ORIGINAL ARTICLE

Accelerated stability study of *Jawarish Jalinoos*

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Abstract

Background and Objective: *Jawarish Jalinoos* (JJ) is a sugar based semisolid formulation used in Unani system of medicine to treat chronic debilitating disorders, geriatric problems, to strengthen the digestive power, etc., since ancient time. Shelf life/stability evaluation of this valuable compound formulation has not been carried out till date. Therefore, authors aim to evaluate the shelf life of JJ under the accelerated stability condition. **Materials and Methods:** Finished product was manufactured in the institute's in-house pharmacy and packed in four air tight polyethylene terephthalate containers. One container was analyzed at baseline and remaining three containers were kept in stability chamber at 40±2°C/75±5% relative humidity. At each pre decided time point, i.e., 1, 3, and 6 months, one container was taken out from stability chamber and evaluated for organoleptic, physicochemical, microbiological parameters, and high performance thin layer chromatography (HPTLC) fingerprinting to assess any changes. **Results:** No significant changes were observed in organoleptic characters. All physicochemical parameters showed <5% change, HPTLC showed minimum difference, microbial load, and specific pathogenic bacteria counts were within WHO guideline's prescribed limit. **Conclusion:** The shelf life of JJ was calculated as minimum 20 months using Grimm's statement. This was in accordance to the drug and cosmetic rule, 1945–161B, Government of India that states the shelf life of *Jawarish* dosage form as 2 years.

Key words: Accelerated stability study, *Jawarish jalinoos*, Shelf life, Unani system of medicine

INTRODUCTION

Jawarish is a sugar or honey based semisolid dosage form which is prepared with one or more single drugs of plant/animal/mineral origin materials; mixed as powder in *qiwam* (syrup) of sugar or honey and used orally.[1] This semi-solid medicinal preparation is in use since ancient time. It is effective, safe, hastens the drug release and absorption of active drug molecules and protect from microbial contamination. However, there are drawbacks associated with the stability of this valuable dosage form. *Jawarish* contains approximately 76% of sugar[1] and if *qiwam* of formulations is not prepared well or its consistency changes under the influence of environment, such medicinal preparation becomes vulnerable to microbial contamination and liable to stale/spoil earlier. Change in organoleptic characteristic, degradation of active drug molecules, altered pharmacological effect, and development of toxic component not only reduce patients compliance but also patient consume such medicine may be at risk too.

Many ancient Unani physicians have discussed the shelf life of *Jawarish* in their respective compilation.[2-7] They expressed the shelf life of drugs and their dosage form on the basis of organoleptic characters such as *rang* (color), *boo* (smell), *maza* (taste), *sakht* (structure), *vazan* (weight), and *zahiri khususiyat* (external features). When all these characters are in equilibrium, the drugs are assumed to be stable and any deviation in these characteristics is considered as drug losing its shelf life.[8] These methods of fixing the shelf life was subjective and solely based on the personal observation which was in accordance with the criterion available in those days. Further Ayurvedic, Siddha, and Unani Drug Technical Advisory Board of India has proposed the shelf life/expiry date for various dosage form on the basis of these classical textual reference and made it mandatory under the Drugs and Cosmetics Rules, 1945, rule 161B (Amendment, dated

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November 24, 2005), Government of India to conspicuously display the date of expiry of Ayurveda, Siddha, and Unani (ASU) medicines on the label of container or package of an Ayurvedic, Siddhas, and Unani drugs, after which they shall not be in circulation. The shelf life of *jawarish* is mentioned 2 years in this statement. However, being an empirical and due to lack of scientific proof these statements may not be acceptable in contemporary era. Therefore, it is very essential and need of the hour to establish the shelf life of all ASU drugs on scientific parameters using modern techniques for stability studies.

