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Original Article

Photostability evaluation of *Jawarishe Jalinoos*

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ABSTRACT

Jawarishe Jalinoos (JJ) is an orally used formulation available in semisolid dosage form, prepared with powdered plant materials mixed in honey or sugar syrup. It has many admirable pharmacological effects and used in Unani medicine to treat various acute and chronic disorders since ancient times. The ICH Harmonised Tripartite Guideline stated that photostability testing should be an essential part of stability testing to confirm that light exposure does not result in an unacceptable change in drugs substance and finished products. To date, the effect of light on JJ is not studied, in this study photostability evaluation of JJ was carried out. The test sample was manufactured with genuine ingredients in the in-door pharmacy of the National Institute of Unani Medicine. JJ was packed in two transparent polyethylene terephthalate airtight containers. The first sample was analysed at zero-day and the second sample was placed in a stability chamber subjected to light challenge with an overall illumination of 1.2 million lux hours combined with near ultraviolet energy of 200-watt hours per square meter by using option 2, along with 30±2°C temperature and relative humidity 70±5%. Analysis of both finished products showed no considerable changes in organoleptic characters. Less than 5% variation was observed in physicochemical parameters. HPTLC fingerprinting showed justifiable variation. Microbial load and specific counts were within the limit prescribed by WHO. As no unacceptable changes were noted in JJ subjecting to light challenge, it is concluded that JJ is a photostable Unani compound formulation.

Keywords *Photostability, Jawarishe Jalinoos, Shelf life, Unani system of medicine*

INTRODUCTION

Environment has unavoidable effects on objects which amplify with the passage of time and storage conditions. Drug materials also degrade with time under the influence of their storage environmental conditions. Their potency and ultimately their efficacy reduce with time. It is also established that light can alter the property of many drug substances and products (Tonnesen, 2004). This photolytic degradation can play an important role in the safety, efficacy and stability of pharmaceutical products (O'Donnell and Bokser, 2006). As a result of photodegradation, there may be a loss of potency of the product which can result in therapeutically inactive drug products as well as the development of toxic degradants (Tonnesen, 2004).

The ICH Harmonized Tripartite Guideline on Stability Testing of New Drug Substances and Products notes that light testing should be an integral part of stress testing. This guideline was implemented in 1996 (Tonnesen, 2004). As per this guideline, intrinsic photostability characteristics of new drug substances and products should be evaluated to demonstrate that, as appropriate, light exposure does not result in unacceptable change. Normally, photostability testing is carried out on a single batch of the material selected (ICH Q1B, 1996). The guideline primarily addresses the generation of photostability information for submission in registration applications for new molecular entities and associated drug products.

Photostability testing is also an essential part of product development and is compulsory to confirm that reasonable product quality is preserved during transportation, storage, dispensing and its practical use (Tonnesen, 2004). Light influences the colour, and active principles in a drug formulation, in addition to the final product or package. Photodegradation characterizes as precipitation of active ingredients, loss in viscosity of the formulation, change in dissolution rate, bleaching or as discolouration and cloudy appearance of products etc. Sunlight may cause interactions between the active drug ingredients and endogenous substrates to modify the drug molecules into toxic molecules. It may also produce unstable oxygen molecule that easily combines with other molecules in a cell, which may further aggravate oxidative damage to the drugs and eventually causes toxicity to human tissues. The major problem attributed to the photodegradation is a complete loss or reduced potency of the drug substance. Light energy in the form of heat triggers

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molecules to accelerate the reaction rate. The drug molecule which encounters light-induced degradation is known as photolabile drugs. Zero-order kinetics is the dominant mechanism in photo-degradation reactions (Welankiwar *et al.*, 2013). Evaluation of the drug substance and product under significant light challenge is performed to assess the light sensitivity of the drug molecules for development and validation purposes and to obtain data required for packaging, transportation and handling (Tonnesen, 2004).

