ORIGINAL ARTICLE

Stability Study of *Bhallataka Kshaudra*: An Ayurvedic Formulation

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Abstract

Introduction: *Bhallataka* (*Semecarpus anacardium* L.), commonly known as "marking nut," has vast applicability in indigenous system of medicine and is indicated in many ailments such as skin disorders, tumors and malignant growth, fungal disease, excessive menstruation, vaginal discharge, fever, hemoptysis, constipation, and intestinal parasites. About 40 formulations of *Bhallataka* are mentioned in *Bharat Bhaishajya Ratnakar*. Acharya Charaka has described ten different formulations of *Bhallataka* in *Rasayana Adhyaya*. *Bhallataka Kshaudra* is among the 10 *Kalpana* of *Bhallataka*. The ratio of *Kshaudra* (Honey):*Bhallataka* oil: *Ghrita* (Ghee) is 1:8:16, respectively, for the preparation of *Bhallataka Kshaudra*. **Objectives:** The stability study is carried out to establish the duration of shelf life of the product under specified storage condition. **Materials and Methods:** Accelerated stability study was carried out under atmospheric condition temperature: 40 ± 2°C and relative humidity: 75 ± 5%. Organoleptic parameters, physicochemical parameters, microbial limits, and heavy-metal analysis were observed studied during the study. **Results:** No significant change was observed in any of the parameters observed during the stability study. Based on the stability data, the shelf life expected to be 4.49 years. **Conclusion:** The extrapolated shelf life of *Bhallataka Kshaudra* is found to be 4.49 years on assessing the analytical parameters. The microbial count and heavy metals were within permissible limits.

Key words: Accelerated stability, *Bhallataka Kshaudra*, Shelf life

INTRODUCTION

Bhallataka (*Semecarpus anacardium* L.), commonly known as "marking nut," has vast applicability in indigenous system of medicine and is indicated in many ailments such as skin disorders, tumors and malignant growth, fungal disease, excessive menstruation, vaginal discharge, fever, hemoptysis, constipation, and intestinal parasites.[1] About 40 formulations of *Bhallataka* are mentioned in *Bharat Bhaishajya Ratnakar*. Acharya Charaka has described 10 different formulations of *Bhallataka* in *Rasayana Adhyaya*. *Bhallataka Kshaudra* is among the 10 *kalpana* of *Bhallataka*. The ratio of *Kshaudra* (Honey):*Bhallataka* oil: *Ghrita* (Ghee) is 1:8:16, respectively, for the preparation of *Bhallataka Kshaudra*. In one of the recent studies, *Bhallataka Kshaudra* prepared as per the classical method has shown antihyperlipidemic activity in cholesterol-fed albino rats. *Bhallataka Kshaudra* has diverse applicability in therapeutics, but the shelf life study of the formulation is not available till the date. The stability study is carried out to find the shelf life period of the product under specified storage condition. Stability is the capability of a specific formulation in a particular container/closure system to remain within its physical, chemical, microbiological, toxicological, and therapeutic specifications. Hence, an attempt has been made to carry out the stability study of *Bhallataka Kshaudra* as per the guidelines.

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Table 1: The organoleptic parameters of *Bhallataka Kshaudra* observed at the initial, 1-, 3-, and 6-month interval of the study

Organoleptic parameters	0 month	1 month	3 months	6 months
Description	Black-colored viscous liquid	Black-colored viscous liquid	Black-colored viscous liquid	Black-colored viscous liquid
Odor	Characteristic	Characteristic	Characteristic	Characteristic

Table 2: Physicochemical parameters of *Bhallataka Kshaudra*

Parameters	0 month	Accelerated stability study		
		1st month	3rd month	6th month
Moisture by KF (%)	0.47	0.61	0.55	1.08
Specific gravity	0.962	0.940	0.923	0.953
Acid value	5.83	4.05	6.63	8.01
Saponification value	147.85	155.83	160.07	212.7
Viscosity by Brookfield (cP)	1620	1250	950	800

KF: Karl Fischer

MATERIALS AND METHODS

Preparation of *Bhallataka* oil

Fresh *Bhallataka* fruits were taken by selecting as *Prashasta* (acceptable) and *Aprashasta* (unacceptable) variety. The fresh *Bhallataka* fruits were immersed in a vessel containing potable water. The fruits which get settled at the bottom were accepted as *Prashasta* variety, whereas those who were floating were rejected as *Aprashasta* variety. The *Prashasta* fruits were then placed in a wooden box filled with *Yava* (barley fruits) and lined with fodder on inner side. The box (or *Yavarashi*) was kept closed for 4 months for the process of curing (*Kala-Prakarsh*). The cured *Bhallataka* fruits were taken out from *Yavarashi*. The cured *Bhallataka* fruits were subjected to cut off the dried pseudocarp for opening of the mouth. The chopped *Bhallataka* fruits were placed in the upper vessel of *Patala Yantra*, and the mouth of the vessel was covered with an iron mesh. The lower vessel was attached to it, and *sandhibandhana* was done using *Kapadamittti* process and it was allowed to dry in the sunlight. The whole arrangement was then placed in a pit and cow dung cakes were placed over it. The fire was ignited and the oil was then allowed to extract out of the fruits. After the completion of the process, the oil was collected in a clean vessel.