Jawarish Jalinoos (JJ) is an important Unani compound formulation of this class. It contains total 18 ingredients of plant origin including *zafran* and *mastagi* as chief constituents.[1] It is extensively used in chronic debilitating disorders, geriatric problems and to strengthen the digestive power. It provides strength to different body organs especially vital organs[9] and has beneficial effects almost on all the human body systems. It has *muqawwie kabid, muqawwie aam, kasir riyah, hazim*,[1] and *muqawwi bah*[10-12] effects. It has an excellent therapeutic efficacy as a general body tonic used in various disorders such as *zofe meda, zofe kabid, nafkhe shikam, khafqan,*[1] *niqras,* phlegmatic disorders, *zofe azae raeesa,* and *daad*.[6,9,11]

Till date, no stability study of JJ is conducted to establish its shelf life, thus the present study was carried out to develop the method and evaluate shelf life of JJ at the accelerated storage condition.

MATERIALS AND METHODS

Procurement of Raw Drugs

The ingredients of formulation were procured from herbalist/raw drug dealer at Bengaluru, Karnataka, India, during the month of June-July 2015. Drugs were identified and authenticated by S. Noorunnisa Begum, Professor, Department of Pharmacognosy, Centre for Repository of Medical Resources, Trans Disciplinary University under FRLHT Bengaluru (accession number 3801, 3802, 3803, 3804, 3805, 3806, 3807, 3808, 3809, 3810, and 3811). All the drugs were stored in air tight containers.

Preparation of JJ

Ingredients of JJ

1. *Asaroon* (*Asarum europaeum* L. Root) 10 g
2. *Chiraita shireen* (Swertia chirayita Roxb. Whole plant) 10 g
3. *Darchini* (Cinnamomum zeylanica Blume. Bark) 10 g
4. *Filfil daraz* (Piper longum L. Fruit) 10 g
5. *Filfil siyah* (Piper nigrum L. Fruit) 10 g
6. *Habbul aas* (*Myrtus communis* L. Fruit) 10 g
7. *Heel khurd* (*Elettaria cardamomum* Maton. Fruit) 10 g
8. *Kulanjan* (*Alpinia galanga* Willd. Rhizome) 10 g
9. *Mastagi* (*Pistacia lentiscus* L. Resin) 25 g
10. *Ood balsam* (*Balsamodendron opobalsamum* L. Wood) 10 g
11. *Qaranfal* (*Syzygium aromaticum* L. Flower buds) 10 g
12. *Qust shirin* (*Saussurea lappa* Decne. Root) 10 g
13. *Saad kufi* (*Cyperus rotundus* L. Rhizome) 10 g
14. *Saleekha* (Cinnamomum aromaticum Nees. Bark) 10 g
15. *Sumbulut teeb* (*Nordostachys jatamansi* DC. Rhizome) 10 g
16. *Zafran* (*Crocus sativus* L. Stigma and style) 10 g
17. *Zanjabeel* (*Zingiber officinale* Rosc. Rhizome) 10 g
18. *Asal* (Honey) 600 g

All the ingredients except *zafran* and *mastagi* were washed with running tap water and spread in the shade to get rid of extra water. Further they were dried in the oven at 60°C for 4–6 h. All the plant materials except *zafran* and *mastagi* were powdered separately in grinder and passed through the sieve size number 80. *Zafran* was made into fine paste with *arq gulab* (rose water) in mortar and pestle and *mastagi* was powdered by grinding slowly in porcelain mortar and pestle. Honey was placed on the fire for about few minutes till it started boiling, its impurities were removed and allowed to cool. Except *zafran* and *mastagi* all powdered ingredients of the formulation were mixed together and incorporated into the honey with continuous stirring. After incorporating the powdered plant material *zafran* paste was added with rigorous mixing. Finally powdered *mastagi* was dissolved in 100 ml of slightly warmed *roghan gao zard* (*ghee*) and sprinkled on *jawarish* and mixed uniformly. After ensuring proper mixing of all the ingredients, JJ was allowed to cool at room temperature. No preservatives were added in the preparation.[1]

Methodology for Accelerated Stability Study

JJ was filled/packed in four transparent, air tight, polyethylene terephthalate (PET) containers of 250 ml capacity, purchased from local market of Bengaluru. In each container 200 g of JJ was filled. Maximum possible attention was paid to avoid any contamination during preparation and packaging. All the necessary information including name of the formulation, date of manufacture, duration and quantum of thermal/humidity challenge, date of commencement, and withdrawal of thermal/humidity challenge were labeled. One container of JJ was used for physicochemical and microbial evaluation just after manufacture (at 0 month). Remaining three containers were placed in stability chamber (Osworld photostability chamber OPSH G-4 1258 with temperature resolution +0.1°C, accuracy of ±0.2°C, uniformity of ±1°C, and maximum humidity deviation ±2%) and subjected to thermal humidity challenge at 40±2°C/75±5% relative humidity (RH) for a period of 6 months. Containers were removed from stability chamber at predetermined time point

