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Shelf Life Evaluation of *Trivrit Avaleha* - A Preliminary Assessment

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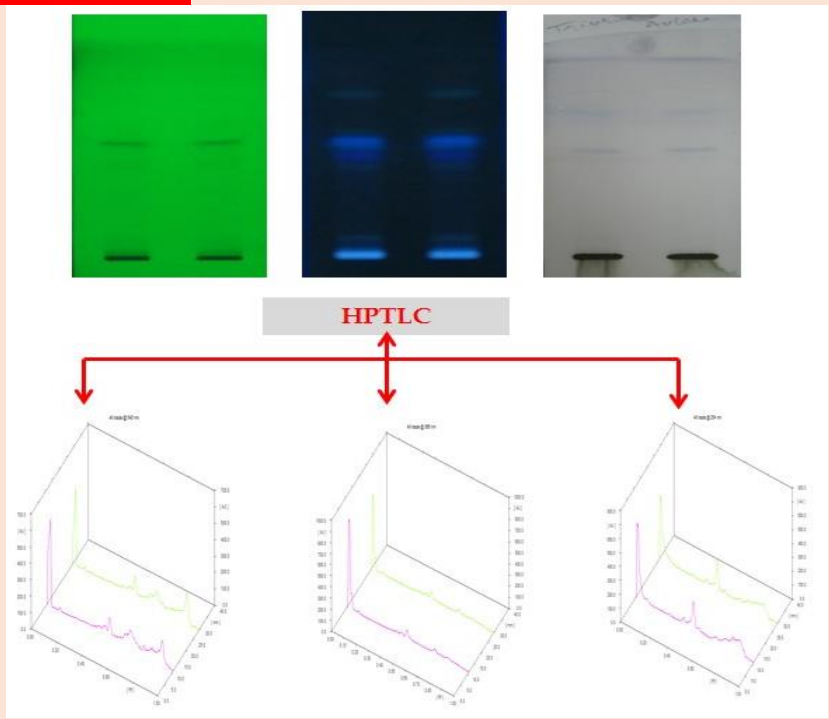
ABSTRACT

Introduction: Potency of drug is termed as *Virya* of *Ausadhi* (effectiveness of drug) and the time period, where it retains the potency is known as *Saviryata Avadhi.* Every product has definite shelf life that depends on physical, chemical, environmental and biological factors. Though, shelf life of various categories of āyurvedic medicines is available; there is a need to revalidate the shelf life of individual formulations by following parameters prevalent in respective scenario. *Trivrit Avaleha* is mentioned in Astanga Hridaya for *Virecana* (therapeutic purgation). Aim of the current study is to evaluate shelf life of *Trivrit Avaleha* (TA) with the help of modern analytical techniques. ***Methods:*** Physico-chemical parameters were measured at 40°C ± 2°C temperature and 75%±5% relative humidity. Analysis was repeated at intervals of 1, 3, and 6 months and average 10% degradation of the test drug samples was calculated and extrapolated to find the shelf life. ***Results:*** Product was found to be free from microbial contamination and heavy metals were within the permissible limits. There were insignificant changes in physico-chemical profiles at different intervals of analysis. On extrapolation of the observations, the shelf life of TA was found to be 1 year and 11 months. ***Conclusion:*** Shelf life of TA is found within the standards of Gazettes of Govt. of India as well as *Sarandhara Samhitā*.

