ORIGINAL ARTICLE

Quality evaluation and standardization of a traditional Unani formulation *Jawarish-e-Amla Sada*

Sunita Shailajan, Gauri Swar, Archana Matani, Bhavesh Tiwari
Herbal Research Lab, Ramnarain Ruia College, Matunga (East), Mumbai, Maharashtra, India

Background: *Jawarish-e-Amla Sada* (JAS) is a traditional Unani formulation commonly used for the clinical treatment of *Zof-e-Meda* (weakness of the stomach), *Zof-e-Kabid* (weakness of the liver), *Zof-e-Qalb* (weakness of the heart), *Khafqan* (palpitation), *Nafkh-e-Shikam* (flatulence in the stomach) and *Is-Hal-e-Safrawi* (biliary diarrhoea). It is a semisolid polyherbal preparation of six medicinal plants. **Aims and Objectives:** In the present research work an attempt has been made to establish the physicochemical, phytochemical and safety profile of JAS which may be useful for its quality control and standardization. **Materials and Methods:** Standardized operating procedure for the preparation of JAS was developed in accordance with National Formulary of Unani Medicine. The formulation was also subjected to preliminary phytochemical and physicochemical evaluation. Chemical characterization of the formulation was carried out on the basis of gallic acid content using a validated high-performance thin layer chromatographic method. Safety of the formulation was affirmed by conducting acute oral toxicity in mice. **Results:** Preliminary phytochemical, physicochemical and chromatographic profile of the formulation was established. The formulation was found safe up to an oral dose of 2 g/kg body weight in mice. **Conclusion:** Findings of the present work can be used as a reference standard by quality control/assurance laboratory of a pharmaceutical firm in order to have a proper quality check over its preparation and processing.

Key words: Gallic acid, high-performance thin layer chromatographic, *Jawarish-e-Amla Sada*, safety study, standardization

INTRODUCTION

In the past decade, there has been renewed attention and interest in the use of traditional medicine globally. Though the Unani formulations are gaining global acceptance due to their amazing clinical efficiency, their quality control and standardization remains an unexplored issue. Hence, the concept of standardization is becoming essential as a means of ensuring a consistent supply of high-quality traditional phytopharmaceutical products.[1]

Jawarish-e-Amla Sada (JAS) is a polyherbal Unani formulation used as a remedy for the clinical treatment of *Zof-e-Meda* (weakness of the stomach), *Zof-e-Kabid* (weakness of the liver), *Zof-e-Qalb* (weakness of the heart), *Khafqan* (palpitation), *Nafkh-e-Shikam* (flatulence in the stomach) and *Is-Hal-e-Safrawi* (biliary diarrhoea). The formulation includes six medicinal herbs namely *Amla khushk, Post-e-Turanj, Sandal Safaid, Mastagi, Dana Heel Khurd* and *Gulnar Farsi* [Tabel 1].[2]

Access this article online	
Quick Response Code:	Website: www.greenpharmacy.info
	DOI: 10.4103/0973-8258.150916

Jawarish-e-Amla Sada is being manufactured by some Unani industries like Rex Remedies Pvt. Ltd. and Hamdard Laboratories, etc. Recently, immunomodulatory and anti-mycobacterial activities of JAS available in the market have been reported.[3,4] Literature survey revealed that the formulation composition of the marketed formulation varies significantly in terms of the number as well as the percent component of ingredients. In order to have uniform quality as well as batch to batch consistency, there is an urgent need to have some reference standards for quality control and standardization of this formulation. Thus, in this study we report the development of physicochemical, phytochemical and safety profile of JAS for its quality assessment.

Jawarish-e-Amla Sada was prepared (in-house) using the standardized raw materials as per the National Formulary of Unani Medicine. The formulation was subjected to qualitative evaluation for the presence of some major group of secondary metabolites. Percent content of reducing sugar as well as crude fiber was also determined. As, *Amla Khushk* (*Emblica officinalis* L.) is a major ingredient of JAS[2] and gallic acid is a bioactive principle of this ingredient;[5] the chromatographic

Address for correspondence: Dr. Sunita Shailajan, Herbal Research Lab, Ramnarain Ruia College, Matunga (East), Mumbai - 400 019, Maharashtra, India. E-mail: sunitashailajan@gmail.com
Received: 02-04-2014; **Accepted:** 30-10-2014

characterization of the formulation was carried out in terms of gallic acid content using a validated high performance thin layer chromatographic (HPTLC) technique. As an application of the developed HPTLC method, JAS manufactured by some Unani industries were also evaluated and compared for their gallic acid content with the formulation prepared in-house. The formulation was also evaluated for its safety in mice as per the OECD guidelines no. 420.

MATERIALS AND METHODS

Plant Materials

Raw materials used for the preparation of JAS were procured from Ratan Gandhi Shop, Pydhonie (Mumbai) and authenticated by Herbal Research Lab, Ramnarain Ruia College. The materials were dried in an oven preset at 45°C, powdered, sieved through an 85-mesh (BSS) sieve and stored in air tight containers. JAS manufactured by Rex Remedies Pvt. Ltd., batch no. 161/7 (M_1) and Hamdard Laboratories, batch no. NO24 (M_2) was procured from the market Mumbai.

