-reducing sugar content was obtained by subtracting reducing sugar from total sugar.

Non reducing sugar = Total sugar – reducing sugar.

9. High Performance Thin Layer Chromatography (HPTLC)

Chromatography is the separation of a mixture into individual components using a stationary phase and mobile phase. Thin Layer Chromatography is a method based on adsorption chromatography. HPTLC is a sophisticated and automated form of TLC.

HPTLC was carried out after making appropriate solvent system with Methanolic extract of *Musta-Triphaladi Avaleha*. On performing HPTLC, visual observation under UV light showed few spots but on analyzing under densitometer at 254nm and 366nm it resulted into 9 and 8 spots respectively. Solvent system – Toluene: Ethyl acetate: Acetic Acid = (7:2:1). Results of HPTLC are given in Table 3.

OBSERVATIONS AND RESULTS

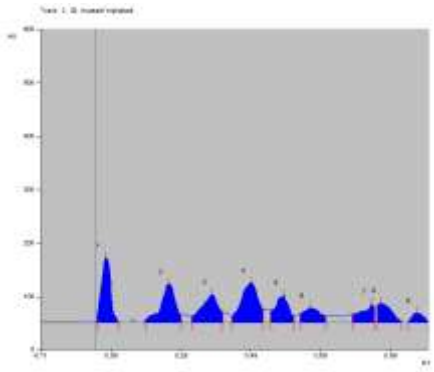
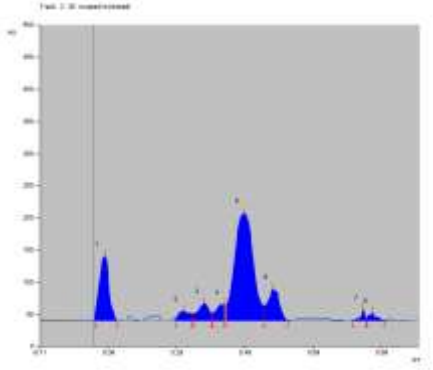
Table 2- Physico-Chemical Parameters of *Musta-Triphaladi Avaleha*

No.	Parameters	*Musta-Triphaladi Avaleha*
1.	Loss on drying at 110°C	11.094 % w/w
2.	Total ash	0.142 g
3.	Acid insoluble ash	0.193% w/w
4.	pH value	6.0
5.	Water soluble extractive	76.29 % w/w
6.	Alcohol soluble extractive	83.65 % w/w
7.	Qualitative Test	
a.	Test for Carbohydrate	Present
b.	Test for Tannins and Phenolic Compounds	Present
c.	Test for Steroid	Present
d.	Test for Cardiac Glycoside	Present
e.	Test for Saponin Glycoside	Absent
f.	Test for Flavonoids	Absent
g.	Test for Alkaloids	Absent
h.	Test for Proteins	Absent
i.	Test for Amino Acids	Absent
8.	Sugar content	
a.	Total sugar	78.74 %
b.	Reducing sugar	49.46 %
c.	Non-reducing sugar	29.28 %

Table 3 – Results of HPTLC of *Musta-Triphaladi Avaleha*

Wave lengths	Short UV (254 nm)	Long UV (366 nm)
No of Spots	**09**	**08**
Max. Rf Value	**0.08, 0.26, 0.38, 0.49, 0.58, 0.66, 0.84, 0.86, 0.97**	**0.08, 0.31, 0.37, 0.43, 0.49, 0.57, 0.84, 0.86,**

Plate 1 – Densitogram of HPTLC of *Musta-Triphaladi Avaleha*

Wave lengths	Short UV (254 nm)	Long UV (366 nm)
		

DISCUSSION

Evaluation of physico-chemical parameters and qualitative analysis helps to identify the presence of specific chemical constituents in a formulation and application of chromatographic techniques helps in recognition of number of ingredients and also to assess the purity by comparing with the standard ones. With the knowledge of availability of certain chemical compounds it can be evaluated specific action of the formula.

Loss on drying at 110^0 c is one of the major factors for assessing the stability of the drugs. Analytical study showed that loss on drying of *Musta-Triphaladi Avaleha* was 11.094%. Higher value of loss on drying indicates that it contains higher moisture level and which may leads to fungal and other microbial growth, hence it reduce the shelf life of the drug. Lesser value suggests the reduced moisture holding capacity which may increase the shelf life of the drug. As loss on drying of *Musta-Triphaladi Avaleha was* comparatively higher proper handling should be done in order to maintain a good shelf life.

Musta-Triphaladi Avaleha showed a higher value of water soluble extractive percentage (76.29%) as it contains water soluble ingredients such as *Sharkara* and honey in higher quantity. Because of *Musta-Triphaladi Avaleha* contains more sugar, alchohol soluble extractive percentage was also comparatively very high (83.65%).

Ash Value of *Musta-Triphaladi Avaleha* was 0.142g. It may due to the many organic herbal ingredients are in *Musta-Triphaladi Avaleha.* 0.193% of acid insoluble ash was found in *Musta-Triphaladi Avaleha*.

As far as pH values are concerned; it was found as 06 in *Musta-Triphaladi Avaleha*. This indicates that the drug is nearly neutral in nature.