(i.e., at the completion of 1, 3, and 6 months) as per ICH guideline. In the present study, a 4th time point at 1 month was an additional time point to monitor any significant change earlier.[13] On completion of thermal/humidity challenge as per predetermined time point JJ was evaluated for various physicochemical and microbial analysis.[13]

Assessment of Parameters

JJ was assessed on the organoleptic characters (appearance, color, odor, and taste), various physicochemical parameters (loss of weight on drying, alcohol and water soluble matter, successive extractives value, pH, viscosity, ash value, total alkaloids, total sugar, and reducing sugar), high performance thin layer chromatography (HPTLC), and total and specific microbial count to derive data for shelf life.

HPTLC analysis was performed on plate size of 20 cm × 10 cm silica gel 60 F_{254} plate. The sample solution was applied on the plate using TLC sample applicator Linomat 5 (CAMAG Switzerland) automated spray-on band, applicator was equipped with 100 µl Hamilton syringe and operated with the following settings: Band length 10 mm, application position Y 12 mm, and number of track 4. Toluene**:**ethyl acetate (9:1) solvent system was used for the development of plates.[14] Before place the plate therein, twin trough chamber was saturated with the solvent system for about 20 min. The developed plates were dried and densitometric scanning was performed in the absorbance mode at UV 200nm, 254 nm, 366 nm, and 550 nm. The plate was further derivatized with anisaldehyde-sulfuric acid, heated at 110°C and evaluated under visible light. Screening was carried out using CAMAG TLC SCANNER-3 operated by win CATS software (V 1.4.2, Camag).[15]

RESULTS AND DISCUSSION

No significant changes were observed in organoleptic characters whereas variations in the other physicochemical parameters were <5% during whole 6-month study period at any time point. Total bacterial and fungal count in JJ were within allowable limits as per the WHO guideline and pathogenic microbes were absent in all test samples [Tables 1].

Organoleptic Characters

JJ was semisolid and homogenous in appearance after preparation and this characteristic persists till 6 month without any solid liquid phase separation, sedimentation, crystallization, caking, or gas formation. Color was blackish brown in bulk and yellow-brown in thin layer; odor moderate, pleasant with dominant saffron character; and taste was sweetish, pungent with slightly bitter at preparation. Bitterness further gradually reduced at 1st, 3rd, and 6th months compared to 0 month samples; however, it was insignificant. Color, odor, and taste were also constant as noted at the time after preparation except slight decrease in bitterness. As JJ preserved all its physical attributes without any significant changes during the 6 months of accelerated stability condition it can be said that JJ was in confirmation to ICH guideline for accelerated stability criteria.

Loss of Weight on Drying

At accelerated stability condition the percentage of change in moisture content at 1st, 3rd, and 6th months was 0.37%, 0.21%, and 2.38%, respectively, when compared with base line sample. The percent of change in loss of weight on drying was highest at the end of 6 months (2.38%). These finding shows that at any time point, change in moisture content of JJ was below 5% which was in agreement with ICH guidelines for stability study.

Insignificant change in percent of loss of weight on drying proves that *qiwam* of JJ was precisely prepared and containers were of good quality and tightly packed to prevent moisture penetration or water evaporation. Further, *qiwam* of this product was prepared with honey, which is a saturated sugar solution[16] and saturated solution prevents microbial growth. Hence, during the study period no significant microbial growth and change in organoleptic characters of JJ was observed.

Ash Value

There was slight variation in ash value of JJ at different time points in the study. At the accelerated stability study total and water soluble ash at 6 months was slightly increased as compared to 0 month and percent of change noted was 1.9% and 3.33%, respectively, while no change was observed in acid insoluble ash. It represents that the percentage of change in total, water soluble, and acid insoluble ash of the JJ at the accelerated stability condition was not more than 5%. Therefore, ash value of JJ meets the acceptance criteria limit of ICH guideline for stability study.