KEYWORDS

Accelerated stability, *Saviryata Avadhi*, Shelf life, *Trivrit Avaleha.*

PICTORAL ABSTRACT



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1. Introduction

Shelf life is the time period during which a drug product is expected to remain within the approved life specification, provided that it is stored under the conditions defined on the container label.[1] Shelf life is the time required for 10% of the material to disappear; it is the time at which molar concentration of the reactant has decreased to 90% of its initial concentration.[2] In āyurvedic literature, shelf life is known as *Saviryatā Avadhi* refers to the time period during which a drug remains potent. The information regarding the concept of shelf life for different dosage forms is found scattered in *Brihatrayi* like Shelf life of *Vati* (tablet) and *Churna* (powder) is one *Prahara* (3 hours) and 2-3 months respectively. *Acārya Caraka* explained that a *āushdhi* (drug) can be utilized for treatment purposes until it maintains its fragrance, color, taste etc.[3] After that Acārya *Sharangadhara*[4] opines that the *Avaleha* start to lose their therapeutic potency after a year, while *Yogaratnakara*[5] opines the period as six months. *Trivrit Avaleha*[6] (TA) is composed of *Trivrit* (*Operculina turpethum* Linn.), *Trijāta* (*Cinnamomum tamala* Nees and Eberm., *Cinnamomum zeylanicum* Breyn., *Elettaria cadamomum* Maton.), *Madhu* (honey) and *Khaṇḍaśarkarā* (sugar candy). It is an important formulations used frequently to induce *Virecana* (therapeutic purgation) by *āyurvedā* physicians. However, no stability profiles of this

formulation are available till date. Considering this, an attempt has been made to evaluate shelf life of TA with the help of modern analytical techniques.[7]

2. Materials and methods

2.1 Collection of raw materials

All the herbal drugs, *Madhu* (honey) and *Khaṇḍaśarkarā* (sugar candy) were procured from the Pharmacy, IPGT & RA, Jamnagar. All the herbal drugs were authenticated in the Pharmacognosy Laboratory, IPGT & RA, Jamnagar. Composition formulation is placed at Table1.

2.2 Pharmaceutical preparation of formulation

Avaleha is prepared by following classical guidelines of *Avaleha*.[8]

2.3 Shelf life evaluation

2.3.1 Sample quantity and packing

Samples were supplied in four transparent airtight food grade plastic containers plastic bottles with transparent screw cap. Each bottle contains 100 grams of TA.

2.3.2 Storage conditions

Accelerated stability study was conducted as per International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guidelines Q1A (R2).[1] Samples were stored at 40±2ºC and Relative Humidity 75±5%.

2.3.3 Frequency of withdrawal

The products were withdrawn from the container and analyzed initially and at a gap of 1, 3 and 6 months.

2.4 Parameters

Basic analytical parameters including moisture %, ash value and water soluble extractive[9] were evaluated at intervals specified earlier. Test for microbial contamination was done initially and at the end of six months of storage by following standard guidelines.[9] Chromatographic profiles (HPTLC) were evaluated under 254, 366 and 540 nm initially and after six months of storage. Accurately weighed 5g of sample was individually soaked in 10 ml methanol on a Vortex for 10 min, heated for 10 min, filtered through Whatman filter paper no. 1 and then concentrate it on water bath up to 2ml. Drying Mode, Temp. & Time: TLC Plate Heater Preheated at 100± 5^0C for 3 minutes; Application Mode: CAMAG Linomat 5 – Applicator; Filtering System: Whatman filter paper No.1; Stationary Phase: MERCK - TLC / HPTLC Silica gel 60 F_{254} on Aluminum sheets; Application (X axis) Start Position: 10mm; Development (Y axis) End Position: 90mm from plate base; Space Between Band: 10mm; Sample Application Volume: 06µL; Development Mode: CAMAG TLC Twin Trough Chamber; Chamber Saturation Time: 30 minutes; Mobile Phase (MP): Toluene: Ethyl acetate: Formic acid (7:3:0.1); Visualization: @254nm, @ 366nm and @ 540 nm (after derivatization); Spray reagent: Anisaldehyde Sulphuric acid reagent; Derivatization mode: CAMAG – Dip tank for about 1 minute[10].