Unani systems of medicine engage in recreation in health care needs. It is an ancient system providing major health care needs to a great section of the population in developing countries. Developed countries are also showing attention to the use of herbal medicines as they suppose it to have fewer or no side effects. According to WHO, over 80% of the world population relies on the traditional systems of medicine mainly of plant origin, to meet up their primary healthcare needs. Therefore, it is need of the hour to establish the quality, safety, efficacy, shelf life and photostability etc. of the finished products used in Unani medicine by subjecting them to strict scientific testing.

Jawarishe Jalinoos (JJ) is a sugar based semi-solid orally used formulation prepared with sugar or honey syrup. JJ is used in Unani medicine for a range of ailments like general weakness (Hafeez, 2005), phlegmatic diseases, melasma (*kalaf*), ring worm (*daad*) (Ghani, 2010), weakness of the principal organs (*zofe aza raeesa*), hepatitis (*warame kabid*), gastric weakness (*zofe meda*), palpitation (*khafqan*) (NFUM Part-I, 2006), flatulence (*nafkhe shikam*), gout (*niqras*) (Ghani, 2010; Khan, 2005), back pain, weakness of bladder (Hafeez, 2005; Kabeeruddin, 2004), indigestion (Kabeeruddin, 2004), halitosis etc. It is also used to treat phlegmatic cough, polyurea, and greying of hair. JJ is also prescribed to increase appetite (Hafeez, 2005) and sexual vigour (Kabeeruddin, 2004).

Like any other herbal formulation, JJ is also influenced by environmental conditions and light. As JJ is a highly praised formulation used in Unani medicine, an attempt was made to establish its photostability.

MATERIAL AND METHODS

Procurement of raw drugs

The plant materials of formulation were purchased from a reliable herbal drug supplier at Bengaluru, Karnataka, India during the month of June-July 2016. Plant materials were identified and authenticated by S. Noorunnisa Begum, Assistant Professor, Dept. of Pharmacognosy, Centre for Repository of Medical Resources (C-RMR), Trans-Disciplinary University (TDU), FRLHT Bengaluru (accession number 3801-17). Specimens of all the ingredients used were deposited in the Drug Museum, National Institute of Unani Medicine, Bengaluru (voucher specimen no. 40/IS/Res/2016) for future reference.

Preparation of JJ

All the ingredients were manually cleaned and excluding *zafran* and *mastagi*, washed with running tap water to remove dirt. They were spread in the shade for a few hours to remove water. Further, the ingredients were placed in the hot air oven at 60°C for 4-6 hours to dry them completely. All the drugs except *zafran* and *mastagi* were powdered separately in an electric pulveriser and passed through the sieve size number 80. *Zafran* was finely ground manually with rose water (*arq gulab*) in mortar and pestle (*kharal*). *Mastagi* was grounded gently in porcelain mortar and pestle to avoid melting. For the preparation of honey syrup (*qiwam*), honey was placed on the low flame till it begins to boil. Its impurities were removed and cooled to room temperature. The powder of all the ingredients was mixed uniformly and entered into honey syrup with continuous stirring. Then, *zafran* paste was incorporated into honey syrup and mixed well. At the last, powdered *mastagi* was dissolved in 100 ml of slightly warmed cow *ghee* (*roghan gao zard*) and sprinkled on the mass and rigorously mixed and JJ was prepared. Prepared JJ was allowed to cool at room temperature (NFUM Part-I, 2006). No preservatives were added in the preparation.

Ingredients of *Jawarishe Jalinoos* (JJ)