Extractive Value

Alcohol and water soluble matter

In this stability study, the percentage of change of alcohol and water-soluble extractive value at the end of 6 months was 1.3% and 1.47%, respectively.

Successive extractive

In the accelerated stability study, the percentage reduction of extractive values in petroleum ether and ethanol was 2.08 and 0.39 whereas in chloroform the extractive value increased by 3.84% at the end of 6 months compared to the value at 0 month/day. All the successive extractive values of JJ at

Table 1: Status of physicochemical and microbial character in ASS of JJ at different time point.

SN	Parameters	Time point			
		0 Month	1 Month	3 Month	6 Month
1	Loss of weight on drying at 105^{0}C (%)	18.84±0.01	18.91±0.00	18.80±0.00	18.39±0.01
2	Ash value (%w/w)				
	Total ash	1.05±0.02	1.06±0.01	1.07±0.01	1.07±0.00
	Water soluble ash	0.30±0.02	0.28±0.01	0.31±0.0	0.31± 0.01
	Acid insoluble ash	0.24± 0.00	0.23±0.0	0.24±0.0	0.24±0.0
3	Alcohol soluble matter (%w/w)	53.39±0.18	52.78±0.43	52.76±0.43	52.65±0.19
4	Water soluble matter (%w/w)	55.70±0.35	55.45±0.54	54.86±0.30	54.88±0.02
5	Successive extractives (%w/w)				
	Petroleum ether	0.48±0.01	0.46±0.01	0.50±0.01	0.47±0.01
	Chloroform	0.52±0.05	0.50±0.01	0.54±0.01	0.54±0.01
	Ethanol	60.22±0.24	60.24±0.03	60.68±0.55	59.98±0.39
6	pH				
	At 1% aqueous solution	5.62±0.01	5.56±0.01	5.48±0.02	5.42±0.01
	At 10% aqueous solution	5.21±0.02	5.18±0.01	5.12±0.00	5.16±0.01
7	Sugar				
	Total sugar	62.6±0.33	61.6±0.33	60.0±0.00	59.7±0.33
	Reducing sugar	58.0±0.00	58.3±0.33	58.6±0.33	59.0±0.57
8	Total alkaloid (%)	0.10	0.10	0.10	0.11
9	*Microbial examination(Cfu/gm/ml)				
	Total bacterial count	27	5	Nil	Nil
	Total fungal count	Nil	Nil	Nil	Nil
10	#Specific pathogen				
	E.coli	Absent	Absent	Absent	Absent
	Salmonella	Absent	Absent	Absent	Absent
	Staph. aureus	Absent	Absent	Absent	Absent
	P. aeruginosa	Absent	Absent	Absent	Absent

*WHO limit for total bacterial count and total fungal count is 10^5 and 10^3 (Cfu/gm/ml) respectively.#WHO limit for specific pathogen- Absent.

accelerated stress condition showed <5% change. Thus, it can be concluded that the product was within the acceptance criteria of ICH guideline for stability study.

Extractive values correspond to the estimated amount of active essential compound. It also provides information about the types of components present in the product. In herbal medicinal products several herbal ingredients are present; therefore, quantitative determination of each active constituent is not practically possible. Hence, this method is employed for the determination of a sum total of active constituents present.[17] Solubility study in different solvents provides information about any adulteration,[18] substitution, or degradation of the drug. These values may be influenced by heat, light, and pH; therefore, these parameters were included in the present stability study.

pH

At accelerated stability condition period the pH among various samples of JJ was in the range of 5.62±00–5.42±0.01 in 1% solution and 5.21±0.02–5.12±0.00 in 10% solution. The percent of change in pH value at any time point was not more than 5% from the initial value. There is certain pH range for herbal products at which they remains stable for a long time and any change in pH may accelerate or decelerate their degradation. This effect of pH is very much influenced by temperature. Low pH has inhibitory effect on the growth of bacteria whereas neutral or alkaline pH for herbal product produces susceptible environment for microbial contamination.[19] Herbal product prepared with sugar/honey syrup has the risk of hydrolysis and inversion and this process is catalyzed by hydrogen ion[20] as well as temperature. Invert sugar formed as a result of inversion has high affinity for water and retains moisture.[21] This moisture may cause change in osmotic pressure and provides suitable medium for microbial growth. Hence, directly or indirectly pH influences stability of herbal products.