3. Results and discussion

The organoleptic characters of the TA are shown in Table 2. No changes in organoleptic characters were found in the formulation at different levels of storage. Physico-chemical characters of TA at initial, 1, 3, 6months interval are shown in Table 2. Microbial growth was found below prescribed limits[9] by the end of 6thmonths (Table 3). Heavy metals were also found to be within the permissible limits at the end of 6th months[9] (Table 4). HPTLC showed 2spots at 254 nm, 4 spots at 366 nm and 4 spots at 540 nm (Figure 1). R_f values recorded were same for both samples (0, 6 months) (Table 5).

No changes in organoleptic characters were found at different levels of storage. TA was found to be brownish black in color with characteristic odor and taste. Color of drug was due to its components. Insignificant differences were observed in basic physico-chemical profiles in the drugs at different stages of analysis. Physicochemical standards such as total ash value and water soluble extractive value are useful in identification and authentication of the plant material. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of inorganic matter and also helps in determining both physiological ash and non-physiological ash. Extractive values help in determining the amount of active constituents and is done on plant materials in particular solvent for which as yet no suitable chemical or biological assay exists.[11] Presence of more moisture content in a sample can create preservation problem. The moisture content was found to be increasing gradually with storage. Microbial count and Heavy metals were within the permissible limits indicating safety and quality of the product.

Table 1. Formulation composition of *Trivrit Avaleha*

Drug	Latin name	Part used	Ratio	Quantity
Trivit	*Operculina turpenthum* Linn.	Dried Root	25 parts	6.25 kg
Tamāla patra	*Cinnamomum tamala* T. Nees.	Dried Leaves	1 part	250 g
Tvak	*Cinnamomum zeylanicum* Blume.	Dried Bark	1 part	250 g
Ela	*Elettaria cadamomum* Maton.	Dried Fruit	1 part	250 g
Madhu	Honey	-	5 parts	1.25 kg
Khandasharkara	Sugar candy	-	10 parts	2.5 kg

Table 2. Organoleptic and physico chemical parameters of *Trivrit Avaleha*

Organoleptic parameters				
	Initial	**1st month**	**3rd month**	**6th month**
Taste	Characteristic	Characteristic	Characteristic	Characteristic
Color	Brownish black	Brownish black	Brownish black	Brownish black
Odor	Characteristic	Characteristic	Characteristic	Characteristic
Consistency	Semi-solid	Semi-solid	Semi-solid	Semi-solid
Physico chemical parameters				
Moisture content(%W/W)	10.96	10.56	12.20	16.66
Total Ash (%W/W)	1.02	0.97	0.95	0.96
Water Soluble Extractive (%W/W)	74.67	73.68	77.25	69.31

Table 3. Total microbial growth in Trivrit Avaleha

Organism	Initial	6 months	Permissible Limits
Total plate count (cfu/g)	<10 cfu/g	<10 cfu/g	10^5/g
Total Yeast and Mould Count	Ab	Ab	10^3/g
E. coli	Ab	Ab	Absent
Pseudomonas aeruginosa	Ab	Ab	Absent
Staphylococcus aureus	Ab	Ab	Absent
Salmonella enteric	Ab	Ab	Absent

cfu: Colony Forming Units, Ab: Absent, g: Grams

Table 4. Heavy metals in *Trivrit Avaleha*

Heavy metals	TA	Permissible limits
Lead	2.647ppm	10 ppm
Cadmium	ND	0.30 ppm
Arsenic	0.425 ppm	3 ppm
Mercury	ND	1 ppm

ppm: parts per million, ND: Not Detected

Table 5. R_f values for methanolic extract of *Trivrit Avaleha*

Drug	Spot	R_f at 254 nm		R_f at 366 nm		R_f at 540 nm	
		Track1 (0 month)	Track2 (6 month)	Track1 (0 month)	Track2 (6 month)	Track1 (0 month)	Track2 (6 month)
TA	1	0.48	0.48	0.10	0.10	0.10	0.10
	2	0.55	0.55	0.48	0.48	0.55	0.55
	3			0.55	0.55	0.64	0.64
	4			0.77	0.77	0.77	0.77

