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ACCELERATED STABILITY STUDY OF KHAMIRAE ABRESHAM SADA

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KEYWORDS:

Accelerated Stability Study, Unani Formulation, Khamirae Abresham Sada, High Performance Thin Layer Chromatography (HPTLC), Organoleptic, Physical, Chemical, Microbiological.

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ABSTRACT

The stability forms an important component of quality assurance program which involves the implementation of systems and procedures that provide a high probability that each dose or package of a pharmaceutical product have homogenous characteristics and properties which ensure both chemical safety and efficacy of that product. Stability studies are paramount requirement for finished pharmaceutical product for its registration, quality control assurance, prediction of shelf life and expiry dates. This study has furnished the feasible methods for evaluating shelf life of Unani Drugs**.** Accelerated Stability Study for 6 months of Unani formulation Khamirae Abresham Sada was done. The drug was prepared, packed and kept in stability chamber at 40^0C and 75% RH and then analyzed for organoleptic, physical, chemical and microbiological parameters. Samples were analyzed at 0, 3 and 6 months. Formulation was assessed for organoleptic characters, physical, chemical and microbiological changes. High Performance Thin Layer Chromatography (HPTLC) with multiple wavelength densitometric scanning was done. The findings showed degradation changes in drug formulation. The present study showed that the shelf life of Khamirae Abresham Sada was found to be 10 months to 1 year. The data collected at Accelerated Stability Study can be extrapolated for intermediate, long term, bracketing and matrixing so that better storage conditions for these two drugs can be recommended.

INTRODUCTION:

Understanding the stability characteristics of drug substances and drug products is a critical activity in drug development and the above quote by Carstensen undergoes the need to learn of potential stability problems as soon as possible during development[1]. World health organization (WHO) has recognized the effectiveness of traditional system of medicine and its safety. According to WHO report, over 80% of the world population relies on traditional medicine largely plant based for their primary healthcare needs. Stability data can also be generated under accelerated atmospheric conditions of temperature, humidity and light, which is referred to as short term stability and the data so obtained is used for predicting shelf-life of the product. Stability testing should be conducted on the dosage form packaged in the container closure system proposed for marketing. With the help of modern analytical techniques like spectrophotometer, HPLC, HPTLC and by employing proper guidelines it is possible to generate a sound stability data of herbal products and predict their shelf-life, which will help in improving global acceptability of herbal products [2]. Stability studies of drugs are necessary for betterment of patients for clinical efficacy of formulations and for drug regulatory requirements. In Unani classical literature Unani Scholars mentioned the different expiration dates of various Unani single and compound formulations. Classical Unani practitioners shared a lot from their experience regarding the identification of crude drugs, their authentication methods and about their clinical efficacy. They also mentioned the method of compound drug formulations, and their possible expiration dates [3].

Material and Methods:

Identification:

The ingredients of the Khamira formulation (Table 1) were purchased from the market by purchasing committee and were identified by Botanist Dr. Sumathi, Research officer, RMR, FRLHT-IAM, Bangalore. pecimens were preserved in the Repository of Medical Resources Herbarium and the Accession numbers are Gule Gaozaban *(Onosma bracteatum)*-2728, Barge Raihan *(Ocimum sanctum)*-2735, Badranjboya *(Mellisa officinalis)*- 2730 ,Tukhme Raihan (*Ocimum sanctum*)-2734, Gule Nilofar *(Nymphaea alba)* -2731, Darunaje Aqrabi *(Doronicum hookeri)* -2737, Gule surkh *(Rosa Damascena)* was cultivated in the herbal garden of NIUM by procuring the saplings from Nursery of Lalbagh Botanical garden, Bangalore. Flowers of the plant were identified by Prof. Dr. Vasundhara, GKVK,Bangalore. All the samples were found to be of good quality.

Preparation of *Khamirae Abresham Sada*:

The Khamira was prepared as described in National Formulary of Unani Medicine under the guidance of Chief Pharmacist of pharmacy unit, NIUM, Bangalore. Ingredients from 1-8 were

boiled with 1 litre of water till 200 ml of decoction was left and filtered, then Qand Safeid was added and boiled till required consistency, until reading of 85 was obtained by Sacchrometer/Refractometer and then Khamira was prepared by continuous stirring till it turned frothy white. Warqe Nuqra and Arqe Gulab were added during stirring and Khamira was prepared [4].

Storage: Container closure system was procured from market. Each drug formulation weighing about 150 gm was packed in 200 gm air tight containers; the containers taken were of plastic material and are transparent in appearance. All precautions were taken while packing these formulations in the containers. Clean and dry containers were used and fitted with air tight lids. The formulations were filled in three stated packs. They were labeled properly. One pack was tested for various parameters at the time of manufacture; other two packs were kept in stability chamber for accelerated stability study. Temperature was regulated at 40°C and relative humidity at 75%. The second pack was opened after 3^{rd} month and studied for various parameters. The third pack was opened after 6^{th} month and studied for various parameters .The procedures were strictly followed according to ICH Tripartite Guidelines.