As JJ was acidic and change in pH during entire study period was <5%, that was supposed to be in favor of product and helpful to prevent bacterial and fungal growth.

Sugar

At accelerated storage condition total sugar decreased and reducing sugar increased gradually at each time point in the study and the percent of change was highest at completion of 6 months by 4.63% and 1.72%, respectively. However, the change among the samples at any time point was not more than 5% from the initial assay. Hence, it can be said that sugar content of JJ was in agreement to ICH guideline for stability study.

Sugar is an influential parameter in the shelf life estimation of herbal product. Concentrated sugar solution creates high osmotic pressure around the microbes and as a result microorganisms present lose their protoplasmic water along the concentration gradient. Their enzymes get inactivated and they lose their ability to propagate. If the sugar concentration is not accurate then situation will be reversed and sugar provides a favorable medium for the growth of microorganisms. Further inversion of sugar is catalyzed by low pH/heat and the invert sugars have high affinity for water and to retain moisture[21] that may offer a favorable condition for molds.[22]

Total alkaloids

Total alkaloid content was 0.10 % at baseline sample of JJ. This finding is in accordance to the previous study carried out by CCRUM.[23] In the present study, total alkaloid content at 1, 3, and 6s month was 0.10%, 0.10%, and 0.11%, respectively. At 1st and 3rd months, no change was observed however, at 6 months 10% change was noted from baseline. Although change in total alkaloid content was noted; nevertheless, product has 90% of potency at any time point in all samples thus it was acceptable.[24]

HPTLC analysis

HPTLC analysis of JJ of ASS at 1 month demonstrated 8, 5, and 6 peaks, at 3 month 5, 4, and 8 peaks, and at 6 months 9, 3, and 8 peaks under 254, 366, and 550 nm (i.e., after spraying area specific resistance), respectively. While comparing with initial sample at 254 nm 3, 6, and 2 peaks were missing at 1, 3, and 6 months, respectively. Likewise at 366 nm 1, 2, and 3 peaks were missing whereas at 550 nm number of peaks was same at each time point except at 1 month where 2 peaks were missing.

Although HPTLC analysis of JJ represents variation in number of peaks, Rf value, peak heights, and area under the peak rest of the physicochemical and microbiological evaluation did not show any considerable variation; hence, it is concluded that JJ was satisfactorily stable during the accelerated stressed condition [Figure 1 and Table 2].

Microbial Analysis

Total bacterial count at 0 month and 1 month was 27 and 5 cfu/g/ml in stability samples but was negative at 3 and 6 months samples. No fungal growth or specific pathogens was detected at any time point. All the test sample of JJ at accelerated stability condition was under the WHO prescribed microbial contamination limit.[25] Thus, it was concluded that JJ was microbiologically stable at accelerated thermal/ humidity condition during study period [Tables 1].

Microbial analysis is an indicator of physical condition of the herbal product. Microbial status of a product is affected by pH, temperature, light, humidity, moisture content, nutritional status, etc. Abba *et al.* stated that high contamination level of herbal products is associated with neutral or alkaline pH whereas at acidic condition bacterial counts were found low.[19] Nutrients of the medium and moisture content provide suitable environment for the growth of organisms and the temperature influences rate of chemical reaction and enzymatic activity. Metabolic rate and growth of microorganisms doubles at each 10°C rise in temperature.[26]