Preparation of *Bhallataka Kshaudra*

Bhallataka Kshaudra was prepared using *Bhallataka* oil, *Kshaudra*, and *Ghrita*. To prepare it, add 320 g of *Ghrita*, 20 g of *Madhu*, and 160 g of *Bhallataka* oil in a porcelain mortar and pestle and triturate it to make a homogeneous mixture.

Storage Condition and Evaluation Parameters

Samples were supplied in four transparent glass bottles with screw cap. Each bottle contains 100 g of *Bhallataka Kshaudra*. An accelerated stability study and real-time stability study were conducted as per the International Conference on Harmonisation guideline Q1A (R2).[2] Storage conditions are mentioned as below:

- Accelerated stability: Temperature: 40 ± 2°C and relative humidity (RH): 75 ± 5%
- The change was observed during 6 months for accelerated stability at an interval of 0, 1, 3, and 6 months.

Table 3: Microbial limit test for *Bhallataka Kshaudra*

Parameters	0 month	6 months
Total plate count (NMT 105 cfu/g)	1654 cfu/g	846 cfu/g
Total yeast and mold count (NMT 103 cfu/g)	Absent	Absent
Salmonella (absent)	Absent	Absent
Staphylococcus aureus (absent)	Absent	Absent
Pseudomonas aeruginosa (absent)	Absent	Absent

NMT: Not more than

The following parameters were considered for evaluation of stability study

i. Organoleptic characters such as color, odor, and taste
ii. Physicochemical parameters such as moisture content (using Karl Fischer titer), specific gravity,[3] acid value,[4] saponification value,[5] and viscosity (using the Brookfield viscometer)
iii. Microbial limit test
iv. Heavy-metal analysis.

Based on the analytical values obtained before and after 6 months of storage, intercept and slope were calculated. Using these data, approximate 10% degradation was calculated and was extrapolated to get shelf life. Real-time aging factors 5 and 3.3 were used for extrapolation of shelf life for climatic

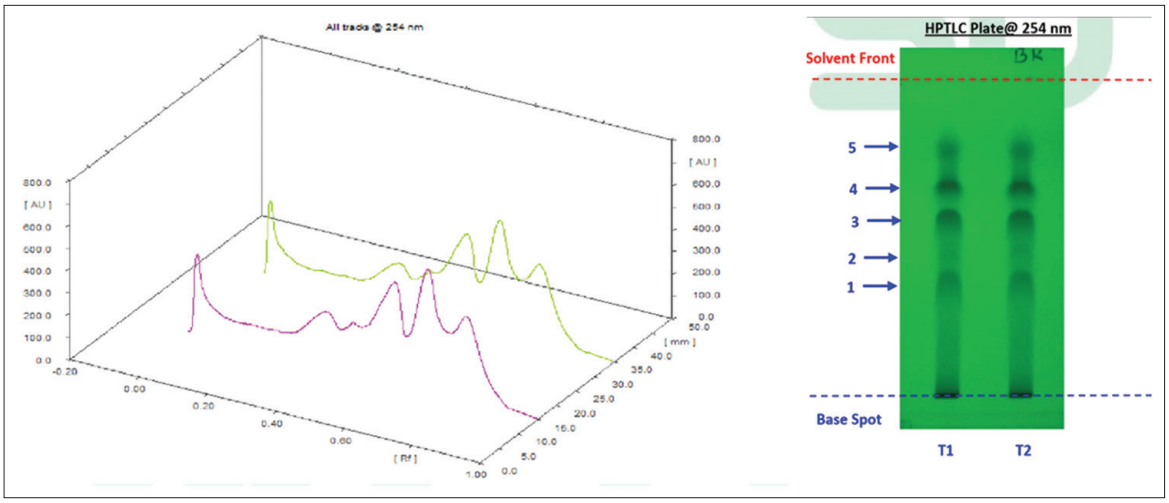

Figure 1: High performance thin layer chromatography chromatogram of *Bhallataka Kshaudra* at 254 nm