1. **Appearance:** Appearance was recorded according to the consistency whether semisolid, semiliquid etc [5-6].
2. **Determination of Color:** The color of the drug formulation was noted by using the Munsell color charts . If any changes occurred were noted [5-11].
3. **Determination of Odor:** The description of this feature sometimes may not be accurate because it depends on individual perception. If the material is expected to be innocuous, a small portion of the sample can be examined by slow and repeated inhalation of air over the material. The strength of the odor like weak, distinct, strong is first determined and then the odor sensations like musty, mouldy, rancid, fruity, aromatic etc were determine [6, 9]**.**
4. **Determination of Taste:** First of all the depth of organoleptic capacity should be tested. This can be done by asking the volunteer to taste serial dilutions of drugs. It should be noted that the volunteers do not taste in ordinary sense. In so doing they would have to score the degree of flavouring, e.g., was it less than present originally, i.e., was the flavour being lost? They would also have to be able to describe the flavour well originally [6, 9]**.**

Physical Parameters:

1. **Determination of pH:**

pH of 1% solution- 1 gm of drug was accurately weighed and dissolved in accurately measured 100 ml of water, then filtered and pH was checked with a standardized glass electrode. pH of 10% solution-10 gm of drug was accurately weighed and dissolved in accurately measured 100 ml of water, then filtered and pH was checked with a standardized glass electrode[6, 12].

2. Determination of Moisture Content: Azeotropic method (Toluene distillation):

The apparatus consists of a glass flask connected by a tube to a cylindrical tube fitted with a graduated receiving tube and a reflux condenser. The receiving tube is graduated in 0.1-ml divisions so that the error of readings does not exceed 0.05 ml. The preferred source of heat is an electric heater with a rheostat control was taken. The upper portion of the flask and the connecting tube was insulated. Receiving tube and the condenser of the apparatus, was thoroughly cleaned, rinsed with water and dried. 75ml of toluene R and about 10 gm of drug was introduced into a dry flask. Flask was heated to distil the liquid over a period of 6 hours, and then allowed for cooling for about 30 minutes and reading of the volume of water to an accuracy of 0.05 ml (first distillation). Accurately a 10 gm drug material was weighed A few pieces of porous porcelain was added and the flask was heated gently for 15 minutes. When boiling began, 2 drops per second was distilled until most of the water has distilled over, then rate of distillation was increased up to about 4 drops per second. As soon as the water was completely distilled, inside of the condenser tube was rinsed with toluene R. the distillation was continued for 5 more minutes, removed from the heat, the receiving tube was allowed to cool at room temperature and droplets of water adhering to the walls of the receiving tube were dislodged by tapping the tube. The water and toluene layers were allowed to separate; the volume of water (second distillation) was noted. Content of water was calculated as percentage using the formula: $\mathbf{100(n_1-n) / w}$

Where, w = the weight in g of the material being examined

n = the number of ml of water obtained in the first distillation

n_1 = the total number of ml of water obtained in both distillations [6,12].

1. Determination of Ash value

Total ash:

4gm of the drug was accurately weighed, in a previously ignited and tarred crucible (Silica). Material was spread in an even layer and ignited by gradually increasing the heat to 500-600°C until it turned white, indicating the absence of carbon. Then it was cooled in a desiccator and weighed. Carbon free ash was not obtained in this manner, so crucible was cooled and residue was moistened with about 2 ml of water. It was dried on a water-bath, then on a hot-plate and ignited to constant weight. Residue was allowed to cool in a desiccator for 30 minutes and then weighed without delay. Content of total ash in mg / gm of drug was calculated [9, 12].

Acid-insoluble ash:

To the crucible containing the total ash, a 25 ml of dilute hydrochloric acid, was added and covered with a watch-glass and boiled gently for 5 minutes. Watch glass was rinsed with 5 ml of hot water and this liquid was added to the crucible. Insoluble matter was collected on an ash less

filter-paper and washed with hot water until the filtrate was neutral. The filter-paper containing the insoluble matter was transferred to the original crucible, then dried on a hot-plate and ignited to constant weight. Residue was allowed to cool in a suitable desiccator for 30 minutes, and then weighed without delay. The content of acid-insoluble ash was calculated in mg/ gm of material[9, 12].

Water-soluble ash:

To the crucible containing the total ash, 25 ml of water was added and boiled for 5 minutes. The insoluble matter was collected on an ashless filter-paper. Washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450°C. The weight of this residue in mg was subtracted from the weight of total ash. The content of water-soluble ash in mg/ gm of material was calculated [9, 12].

Physicochemical Parameters

1. **Determination of Aqueous Extractive Value:**

4gm of drug material was accurately weighed, in a glass-stoppered conical flask. Macerated with 100 ml of the water for 6 hours, shaking frequently, was then allowed to stand for 18 hours. Then rapidly filtered taking care not to lose any solvent and then 25 ml of the filtrate was transferred to a tarred flat-bottomed dish and evaporated to dryness on a water-bath, then dried at 105°C for 6 hours, cooled in a desiccator for 30 minutes and was weighed without delay. The content of extractable matter was calculated in mg/ gm of material [6, 9, 12].