1	*Asaroon (Asarum europaeum L. Root)*	30 gm
2	*Chiraita shireen* (*Swertia chirayita Roxb. Whole plant*)	30 gm
3	*Darchini* (*Cinnamomum zeylanica Blume. Bark*)	30 gm
4	*Filfil daraz* (*Piper longum L. Fruit*)	30 gm
5	*Filfil siyah* (*Piper nigrum L. Fruit*)	30 gm
6	*Habbul aas* (*Myrtus communis* L. Fruit)	30 gm
7	*Heel khurd* (*Elettaria cardamomum* Maton. Fruit)	30 gm
8	*Khulanjan* (*Alpinia galangal* Willd. Rhizome)	30 gm
9	*Mastagi* (*Pistacia lentiscus* L. Resin)	75 gm
10	*Ood balsan* (*Balsamodendron opobalsamum* L. Wood)	30 gm
11	*Qaranfal* (*Syzygium aromaticum* L. Flower buds)	30 gm
12	*Qust shirin* (*Saussurea lappa* Decne. Root)	30 gm
13	*Saad kufi* (*Cyperus rotundus* L. Rhizome)	30 gm
14	*Saleekha* (*Cinnamomum aromaticum Nees.* Bark)	30 gm
15	*Sumbulut teeb* (*Nordostachys jatamansi* DC. Rhizome)	30 gm
16	*Zafran* (*Crocus sativus* L. Stigma and style)	30 gm
17	*Zanjabeel* (*Zingiber officinale* Rosc. Rhizome)	30 gm
18	*Asal* (Honey)	1800 gm

Storage of JJ

JJ was filled/packed in transparent, airtight, polyethylene terephthalate (PET) containers of 250ml capacity, purchased from the local market of Bengaluru. In each container, 200gm of JJ was filled. Maximum possible attention was paid to avoid any contamination during preparation and packaging.

Photostability testing

JJ packed in PET containers were subjected to the light challenge with overall illumination of 1.2 million lux hours and integrated near ultraviolet energy of 200watt hours per square meter by using option 2 as per the ICH guideline Q1B. A cool white fluorescent lamp emitting light energy comparable to that stated in ISO 10977(1993) and near UV fluorescent lamp producing a spectral distribution, 320-400 nm with an extreme energy discharge between 350nm and 370nm and a significant quantity of UV light in the band of 320 to 360nm and 360 to 400 nm were utilized (ICH Q1B, 1996).

Exposure time for white fluorescent and UV light was calculated for photostability chamber model-Osworld photostability chamber OPSH G-4 1258 as follows.

Calculation of exposure time for white fluorescent light

Duration of calibration	1 hour
Average light intensity observed in stability chamber	11100 lux
Required light intensity	1.2 million lux hour
Exposure required in hours	1.2×1000000/11100 =108
Exposure required in days	108/24=4.5 Days **(4 days 12 hours)**

Calculation of exposure time for UV light

Duration of calibration	1 hour
Average light intensity observed in stability chamber	887µW/cm^2/1000000=0.000887 W/cm^2 or 0.000887W/cm^2×10000=8.87 W/cm^2
Required light intensity	200 W/m^2
Exposure required in hours	200 W/m^2/8.87 W/m^2=22.5
Exposure required in days	22.547/24=**22 hours and 13 minutes**

To achieve the overall illumination of 1.2 million lux hours and integrate near ultraviolet energy of 200-watt hours per square meter, JJ was placed in a photostability chamber. At the commencement, both UV lamp and white fluorescent lamp were concurrently switched on. After completion of 22 hours and 13 minutes, the UV lamp was switched off and the fluorescent lamp was kept on to complete a total duration of 4 days and 12 hours. During the light challenge temperature and humidity in the stability, the chamber was regulated at 30±2°C/70±5%RH (ICH Q1B, 1996).

Assessment parameters

Before and after the light challenge JJ was evaluated for organoleptic characters (appearance, colour, odour and taste) and various physicochemical parameters viz. loss of weight on drying, alcohol and water-soluble matter, successive extractives value, pH, viscosity, ash value, total sugar (Total Carbohydrate protocol), reducing sugar (Baskan *et al.,* 2016), total alkaloids, HPTLC and total and specific microbial contamination.