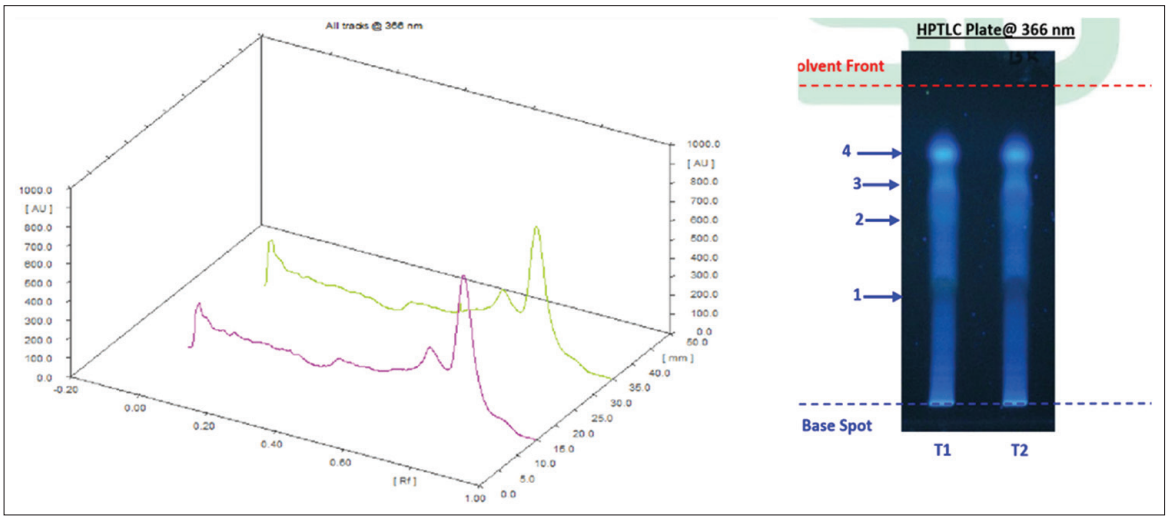

Figure 2: High-performance thin-layer chromatography chromatogram of *Bhallataka Kshaudra* at 366 nm

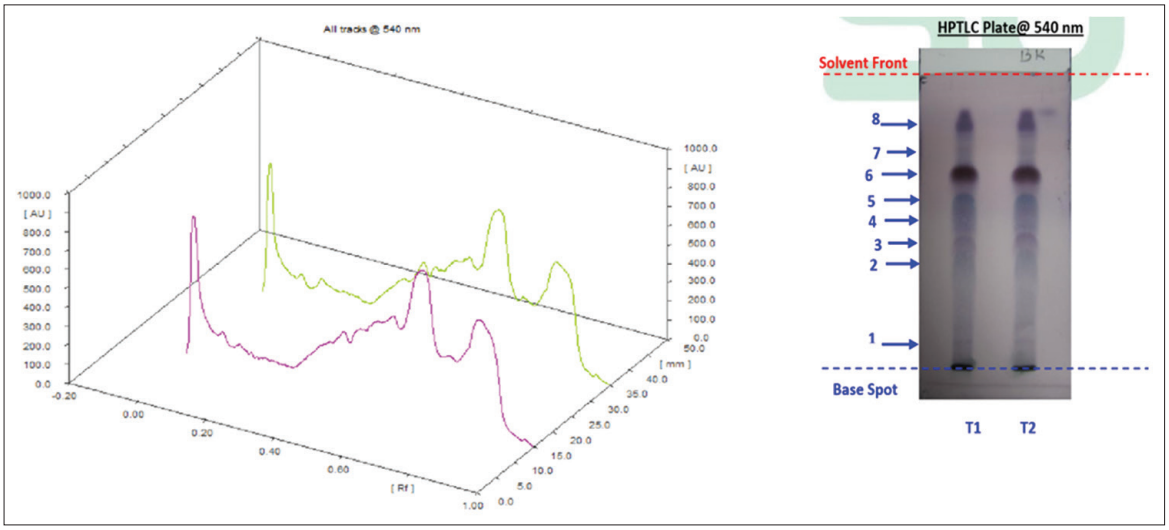

Figure 3: High-performance thin-layer chromatography chromatogram of *Bhallataka Kshaudra* at 540 nm

Zone I and II countries and climatic Zone III and IV countries, respectively. The ambient temperature and humidity for Zone I and II countries are 21°C/45% RH and 25°C/60% RH, respectively, whereas 30°C/35% RH and 30°C/70% RH are

Table 4: R_f values recorded at 0-month and 6-month interval

R_f @ 254 nm			R_f @ 366 nm			R_f @ 540 nm		
Spot number	Track-1	Track-2	Spot number	Track-1	Track-2	Spot number	Track-1	Track-2
1	0.36	0.36	1	0.34	0.34	1	0.09	0.09
2	0.45	0.45	2	0.57	0.57	2	0.36	0.36
3	0.57	0.57	3	0.68	0.68	3	0.45	0.45
4	0.67	0.67	4	0.77	0.77	4	0.53	0.53
5	0.77	0.77				5	0.59	0.59
6						6	0.67	0.67
7						7	0.75	0.75
8						8	0.84	0.84

Track-1: *Bhallataka Kshaudra* (0 month), Track-2: *Bhallataka Kshaudra* (6 months)

the respective temperature and humidity for Zone III and IV countries. India comes under climatic Zone III and IV.