2. **Determination of Alcohol Extractive Value:**

4gm of drug material, accurately weighed, was placed in a glass-stoppered conical flask. Macerated with 100ml of the Ethanol for 6 hours, by shaking frequently, and then was allowed to stand for 18 hours. Then rapidly filtered taking care not to lose any solvent then 25 ml of filtrate was transferred to a tarred flat-bottomed dish and evaporated to dryness on a water-bath. Dried at 105°C for 6 hours,cooled in a desiccator for 30 minutes and weighed without delay. The content of extractable matter in mg / gm of material was calculated [6, 9, 12].

3. **Determination of tannins**

5 gm of drug was accurately weighed and placed, into a conical flask. 150 ml of water was added and heated over water-bath for 30 minutes. The mixture was cooled and transferred to a 250-ml volumetric flask and diluted to 200ml volume with water. The solid material was allowed to settle and filtered through a filter-paper, discarding the first 50ml of the filtrate. The total amount of material that is extractable into water was determined by evaporating 50ml of the extract to dryness; the residue was dried in an oven at 105°C for 4 hours and weighed (T_1).

The amount of drug not bound to hide powder that is extractable into water was determined for which 80 ml of the extract was taken, 6g of hide powder was added and then shaken well for 60 minutes then filtered and 50 ml of the clear filtrate was evaporated to dryness. The residue was dried in an oven at 105°C and weighed (T_2).

To determine the solubility of hide powder, 6g of hide powder R was taken, 80 ml of water was added and shaken well for 60 minutes than filtered and 50ml of the clear filtrate was evaporated to dryness. The residue was dried in an oven at 105°C and weighed (T_0). The quantity of tannins as a percentage was calculated using the following formula:

$T_1-(T_2-T_0)$ X 500/ w

W= Is the weight of the drug [9, 12].

4. Estimation of Total Alkaloids

5 gm of the drug was taken in a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added to the sample. The mixture was covered and allowed to stand for 4 hrs. The mixture was then filtered and the extract was allowed to become concentrated in a water bath until it reached 1/4th of the original volume. Concentrated ammonium hydroxide was added until the precipitation was complete.

The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue, so obtained was alkaloid, which was dried and weighed [13].

5. Isolation of Glycosides

The glycosides content of the extracts was determined by dissolving 10g of the extracts in 100ml of 50% H_2SO_4 in test tubes. The mixture was heated in boiling water for 15minutes and 10ml of Fehling solution added, and the mixture boiled. A red precipitate in each extract tested, indicated the presence of glycosides. The percentage glycoside was calculated [14].

6. HPTLC Fingerprinting of Unani formulation KGS

Preparation of sample solution: 10g given formulation was dissolved in 100ml of HPLC water. Solution was then extracted with Dichloromethane (3x100ml) using separating funnel. DCM fraction was concentrated to dryness and dissolved in 5ml of DCM and used for TLC application.

Chromatographic conditions: Analysis was performed on 2.5x 10cm silica gel 60 F_{254} plates. sample solutions was applied using Linomat 5 (Camag Switzerland) automated spray on band applicator equipped with a 100µl Hamilton syringe and operated with the following settings: Band length 8mm, distance from the plate edge 12.5mm, and distance from the bottom of the plate 10mm.

Development of the plate was carried out allowing 20min for saturation of the twin trough chamber (Camag Switzerland) at room temperature. Solvent system used was Toluene: ethyl acetate: formic acid (7:2.5:0.5) and migration was 8cm. After development the plate was evaluated under UV 254 and 366 nm, the plate was derivatised with Vanillin sulphuric acid and kept in oven at 110°C and evaluated under visible light using CAMAG TLC Visualiser and scanned using CAMAG TLC SCANNER 3 [15].

Microbiological Assay

Enumeration of micro organisms (bacteria/fungi) by serial dilution agar plate method

Requirements: Sample, 1%Nacl, 9ml dilution blanks, sterile 1ml pipettes, sterile petri plates, nutrient agar, potato dextrose agar medium.

Procedure: 50ml each of nutrient agar medium and potato dextrose agar medium was prepared. 1%Nacl was prepared and 10ml was suspended to first test tube and 9ml saline was suspended to the remaining tubes labelled as (10^{-1} to 10^{-6}) each. The above media and tubes containing saline were autoclaved at 121^{o}C for 15mins. After autoclaving contents were brought to laminar air flow and all the tubes containing saline were cooled. 1gm of sample was weighed and transferred to the first tube containing 1%Nacl and mixed gently for uniform suspension. It was allowed to stand for some time. 1ml of suspension was transferred from the first tube to the next tube labelled as 10^{-1}. Further the dilutions were made till the tube labelled as 10^{-6}. Once the dilutions were done 0.1ml of suspension was taken from tube labelled as10^{-1} and plated on sterile petri plate. In the same way 0.1ml of suspension was taken from tubes labelled as 10^{-3} and 10^{-5} each and plated on 2 sterile petri plates. Once the plating was done a thin layer of media cooled to 45^{o}C was poured into the plates containing sample and plates were gently rotated for uniform distribution of cells.