Total alkaloid

Total alkaloid estimation was carried out by HPLC method using atropine as standard. To prepare a standard sample 10mg of atropine was dissolved in 25ml of mobile phase i.e., methanol: water (4:6). To prepare the test sample, a specific quantity of test drug was dissolved in 20 ml of mobile phase, subjected to centrifuge and supernatant was separated and filtered out. The filtrate was used for HPLC analysis. Ultra-fast liquid chromatography system (Waters 510) isocratic with stationary phase column C18-5µn, (length 250mm, diameter 4.6mm) and *Rheodyne* manual injector were used for analysis. Injection volume was 20 µl, flow rate 1 ml/minute using mobile phase methanol: water (4:6 ratio), the pressure of 1300 PSI with a run time of 10 minutes were adopted to run the HPLC. Analysis was carried out at wavelength 274nm by UV detector using IRIS-HPLC SPECTRAL PROCESSING SOFTWARE 32. Qualitative analysis of alkaloid in the test sample was done by equating retention time of atropine (2.46 minutes) and quantitative estimation was carried out by comparison of area under the peak in densitogram of atropine (taken as 100%) and test sample.

HPTLC fingerprinting

JJ was extracted in HPLC grade absolute alcohol using Soxhlet apparatus. The dry extract was dissolved in the mobile phase [Toluene: Ethyl acetate (9:1)] and filtered for application. HPTLC analysis was performed on a plate size of 20×10 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 100µl Hamilton syringe and operated with the settings of band length 10mm, application position Y 12 mm, number of track 4.

Toluene

Ethyl acetate (9:1) was used as a solvent system for plate development (Meena *et al.,* 2013). The twin trough chamber was saturated with the stated solvent system for about 20 minutes and the plate was placed within for development. Densitometric scanning of dried developed plates was carried out at UV 254nm, 366nm, and 550nm. The plate was derivatised with anisaldehyde-sulphuric acid, heated at 110ºC and evaluated under visible light using CAMAG TLC VISUALISER and screened using CAMAG TLC SCANNER 3 operated by win CATS software (V 1.4.2, Camag) (Sethi, 1996).

Microbial analysis

Microbial contamination in the test samples was analysed for the total bacterial count, total fungal count using the pour plate method. The presence of specific pathogenic bacteria, viz *E. coli*, *Salmonella, Staphylococcus aureus, Pseudomonas aeruginosa* were carried out by conventional culturing and colony counting method (Quality control methods for herbal materials, 2011).

RESULTS AND DISCUSSION

All the analytical data JJ of post light challenge sample when compared with the baseline challenge sample did not show any considerable changes.

Organoleptic characters of *Jawarishe Jalinoos* at baseline and post light challenge

Organoleptic characters did not show any significant changes in the appearance, colour, odour, and taste in the baseline and photostability sample of JJ. (Table 1)

Table 1. Organoleptic characters of *Jawarishe Jalinoos* at baseline and post light challenge

S. No.	Organoleptic description	Baseline sample JJ	Photostability sample JJ
1	Appearance	Semi-solid Homogenous	Semi-solid Homogenous
2	Colour	**Bulk** Blackish Brown Pantone 476, **Thin layer** Yellow Brown Pantone 725	**Bulk** Blackish Brown Pantone 476, **Thin layer** Yellow Brown Pantone 725
3	Odour	Moderate, pleasant, (dominant with saffron smell)	Moderate, pleasant, (dominant with saffron smell)
4	Taste	Sweetish, Pungent, Slightly bitter	Sweetish, Pungent, Slightly bitter

Physicochemical parameters of *Jawarishe Jalinoos* at baseline and post light challenge

Physicochemical parameters viz., loss of weight on drying at 105°C, ash value (total ash, water soluble ash, acid insoluble ash), alcohol soluble matter, water soluble matter, successive extractives (in petroleum ether, chloroform and ethanol) pH at 1% and 10% aqueous solution, and total and reducing sugar exhibited less than 5% change at post light challenge sample values in comparison to baseline challenge. However, the total alkaloid showed a 10% reduction after photostability testing (Table 2). In this view, analysis and comparison of baseline and post light challenge data showed that changes in physicochemical parameters and chromatographic fingerprinting were less than 25 per cent and changes in total alkaloidal content were only 10%. Therefore, the authors infer those changes in JJ due to light challenges is not noteworthy and confirmed that JJ is photostable as per the ICH guideline. ICH guideline describes a limit of 5% change in assay from its initial value is acceptable and any surpass in it is taken as failing to meet the acceptance criteria. Appearance and physical attributes are subjectively evaluated and should not exhibit significant change for acceptance. However, O'Donnell and Bokser (2006) mentioned that 90% of labelled potency is considered as the minimum acceptable potency level for any drug. Further, as per the Ayurvedic Pharmacopoeia of India, physicochemical parameters shall not vary beyond 25 per cent of the initial value and ±15 per cent change from the initial assay value if the drug is analyzed for its active compound is acceptable to confirm the stability of the ASU drug.