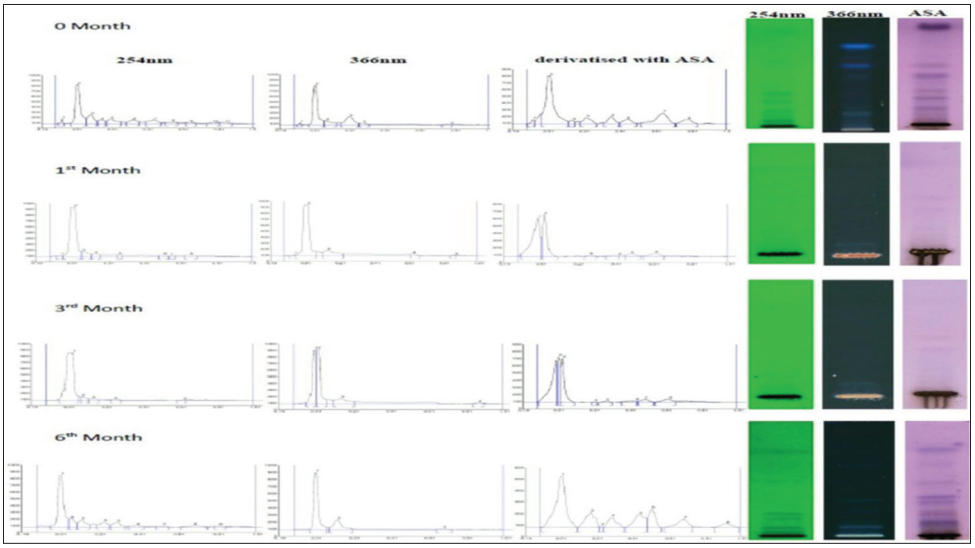

Figure 1: High-performance thin-layer chromatography fingerprint of accelerated stability study of *Jawarish Jalinoos* at 0, 1st, 3rd, and 6th months

Table 2: HPTLC analysis of ASS of JJ at 0, 1st, 3rd, and 6th months

Sample	Under UV 254 nm				Under UV 366 nm				Under UV 550 nm			
	No. of peaks	Rf value (max)	Area (%)	Height (max)	No. of peaks	Rf value (max)	Area (%)	Height (max)	No. of peaks	Rf value (max)	Area (%)	Height (max)
ASSJ-0M	1	–0.08	2.02	93.9	1	–0.08	0.98	38.1	1	–0.07	1.78	64.6
	2	0.01	43.25	747.9	2	0.01	60.69	728.1	2	0.02	53.92	719.2
	3	0.09	13.45	177.2	3	0.07	10.30	75.8	3	0.14	2.17	50.7
	4	0.14	5.39	73.3	4	0.21	23.25	147.2	4	0.22	6.63	92.4
	5	0.2	8.94	87.8	5	0.28	3.48	30.9	5	0.36	6.8	107.2
	6	0.33	7.10	76.7	6	0.78	1.29	11.3	6	0.45	5.45	66.2
	7	0.44	7.85	72.8					7	0.65	17.33	159.2
	8	0.54	3.49	38.1					8	0.78	5.94	63.1
	9	0.64	2.64	27.9								
	10	0.77	2.78	27.1								
	11	0.85	3.09	29.7								
ASSJ–1M	1	–0.05	1.30	24.3	1	–0.05	0.84	20.8	1	–0.01	53.65	532.8
	2	0.02	79.4	849.3	2	0.02	80.27	850.7	2	0.03	33.68	577.7
	3	0.06	7.12	89.3	3	0.13	16.02	90.9	3	0.27	1.38	18.5
	4	0.13	4.27	47.4	4	0.63	2.17	28.2	4	0.42	1.94	17.5
	5	0.27	1.74	32.0	5	0.87	0.71	12.8	5	0.49	3.94	35.6
	6	0.51	2.27	24.1					6	0.62	5.41	42.0
	7	0.55	0.91	14.3								
	8	0.66	2.99	25.6								
ASSJ-3M	1	0.03	88.85	780.2	1	0.00	36.96	821.5	1	–0.01	41.34	591.0
	2	0.07	4.76	62.4	2	0.02	48.13	826.6	2	0.01	21.38	641.2
	3	0.13	3.42	30.7	3	0.14	13.68	80.8	3	0.03	23.37	616.0
	4	0.25	2.05	16.9	4	0.87	1.24	13.4	4	0.21	0.39	10.3
	5	0.63	0.93	10.3					5	0.27	1.09	15.2
									6	0.44	2.67	21.4
									7	0.49	4.30	41.4
									8	0.63	5.48	46.0
ASSJ-6M	1	0.01	58.65	766.4	1	0.00	73.47	809.6	1	0.01	45.45	448.2
	2	0.06	8.32	124.4	2	0.12	24.02	141.1	2	0.18	12.99	133.6
	3	0.11	9.81	104.8	3	0.68	2.52	14.3	3	0.23	0.33	14.8
	4	0.23	7.22	76.4					4	0.29	7.68	91.0
	5	0.29	6.71	68.4					5	0.44	10.95	111.9
	6	0.39	2.60	20.1					6	0.51	11.15	159.3
	7	0.53	1.50	14.1					7	0.69	7.85	72.0
	8	0.68	3.60	20.8					8	0.92	3.61	31.1
	9	0.81	1.60	17.2								