For Bacteria—Nutrient agar media was poured and after solidification the plates were incubated at 37^{o}c for 24 hours in inverted position.

For Fungi---Potato dextrose agar media was poured and after solidification the plates were incubated at room temperature for 5days [16].

Results and Discussion:

The observations from the results of stability studies of KAS showed that there were considerable changes in organoleptic properties from the 3rd month onwards. Appearance of the drug was in semisolid form. At 0 month it was semisolid in appearance with no changes at 3rd month it was semisolid with little caking and at 6th month their was phase separation. Colour of the drug formulated matched YR colour of Munsell colour charts, YR is yellow red colour. Colour changes were noticed at 3rd month, it changed from 7.5YR6/8 at 0 month to 7.5YR5/8 at 3rd month and to 7.5YR4/8 at 6th month [17-18]. Results are shown in table 2.

The odour of the KAS was distinct aroma of rose at 0 month and its aromatic distinctness prominently changed at 3rd month onwards with top note changing to moderate note [17-18]. There were slight changes in the taste which changed considerably after 3rd month on wards Results are shown in table 2

The results from organoleptic observations indicated that the KAS showed significant changes after 3rd month. Moisture content increased from 0 month to 6th month i.e. 25% at 0 month, 26.5% at 3rd month and 27.5% at 6th month. It showed that there was 6 % difference in moisture content from 0 month to 3rd month and 3.6 % difference in moisture content from 3rd to 6th month. It implies that there was 10% increase in moisture from 0 month to 6th month. Thus,this indicates that the package material was not of standard quality, otherwise moisture content must not fluctuate too much [18,19,20]. The pH of the KAS, in 10% solution, progressively decreased from 4.81% at 25^{0}C at 0 month to 4.7% at 3rd month and 4.3% at 6th month ; there was 2% difference in pH from 0 month to 3rd month and 8% from 3rd month to 6th month. pH of 1 % solution at 0, 3rd and 6th month was 5.47%, 5.30% and 4.9% respectively; there was 3% difference in pH from 0 month to 3rd month and 8% difference from 3rd to 6th month. This implies there was 10% difference in pH from 0 to 6th month [18]. Therefore increase in pH implies that there were serious chemical changes occurring in the compound [17-18]. Results are shown in table 2

However there were no changes noticed in the Ash value at 0, 3rd and 6th month. Thus it showed no trace metal contamination [19-20]. Results are shown in table 2

When the chemical stability was assessed, the following changes were observed: Firstly, Aqueous extractive value showed changes from 0 to 6th month that is aq. extractive value was 8% at 0 month, 7.6% at 3rd month and 7.2% at 6th month. It denotes that there was 5% degradation from 0 to 3rd month and 5% degradation from 3rd to 6th month. It implies that from 0 month to 6th month there was 10.5% degradation [17-18]. Results are shown in table 2

Alcohol extractive value showed changes from 0 to 6th month as follows; it was 4.5% at 0 month, 4.3 % at 3rd month and 4% at 6th month. It denotes that there was 4% degradation from 0 to 3rd month and 6% degradation from 3rd to 6th month. It implies that from 0 month to 6th month there was 11% degradation. It is likely, that there was decomposition or degradation of chemical constituent in the drug. Results are shown in table 2

The alkaloid decomposition to the extent of chemical stability showed 2% reduction of total alkaloids occurred at 3rd month of the study and at 6th month, it was 12.9%. In KAS the glycoside degradation was as follows at 0 month it was 0.82%, at 3rd month it was 0.78% and at 6th month it was 0.73%; it implies that there was 4.8% degradation in glycosides from 0 to 3rd month and from 3rd to 6th month it showed 6.4% degradation. It can be assessed that from 0 month to 6th month

there was 10.9% degradation in glycosides [17-18]. Results are shown in table 2.

While tannins have also shown to have degradation that is at 0 month, tannins content was 0.4%, at 3rd month it was 0.38% and at 6th month it was 0.36%; the degradation from 0 month to 3rd month was 5% and from 3rd month to 6th month it was 5.2%. It implies that degradation from 0 month to 6th month was about 10%. Results are shown in table 2

Thus, all the chemical tests showed degradation more than the acceptance criteria i.e. above 5%. [17-18]. Microbial study showed no bacterial load after 24 hours incubation in Agar nutrient media at 37^0C, at 0, 3 and 6 months and no fungal load after incubation at room temperature for 5 days. The zero viable counts reflect that the drug is safe microbiologically. Hence it can be concluded that KAS is safe for consumption after 6 months [19-20].Results are shown in table no. 2 and figures 5 to 10.