Comparative HPTLC profile of *Jawarishe Jalinoos* at baseline and post light challenge

HPTLC fingerprinting shows justified variation summarized in table 3 and Fig. 1 and 2.

Microbial analysis: The total bacterial and fungal count were within the WHO prescribed limit. The specific pathogenic bacteria, viz E. coli, Salmonella, Staphylococcus aureus, Pseudomonas aeruginosa were absent (Table 2).

Retention time and area under the peak in HPLC for total alkaloid in *Jawarishe Jalinoos* at baseline and *Jawarishe Jalinoos* post light challenge

Table 4 summarises the retention time and area under the peak in HPLC for total alkaloid in JJ at baseline and post light challenge.

Stability study of drug substances and drug products is carried out using heat, humidity and light challenges. Many stability studies of herbs and their finished products were also carried out and published in reputed journals, using thermal and humidity challenges. Although the effect of sunlight on various materials including herbal drugs was observed in day to day life and the ICH guideline on stability testing also mentioned that photostability should be an integral part of stress testing, however, to date no photostability study is carried out on herbs and their finished products. Likewise, no specific method for the application of light challenge and parameters for photostability evaluation were discussed in any stability study guidelines for traditional medicinal products.

To the best of the author's knowledge, no published data on photostability study of herbs and their finished product are available to compare and interpret them with the present study. This is the first kind of its study where a Unani compound formulation was subjected to the light challenge as per the photostability guideline provided for conventional drug products. Furthermore, quality control parameters for herbal were used to assess any significant change.

Table 2. Physicochemical parameters of at baseline and post light challenge

S. No.	Parameters	Baseline sample JJ	Photostability sample JJ
1	Loss of weight on drying at 105°C (%)	18.84±0.01	18.46±0.00
2	Ash value (%w/w)		
	• Total ash	1.05±0.02	1±0.00
	• Water soluble ash	0.30±0.02	0.31±0.00
	• Acid insoluble ash	0.24± 0.00	0.23±0.00
3	Alcohol soluble matter (%w/w)	53.39±0.18	55.47±0.06
4	Water soluble matter (%w/w)	55.70±0.35	55.33±0.18
5	Successive extractives (%w/w)		
	• Petroleum ether	0.48±0.01	0.50±0.00
	• Chloroform	0.52±0.05	0.55±0.00
	• Ethanol	60.22±0.24	60.31±0.21
6	pH		
	• At 1% aqueous solution	5.62±0.01	5.36±0.02
	• At 10% aqueous solution	5.21±0.02	4.95±0.02
7	Sugar (%)		
	• Total sugar	62.6±0.33	62.0±0.00
	• Reducing sugar	58.0±0.00	58.6±0.33
8	Total alkaloid (%)	0.11	0.10
9	Microbial examination (Cfu/gm/ml)		
	• Total bacterial count	27	21
	• Total fungal count	Nil	Nil
10	Specific pathogen		
	• *E. coli*	Absent	Absent
	• *Salmonella*	Absent	Absent
	• *Staph. aureus*	Absent	Absent
	• *P. aeruginosa*	Absent	Absent