Table 6.Intercept and slope of *Trivrit Avaleha* for different parameters

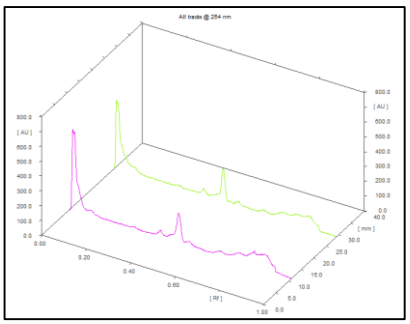
Parameters	Intercept	Slope
Loss on Drying	10.063	1.010
Water Soluble Extractive	75.63	0.76
Ash Value	0.995	0.0081

Figure 1. Comparative HPTLC of methanolic extract of *Trivrit Avaleha*

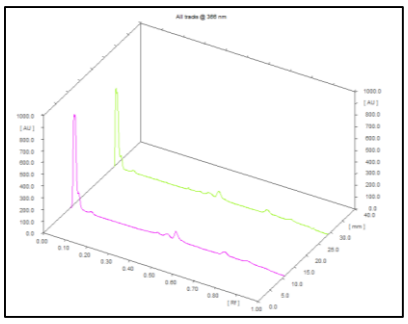
Under short UV

Under long UV

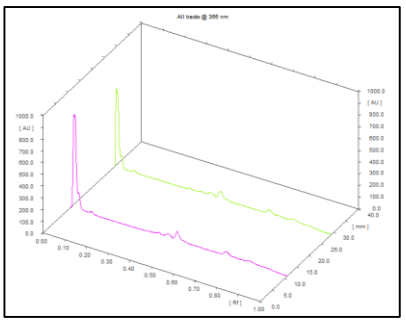
Under white light after derivatisation



At 254nm



At 366nm



At 540nm

Solvent System - Toluene: Ethyl acetate: Formic acid (7:3:0.1)

Table 7. Approximate period (in month) for 10% degradation of *Trivrit Avaleha*

Parameters	Initial	10% Degradation	Approximate Months required for 10% degradation
Loss on drying	10.94	9.846	0.21
Water soluble extractive	74.67	67.203	11.09
Ash Value	1.02	0.918	9.5
Mean months			**6.93333**

Table 8. Extrapolation of Shelf life

Drug	Mean Months for 10% degradation	Multiplication Factor	Shelf life Months	Shelf life Years
Trivrit Avaleha	6.93333	3.33	23.088	1 year 11 months

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Shelf life of *Avaleha* according to classical texts varies from 6 to 12 months, while Rule 161-B of Drugs and Cosmetics Act mentioned it as three years.[12] In current study; Shelf life of TA is found to be 1 year and 11 months. Earlier study, *Shirishāvaleha* showed 2 years 6 months (with *Kanji*) and 1 year 4 months (with water),[13] *Kamsaharitaki Avaleha*[14] showed 1 year 6 months, *Shirishashwagandhādi Avaleha*[15] showed 8 years 7 months. Findings of earlier studies make corroborate to results of TA except *Shirishashwagandhādi Avaleha* that is found more stable, possibly due to the presence of metallic components in that. This indicates that the shelf life of *Avalehas* with metallic composition will be comparatively higher than the ones with pure herbal part (Table 6 to 8).

4. Conclusion

On extrapolation of the observations the Shelf life of TA is found to be 1 year and 11 months. Findings of earlier studies are in support of current observations. Similar R_F values obtained in HPTLC analysis of TA initially and after six months showed minimum deterioration of the product. Studies involving other *Avalehas* are needed to substantiate the observations of the current study. The current work is preliminary in nature involving a few parameters. Extensive studies may be taken-up to revalidate current observations.

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CONTRIBUTORS Dr. Shivshankar contributed to design, literature study and data acquisition. Dr. Shweta contributed to the conceptualization of the topic, data analysis and manuscript editing. Dr. Galib contributed to the manuscript review, intellectual content, design and literature study. Dr. Dei contributed to the manuscript review and analysis of study.

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