HPTLC fingerprinting of KAS: Different compounds absorb light at different wavelength. In this case peaks were found at 280nm with maximum peak area, few peaks were detected with low intensity at UV 360nm. Peaks were not found at visible region. At 0 month, 4 peaks were noticed i.e. at Rf 0.16 area was 11212.80 with height 544.60, at Rf 0.22 area was 2513.10 with height 131.60, at Rf 0.66 area was 6221.90 with height 220.10 and at Rf 0.77 area was 634.00 and height was 1.10, whereas in the 3rd month 4 peaks were noticed at Rf 0.16 area was 18456.80 with height 699.40 , at Rf 0.24 area was 2743.60 with height 153.90, at Rf 0.70 area was 3068.3 with height 102.70, at Rf 0.79 area was 849.70 with height 29.20. This implies that at Rf 0.16 , 0.22 and 0.77 there was increase in height and area, which shows that there may be formation of degradation products , while at 0.66 there was decrease in area and height which shows that there was decrease in concentration of the constituent, while at 6th month 3 peaks were seen at Rf 0.16 area was 24344.10 with height 767.90, at Rf 0.22 area was 2738.40 with height 147.20, and at 0.71 area was 1245.70 with height 49.70, this implies that at Rf 0.16,0.22 and 0.71 there was formation of degradation products and there was loss of one constituent at Rf 0.77. This study showed that there is significant change in Rf values, area and height from 0 month to 6th month. Results are shown in table 3 and figures 1 to 4.

Accelerated Stability study showed that there was significant difference in values from 3rd month to 6th month and all the readings were above limit of 5% degradation which is not in acceptance criterion for stability of the drugs but not much changes were noticed from 0 to 3th month when compared with standard ICH guidelines, table for "Extension of the International Conference on Harmonization Tripartite Guideline for stability testing of new drug substances and products to Countries of Climatic Zone iii and iv" it has been stated that 6 month Accelerated study gives a

provisional shelf life of 20 months at 30^0C [21].As the study shows very little degradation between 0 to 3rd month but more that 5% degradation after 3rd month .Thus a provisional shelf life of 10 months to 12 months can be expected. Whereas Unani physicians have noticed the age of the Khamira for 3 years and in Gazzette of India draft rule 161B states that Stability of Khamira is 3 years [22-23], but the stability study disproves the claims. This does not validate the claim of Unani physician's empirical observations.

Table 1 showing Ingredients of the drug:

Sl.no.	Ingredient	Botanical name	Quantity
1.	Abresham	*(Bombyx mori*	150 gm
2.	Gule Gauzaban	*Onosma bracteatum*	10 gm
3.	Barge Raihan	*Ocimum sanctum* Linn	10 gm
4.	Badranjboya	*Melissa officinalis*	10 gm
5.	Tukhme Raihan	*Ocimum sanctum* Linn	10 gm
6.	Gule Nilofar	*Nymphaea alba*	10 gm
7.	Sandal Safaid	*Santalum album*	10 gm
8.	Darunaj Aqrabi	*Doronicum hokeri* Hook.f.	5 gm
9.	Arqe Gulab	Distillate of *Rosa damascena* Mill.	600 ml
10.	Aab	Water	1 lt
11.	Qand Safaid	*Saccharum officinarum*	1 kg
12.	Warqe Nuqra	Silver Foil	Q.S

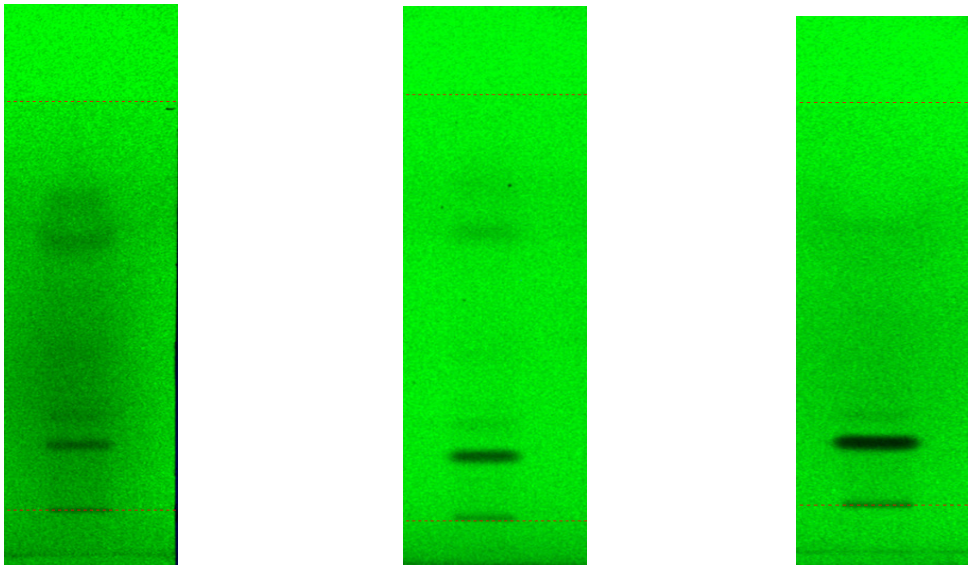
Table 2 showing Physical, Chemical ,Microbiological parameters:

Sl. No.	Parameters	0 month	3rd month	6th month
1.	**Organoleptic parameters**			
a.	*Appearance*	Semisolid	Semisolid Caking	Semisolid Caking with Phase separation
b.	*Color*	7.5YR6/8	7.5YR5/8	**7.5YR4/8**
c.	*Odor*	Molasses	Molasses	Molasses
d.	*Taste*	Sweet	Sweet & sour	Sweet & Sour
2.	**Physical parameters**			
a.	*pH*	1% -5.47 10% - 4.81	1%- 5.30 10%- 4.7	1%-4.9 10%-4.3
b.	*Moisture content*	25%	26.5%	27.5%
C	*Ash Value* *Total ash* *Acid insoluble ash* *Water soluble ash*	 0.90% 0.68% 0.50%	 0.89% 0.68% 0.50%	 0.89% 0.68% 0.50%

3.	**Chemical parameters**			
a.	*Aqueous extractive value*	8%	7.6%	7.2%
b.	*Alcohol extractive value*	4.5%	4.3%	4%
c.	*Alkaloids*	0.31%	0.29%	0.27%
d.	*Glycosides*	0.82%	0.78%	0.73%
e.	*Tannins*	0.4%	0.38%	0.36%
4.	**Microbial load**			
a.	*Bacterial count*	No load after 24 hours of incubation at 37^0C	No load after 24 hours of incubation at 37^0C	No load after 24 hours of incubation at 37^0C
b.	*Fungal count*	No load after 5 days of incubation at room temp	No load after 5 days of incubation at room temp	No load after 5 days of incubation at room temp

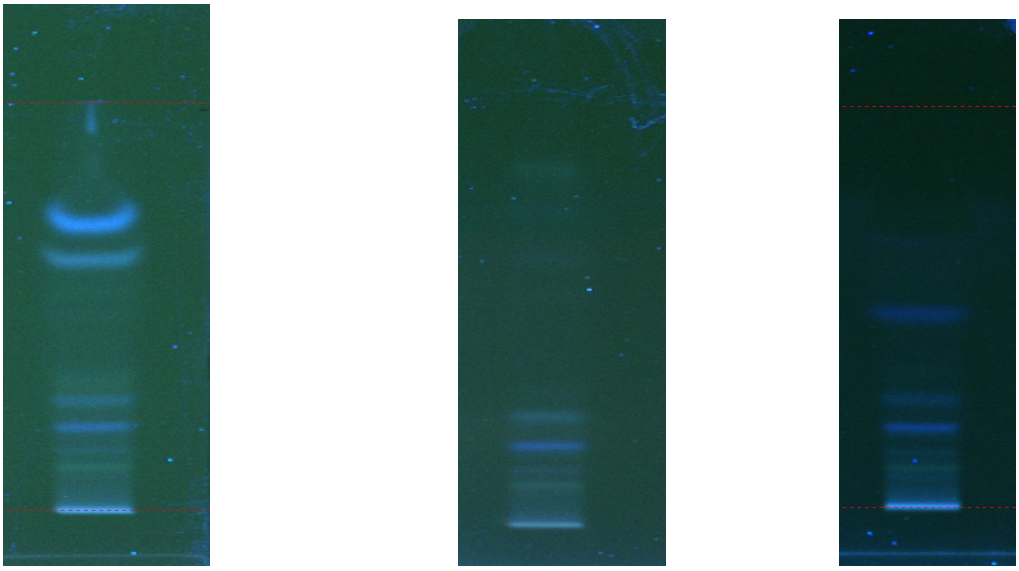
HPTLC profile:

Fig.1: HPTLC profile of Khamirae Abresham Sada under UV 254nm.



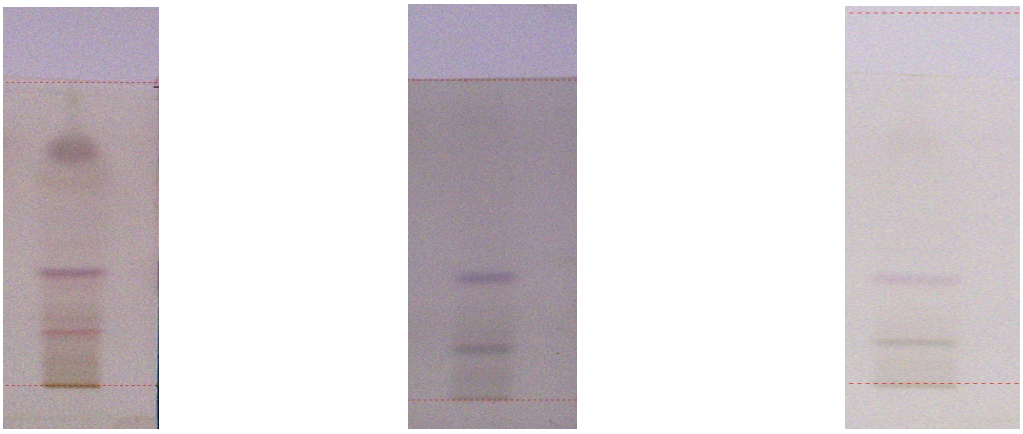
0 month 3rd month 6th month

Fig. 2: HPTLC profile of Khamirae Abresham Sada under UV 366nm.