Qualitative study showed that presence of Tannins in the test drug, *Musta-Triphaladi Avaleha*. It is known fact that Tannins inhibit the excess GIT absorption of iron.[20] There is an excessive iron overload in thalassemic patients' body due to chronic blood transfusion as well as due to increased GIT absorption. Hence tannin helps to prevent iron overload in thalassemia patients' body. In acidic media in stomach, tannins are subjected to hydrolysis and then smaller molecules of them have antioxidant property. It helps to increase general health and quality of life of the thalassemia patients.

Many phenolic compounds in plants are good sources of natural antioxidants. It is a great interest in recent years that many phenolic compounds in foods have inhibitory effects on mutagenesis and carcinogenesis.[21] As *Musta-Triphaladi Avaleha* contains phenols it can be said that drug has the antioxidant property which helps to increase general health and quality of life of the thalassemia patients.

As *Musta-Triphaladi Avaleha* contains *Sharkara* and honey in higher quantity, under qualitative study it was found that presence of carbohydrates there. Carbohydrates provide the body with glucose, which is converted to energy used to support bodily functions and physical activity.[22] General weakness is one of the main symptoms in thalassemia. So trial drug reduces the weakness of the body and provides energy.

Presence of steroids was also found in the trial drug. The food one eat contains copious amounts of the building blocks of human steroids. These precursor chemicals include pregnenolone, androstenedione, hydroxyprogesterone, dehydroepiandrosterone (DHEA), dihydrotestosterone, androsterone, 17-alpha-estradiol, and estriol. The human body turns these chemicals into a wide variety of sex and growth hormones, giving food an intimate connection with human health.[23] Due to the affected multi systems in the body patients' growth is diminished. Hence presence of steroids is plays an important role here.

Qualitative tests showed that presence of Cardiac Glycosides in *Musta-Triphaladi Avaleha*. The main action of the Cardiac Glycosides is to increase the force

of cardiac contraction. The glycosides enhance vagal tone over the heart which slows the heart rate.[24]

At HPTLC study the color and rf values of resolved spots were noted.[25] In *Musta-Triphaladi Avaleha* in wave lengths of 254 nm and 366 nm total 09 and 08 points were observed respectively. Increase in No. of spots at long UV radiation indicating difference of UV light responding componsents mainly dine type systems showing un-saturation in compounds present in *Musta-Triphaladi Avaleha.*

CONCLUSION

Priliminary physicochemical parameters of *Musta-Triphaladi Avaleha* are with in the standared range. Thus the study proves the quality of the final product.

REFERANCE

1. www.pharmatutor.org/pharma-analysis (03.04.2016).
2. Shailesh R. Rajgolkar *et al*, A clinical study on *Beejadushtijanya Pandu* (Thalassemia Major) in children and its management with *Musta-Triphaladi Avaleha.* Dept of Kaumarbhritya, IPGT & RA, GAU, Jamnagar, 2011– 2014.
3. Ayurvedic Pharmacopoeia of India PDF-1, Govt. of India, Ministry of health and family welfare, Delhi, 2007; 5: appendix-2.2.9., p. no. 214.
4. Ibidem, Ayurvedic Pharmacopoeia of India, (1): p. no. 213.
5. Anonymous, Indian Pharmacopoeia, Government of India, Ministry of Health and Family welfare, New Delhi, 1996, Volume V, Appendix 2.2.4.
6. Ibidem, Ayurvedic Pharmacopoeia of India, (1): p. no. 230.
7. Ibidem, Ayurvedic Pharmacopoeia of India, (1): p. no. 214.
8. Ibidem, Ayurvedic Pharmacopoeia of India, (1): p. no. 214.
9. Practical Pharmacognosy, Khandelwal K. R., Nirali Prakashan, Pune, 4th edition, 1998; p. no. 149.
10. Ibidem, Practical Pharmacognosy, (6): p. no. 151.
11. Ibidem, Practical Pharmacognosy, (6): p. no. 150.
12. Ibidem, Practical Pharmacognosy, (6): p. no. 152.
13. Ibidem, Practical Pharmacognosy, (6): p. no. 152.
14. Ibidem, Practical Pharmacognosy, (6): p. no. 153.
15. Ibidem, Practical Pharmacognosy, (6): p. no. 153.
16. Ibidem, Practical Pharmacognosy, (6): p. no. 153.
17. Ibidem, Practical Pharmacognosy, (6): p. no. 151.
18. Anonymus, (1987), Pharmacopoeial standards for Ayurvedic formulation, CCRAS, Ministry of health and family welfare, Delhi.
19. Ibidem Ayurvedic Pharmacopoeia of India, (1): p. no. 218.
20. Wintrobe's Clinical Hematology (Nutritional factors in the production and function Of RBCs-Ascorbic Acid).
21. http://pubs.acs.org/doi/pdf/10.1021/bk-1992-0506.ch001 (01.06.2016).
22. http://www.hsph.harvard.edu/nutritionsource/carbohydrates/ (01.06.2016).
23. http://www.steadyhealth.com/articles/natural-steroids-in-food (01.06.2016).
24. http://www2.nau.edu/~daa/lecture/chfmeds.htm (01.06.2016).
25. Anonymous. The Ayurvedic Pharmacopeia of India. Part 2, vol 2, Appendices 1st ed. New Delhi: Govt of India Publication, 2008; 233-5.