ASS: Accelerated stability study, JJ: *Jawarish Jalinoos*

In the present study, the probable factors as to why JJ retained its microbial status of minimum microbial growth was seems to be the excellent *qiwam,* cleaning and washing of the ingredients of test drug formulation and subsequent drying at 60°C, good quality air tight containers, low acidic pH, etc. Apart from this honey itself, possesses antimicrobial activity[27] that might have killed and prevented microbial contamination in JJ.

To assess the physicochemical parameters of the test drug formulation, 5% variation limit was fixed in present study as per ICH Harmonized Tripartite Guideline.[13] As per final draft. Shelf life recommendations for supplements guidelines for manufacturers, 90% of labeled potency is commonly considered as the minimum acceptable potency level.[24] The results of physicochemical parameters of stability study sample exhibited <5% variation from initial essay value except total alkaloid. Hence, it was concluded that JJ retained stability under accelerated storage conditions and confirms to ICH guideline for stability study.

A number of studies are available to predict the shelf life of product tested at accelerated storage conditions. Joel Devis (Joel Devis rule) stated that stability testing of drug at 40°C/75% RH for the period of 3 months is equivalent to 24 months of shelf life at room temperature, i.e., 25°C.[28] As per this recommendation, JJ will be stable for 4 years at room temperature.

In another proposal discussed in "Shelf-life Recommendations for Supplements Guidelines for Manufacturers" if only one temperature point 10°C above the ambient temperature is used for shelf life recommendation it provides an estimated shelf life of ×2 accelerated storage time period.[29] According to this guideline, JJ will be stable for 1 year.

Another important contribution in this regard is the work of W. Grimm. He proposed predictive factor of 3.3 for zone 4 (to which India is included) for the estimation of shelf life period using accelerated stability study period.[30] This is the most popular concept for estimation of shelf life. As the JJ was stable at accelerated stability condition of 40°C/75% for the period of 6 months; hence, its minimum shelf life at room temperature will be calculated as: 6×3.3=19.8 = 2 years (round off to nearest year).

The results from the Grimm's work can be further verified by Indian Drugs and Cosmetics Rules 161 B (Amendment) 2005, in which the shelf life of *jawarish* is mentioned as two years. However, shelf life calculated in this study is in contrast to the comments of *Hari Chand Multani* who stated the shelf life of *jawarish* as 3–5 years.[31]

Strength

This is the first study of its kind, where JJ was evaluated for its shelf life under accelerated stability storage condition and evaluated on organoleptic and various physicochemical, microbiological parameters, in addition HPTLC finger printing was also carried out to evaluate its stability/shelf life.

LIMITATIONS AND FURTHER RECOMMENDATIONS

Accelerated stability data of JJ should be further supported by real time or long-term stability studies as accelerated stability studies alone cannot predict the exact shelf life of drug. Biologically active molecule in the formulation should be identified and its variation under thermal/humidity and light conditions with time should be evaluated. Simultaneously, if any degradation product is formed it should be detected and identified for toxicity by appropriate techniques.

CONCLUSION

All physicochemical parameters of stability sample of JJ showed <5% variation from initial value at any time point and microbial load with specific pathogen values was as per the acceptance criteria of the WHO. Thus, product was stable during accelerated thermal/humidity condition and result meets the specification for acceptance criteria of ICH guideline. Further, according to Grimm's method of calculation, shelf life of JJ was found to be 20 months at room temperature, i.e., 30°C/70% RH (climatic zone IV). Next to this, ICH harmonized tripartite guide line recommends that when no significant change was observed beyond the accelerated testing period, shelf life will depend on long-term or real time stability data. Hence, further long-term or real time stability study should be carried out.

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