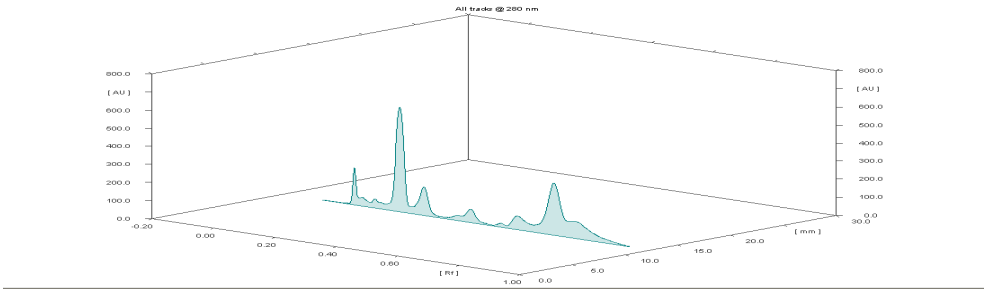
0 month 3rd month 6th month

Fig. 3: HPTLC profile of Khamirae Abresham Sada after derivitised with vanillin sulphuric acid under white light.

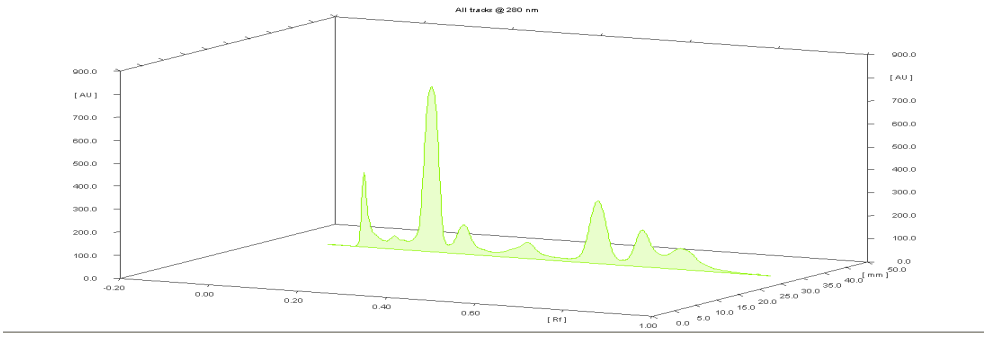


0 month 3rd month 6th month

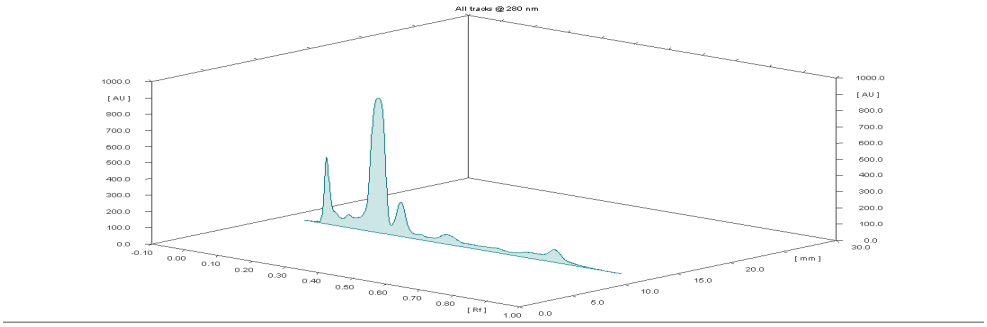
Fig.4: HPTLC densitometric scan of Khamirae Abresham Sada extract at UV 280nm.



0 month



3rd month



6th month

R_f values of Khamirae Abresham Sada extract in Toluene: ethyl acetate: formic acid (7:2.5:0.5) mobile phase.

Month	Under UV 254nm		Under UV 366nm		After spraying vanillin sulphuric acid.		No. of Peaks at 280nm and their peak area and height			
	Rf value	Colour	Rf value	Colour	Rf value	Colour	No. of peaks	Rf value	Area	Height
0 month	0.16	Dark	0.10	green	0.18	Pink	4 peaks	0.16	11212.80	544.60
	0.22	Light	0.14	Blue	0.38	Pink		0.22	2513.10	131.60
	0.66	Light	0.20	Blue	0.79	Pink		0.66	6221.90	220.10
	0.77	Light	0.27	Blue				0.77	634.00	1.10
			0.61	Blue						
			0.7	Blue						
3rd month	0.16	Dark	0.11	green	0.16	Pink	4 peaks	0.16	18456.80	699.40
	0.24	Light	0.14	Blue	0.39	Pink		0.24	2743.60	153.90
	0.70	Light	0.20	Blue				0.70	3068.3	102.70
	0.71	Light	0.27	Blue				0.79	849.70	29.20
6th month	0.16	Dark	0.10	green	0.16	Pink	3 peaks	0.16	24344.10	767.90
	0.23	Light	0.14	Blue	0.37	Pink		0.22	2738.40	147.20
	0.70	Light	0.20	Blue				0.71	1245.70	49.70
			0.27	Blue						
			0.49	blue						