RESULTS

In the accelerated stability study, temperature: 40 ± 2°C and RH: 75 ± 5% was maintained up to 6 months. The product was analyzed on 0, 1, 3, and 6 months. No change was observed in the organoleptic features of the *Kshaudra* [Table 1]. The results of different physicochemical parameters are given in Table 2. Test for microbial growth was conducted at the initiation of the study and at the end of 6 months [Table 3]. High-performance thin-layer chromatography profile represented in Figures 1-3 that showed 5 spots at 254 nm, 4 spots at 366 nm, and 8 spots at 540 nm, respectively. The R_f values were recorded for the sample at the initiation of the study and at the end of the 6th month [Table 4].

Number of months when degradation occurred was calculated using the following formula:

$$\text{Months when 10\% degradation occurs} = \frac{\left(\begin{array}{l}\text{0 month assay value} \\ -\left[\begin{array}{l}\text{0 month assay} \\ \text{value} \times 10/100\end{array}\right]\end{array}\right) - \text{Intercept}}{\text{Slope}}$$

Based on these values, intercept, slope, and approximate time for 10% degradation were calculated. The approximate time for 10% degradation for *Bhallataka Kshaudra* was 16.1976 months [Table 5]. As India comes under climatic Zone III, multiplication factor 3.3 was used for extrapolation of shelf life [Table 6]. Thus, the shelf life of *Bhallataka Kshaudra* was found to be 4.49 years [Table 7].

DISCUSSION

Stability studies determine the time when a product is considered to be safe and effective under a relevant storage condition. The main factors affecting the shelf life are derivation of the drug, dosage forms, environmental factors (humidity, temperature, and light), microbial contamination, storage conditions, and packaging system, etc. It is aimed at assuring that the product remains within specifications established to ensure its identity, strength, quality, and purity. The stability data on any dosage form include selected parameters that together form the stability profile. This stability profile is the basis for assigning the storage conditions and shelf life to pharmaceutical products. The design of the stability program for the finished product should be based on the knowledge of the behavior and properties of the drug substance and the dosage form.[6] On the basis of evaluated data regarding shelf life of *Bhallataka Kshaudra*, there was increase in moisture content, acid value, and saponification value in due course of time. The presence of *Ghrita* and *Madhu* can alter the shelf life by contributing to higher moisture content, thereby altering parameters as well. There was insignificant increase in the specific gravity of the formulation. The increase in moisture content causes hydrolysis and aids in the degradation, leading to increase in peroxide value. The hydrolytic degradation leads to break down of long-chain fatty acids into short-chain fatty acids. High saponification value of fats and oils are due to the predominantly high proportion of shorter carbon chain lengths of fatty acids. There was no considerable change observed in organoleptic characters and microbial load even after 6-month accelerate study. On the basis of available data from the accelerated stability study, it can be extrapolated that shelf life of *Bhallataka Kshaudra* is 4.49 years for countries

Table 5: Intercept and slope of *Bhallataka Kshaudra*

Parameters	Intercept	Slope
Moisture (% w/w)	0.4436	0.0936
Specific gravity	0.9466	0.00086
Acid value	4.8562	0.5095
Saponification value	142.789	10.5293
Viscosity	1470.476	126.19
TPC	1654	134.66

TPC: Total phenolic content

Table 6: Approximate period for 10% degradation in *Bhallataka Kshaudra*

Parameters	Initial	10% degradation	Approximate months required for 10% degradation
Moisture by KF (%)	0.47	0.047	0.22
Specific gravity	0.962	0.0962	93.95
Acid value	5.83	0.583	0.7670
Saponification value	147.85	14.785	0.923
Viscosity by Brookfield (cP)	1620	162	0.098
Total plate count	1654	165.40	1.228
Mean months			16.1976

KF: Karl Fischer

Table 7: Extrapolation of shelf life of *Bhallataka Kshaudra*

Drug	Months	Multiplication factor	Shelf life	
			Months	Years
Bhallataka Kshaudra	16.1976	3.33	53.93	4.49

which come under climatic Zone I and II and 16.60 months (1.38 years) for countries which come under climatic Zone III and IV.

CONCLUSION

The extrapolated shelf life of *Bhallataka Kshaudra* is found to be 4.49 years on assessing the analytical parameters such as moisture content, acid value, peroxide value, saponification value, and viscosity. The microbial count and heavy metals were within permissible limits. This study establishes the shelf life and stability parameters which are helpful in the proper use of *Bhallataka Kshaudra* in the management of different disorders.

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