Table 3. Comparative HPTLC profile of at baseline and post light challenge

Baseline sample JJ				Photostability sample JJ			
Peak No.	**Rf value**	**Area (%)**	**Height**	**Peak No.**	**Rf value**	**Area (%)**	**Height**
Under UV 254 nm							
1	-0.08	2.02	93.9	1	-0.08	1.26	33.4
2	0.01	43.25	747.9	2	0.00	62.85	710.2
3	0.09	13.45	177.2	3	0.07	13.66	95.5
4	0.14	5.39	73.3	4	0.19	6.71	37.4
5	0.2	8.94	87.8	5	0.32	5.23	29.6
6	0.33	7.10	76.7	6	0.43	4.88	26.3
7	0.44	7.85	72.8	7	0.54	2.22	17.3
8	0.54	3.49	38.1	8	0.56	1.71	16.6
9	0.64	2.64	27.9	9	0.84	1.48	11.4
10	0.77	2.78	27.1				
11	0.85	3.09	29.7				
Under UV 366 nm							
1	-0.08	0.98	38.1	1	-0.08	0.73	15.0
2	0.01	60.69	728.1	2	0.00	76.69	713.0
3	0.07	10.30	75.8	3	0.20	19.27	82.9
4	0.21	23.25	147.2	4	0.27	3.30	21.9
5	0.28	3.48	30.9				
6	0.78	1.29	11.3				
Under UV 550 nm							
1	-0.07	1.78	64.6	1	-0.05	6.83	122.3
2	0.02	53.92	719.2	2	0.00	64.02	740.4
3	0.14	2.17	50.7	3	0.13	1.87	29.0
4	0.22	6.63	92.4	4	0.20	4.25	52.8
5	0.36	6.8	107.2	5	0.34	4.07	53.2
6	0.45	5.45	66.2	6	0.43	2.84	27.7
7	0.65	17.33	159.2	7	0.63	11.76	74.8
8	0.78	5.94	63.1	8	0.77	4.36	34.4

Table 4. Retention time and area under the peak in HPLC for total alkaloid in at baseline and post light challenge sample

No. of peak	**Retention time(min.)**	**Area (mVs)**	**Area (%)**
Standard (pure atropine)			
1	2.460	83.516	100
	Total	83.516	100
Baseline Sample			
1	2.327	98.402	49.9
2*	**2.460**	**26.845**	**18.9**
3	3.230	20.523	10.4
4	3.947	33.879	17.2
5	5.083	17.443	8.9
	Total	197.092	100.0
Post light challenge			
1	1.160	6.731	5.9
2	2.273	42.678	37.7
3*	**2.460**	**21.381**	**13.6**
4	3.147	17.222	15.2
5	3.853	16.727	14.8
6	4.770	1.440	1.3
7	4.977	7.146	6.3
	Total	113.324	100.0