Microbiological assay:

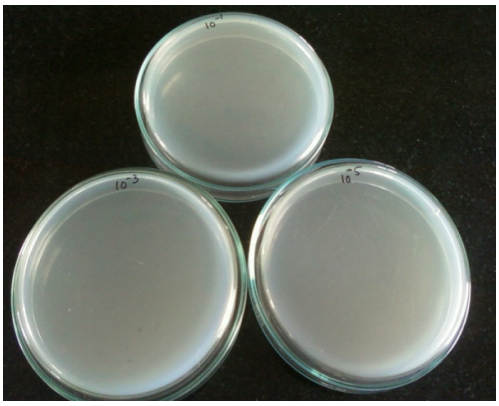


Fig no.5 Bacterial load (0 month)

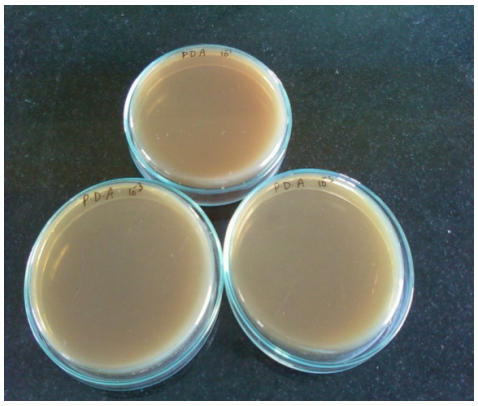


Fig no.6 Fungal load(0 month)

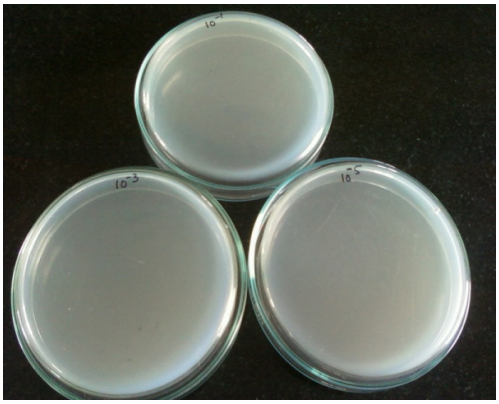


Fig no.7 Bacterial load (3rd month)

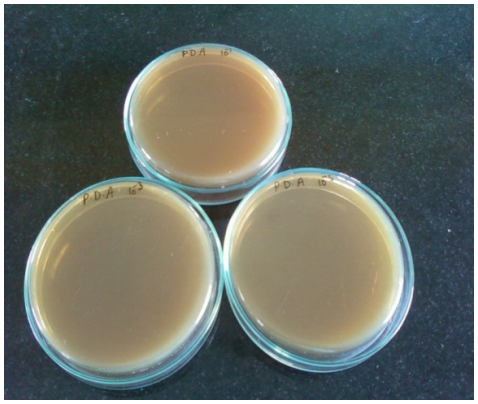


Fig no.8 Fungal load(3rd month)

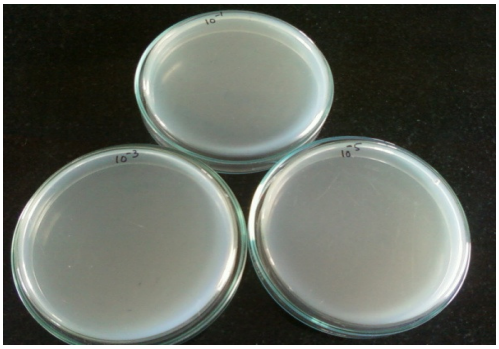


Fig no.9 Bacterial load (6th month)

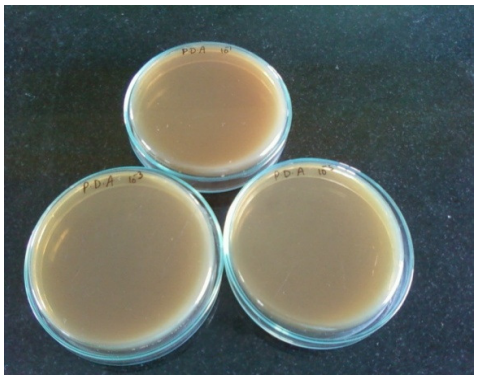


Fig no.10. Fungal load(6th month)

CONCLUSION:

The study showed that the drug KGS was stable from 10 months to one year period, Physical parameters ,Chemical parameters showed degradation more than 5 % which is more than the acceptance criteria according to ICH Guidelines.

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