*Peak designated for the presence of alkaloid

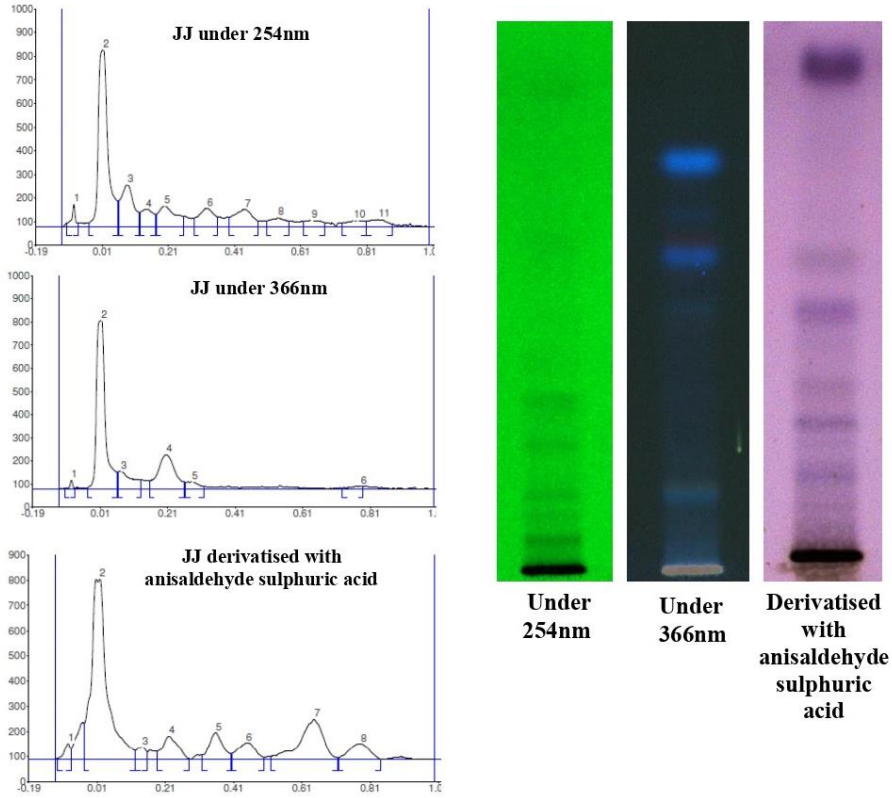

Fig.1 HPTLC fingerprinting of at baseline

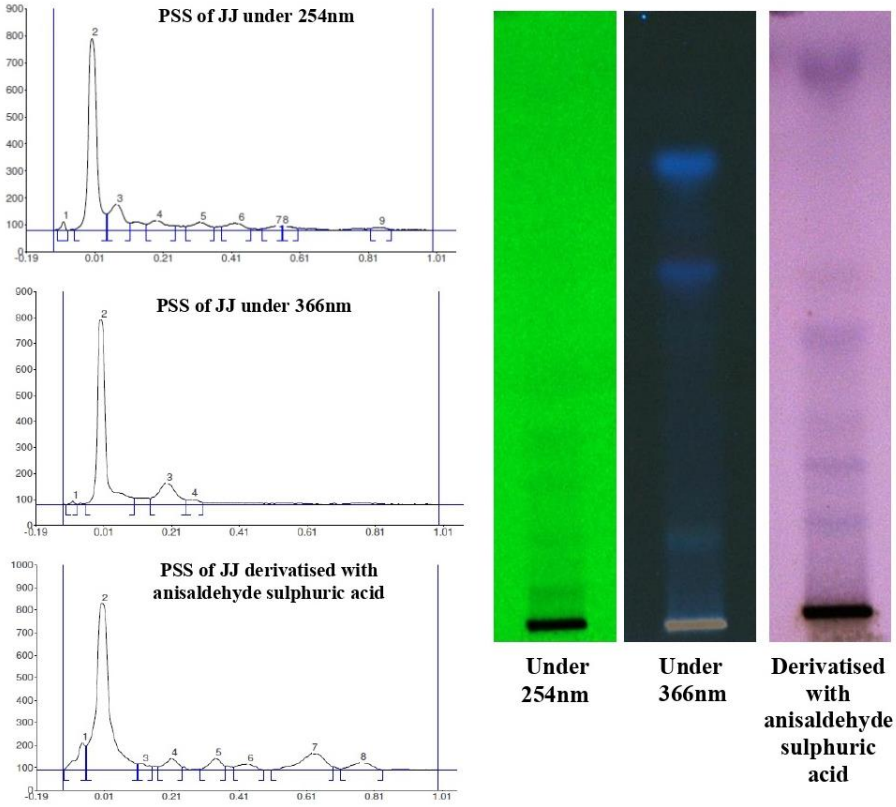

Fig.2 HPTLC fingerprinting of post light challenge

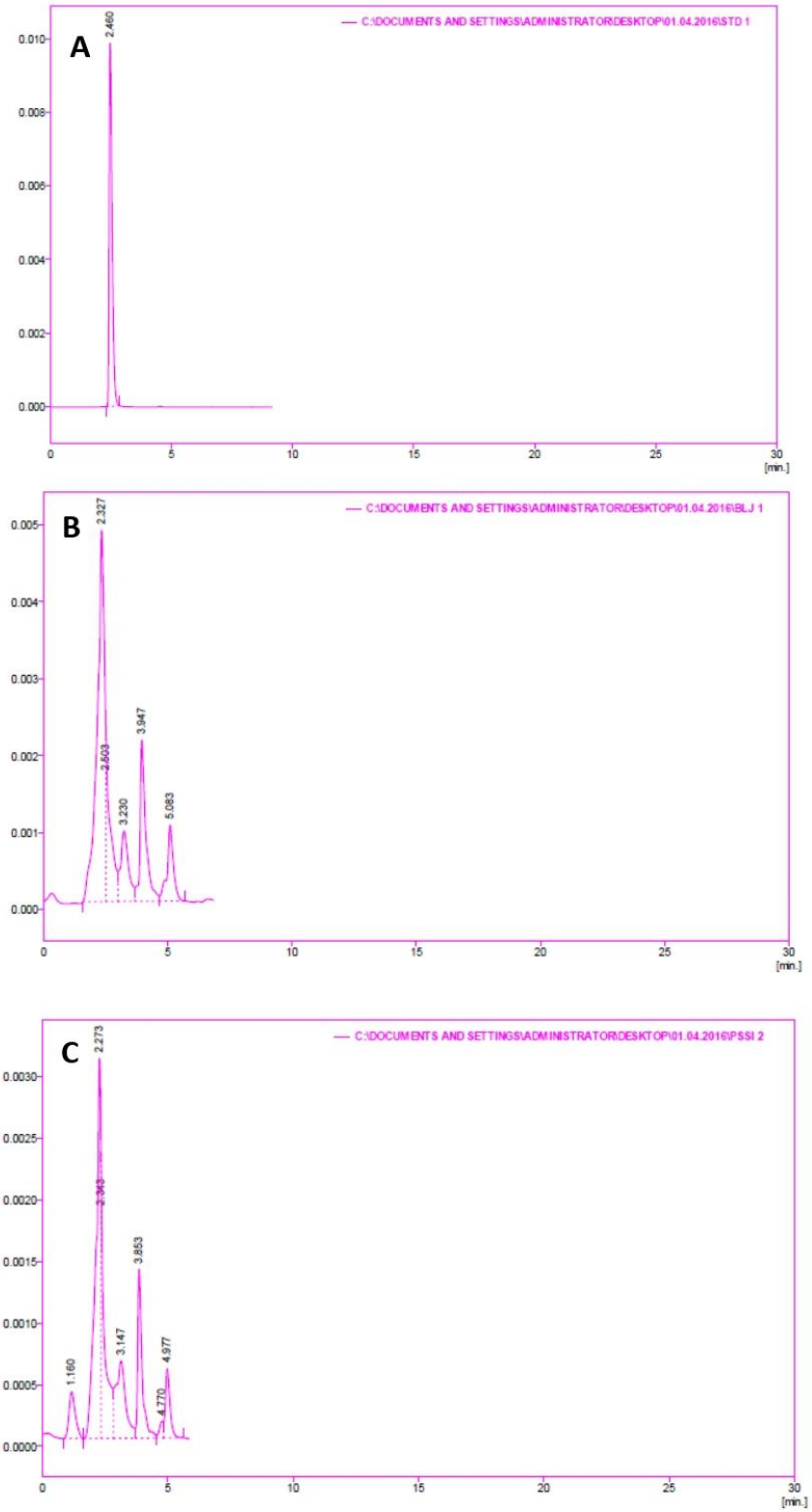

Fig 3. Total alkaloid analysis of (A) standard atropine, (B) baseline sample of and (C) light challenged sample of using HPLC

CONCLUSION

In the view of no obvious changes on the light challenge, the authors concluded that the JJ is stable under photo stress conditions and does not require any specialised light protection packaging. However, it is recommended that JJ must be evaluated for long term and real-time thermal/humidity stability to complete the stability evidence

on how the quality of JJ varies with time under the influence of a variety of environmental factors.

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CONFLICT OF INTEREST-

The authors declare that there is no conflict of interest.

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