Preparation of *Jawarish-e-Amla Sada*

Raw materials complying the pharmacopoeial quality and quantity were subjected to the preparation of JAS as per the composition [Table 1] mentioned in the classical reference.[2] The standard operating procedures for the preparation of JAS involved following steps:

Preparation of Qiwam (Base for Jawarish) and Powdered Mixture

The base for preparation of JAS (*Qiwam*) was prepared using *Aab*, *Qand Safaid* and *Asal*. Accurately weighed *Qand Safaid* (375 g) was dissolved in 375 mL of *Aab* and the mixture was filtered through a muslin cloth to remove all the foreign matter and particulate impurities present in it (if any). The final filtered solution and *Asal* (375 mL) were mixed and heated together in a vessel on a mild flame with continuous stirring till the desired thickness and consistency of one *Tar* (thread) was attained. Powder of each plant based ingredient of JAS was weighed separately as per the formulation composition [Table 1] and mixed thoroughly.

Table 1: Formula composition of JAS

Ingredients		Quantity
Unani name	**Botanical identity**	**(g or mL)**
Amla Khushk	Pericarp of *Emblica officinalis* L.	50
Post-e-Turanj	Fruit peel of *Citrus medica* L.	10
Sandal Safaid	Stem of *Santalum album* L.	10
Mastagi	Gum resin of *Pistacia lentiscus* L.	5
Dana Heel Khurd	Seeds of *Elettaria cardamomum* L. Maton	5
Gulnar farsi	Flowers of *Punica granatum* L.	5
Qand safaid	Sugar	375
Asal	Honey	375
Aab	Water	375

JAS – *Jawarish-e-Amla Sada*

Preparation of The Formulation

The powdered mixture was added intermittently to the *Qiwam* with constant stirring till a homogenized semisolid mixture JAS was obtained. The final product was cooled to room temperature and stored in an airtight glass container.

Standard and Reagents

Analytical grade solvents and chemicals were procured from Merck Specialties Pvt. Ltd., Mumbai, India. Standard Gallic acid (≥98% purity, Figure 1) was procured from Sigma-Aldrich Chemical Company, Germany.

Quality Evaluation of JAS

Physicochemical Evaluation

Crude fiber and reducing sugar content of JAS was determined using Dutch method[6] and Dinitrosalicylic acid (DNSA) method[7] respectively.

Preliminary Phytochemical Evaluation

Phytochemical screening of some major secondary metabolites (flavonoids, essential oils, tannins, glycosides, alkaloids and resins) in JAS was carried out by performing preliminary phytochemical tests as per the method reported.[8]

Chromatographic Evaluation

Extraction of Phytochemical Constituents from *Jawarish-e-Amla Sada* and its Ingredients

In order to extract phenolic compound gallic acid from JAS (in-house and marketed) and its ingredients, samples (1.0 g) were extracted with methanol (10.0 mL), vortex mixed for 1 min and kept standing overnight followed by filtration through Whatman filter paper No. 1. The filtrates were subjected to HPTLC analysis for optimized separation of gallic acid.

Preparation of Standard Stock Solution, Calibrant and Quality Control Samples

Standard stock solution of gallic acid (1000 µg/mL) was prepared in methanol. Serial dilution of the stock solution in methanol was carried out in order to prepare seven calibrant samples (20.0–150.0 µg/mL) and three quality

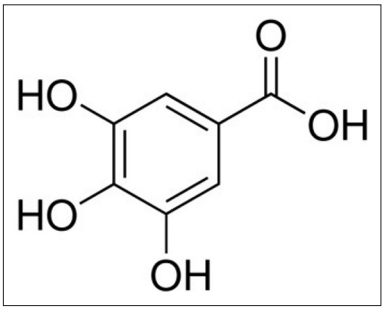

Figure 1: Structure of gallic acid

control samples (LQC-25.0 µg/mL, MQC-55.0 µg/mL and HQC-120.0 µg/mL).

Optimized Chromatographic Conditions for Quantitation of Gallic Acid From *Jawarish-e-Amla Sada* and its Ingredients

The HPTLC system consisted of CAMAG TLC scanner 4 supported by win CATS software version 1.4.7 equipped with CAMAG linomat 5 sample spotter and CAMAG reprostar 3 system for photo documentation. A Denver analytical balance (Goettingen, Germany) was used to weigh the standard and samples. Chromatographic separation of the phytochemical constituents was achieved on TLC plates (E. Merck) precoated with silica gel 60 F_{254} (0.2 mm thickness) on aluminum sheet support. To separate gallic acid from JAS (in-house and marketed) and its ingredients, samples (10.0 µL) and gallic acid standard (100.0 µg/mL, 10.0 µL) were spotted on TLC plate as bands (8.0 mm wide) at a distance of 15.0 mm from the edges under similar instrumental conditions. Plate was developed up to a distance of 85.0 mm in CAMAG twin trough glass chamber presaturated with mobile phase toluene: ethyl acetate: formic acid (2:7:1, v/v/v) for 15 min. The plate was scanned and photo documented at 254 nm. The method was validated as per ICH guidelines.[9]

Safety Evaluation

Acute oral toxicity of JAS (aqueous slurry) was conducted, and the results were compared with the animals of the control group treated with distilled water (guidelines: OECD test guideline: 420,[10] animals: Swiss albino mice, female (20–25 g), *n* = 6/group, parameters observed: Cage side observations, change in body weight along with food and water intake and mortality).

Statistical Analysis

Microsoft Excel-2007 (Microsoft Corporation) was used to determine the mean, standard deviation, relative standard deviation and mean difference during the analysis.

RESULTS AND DISCUSSION

Quality assurance is an integral part of all systems of medicine to ensure quality medicament. Thus, there is an urgent need to evaluate parameters that can be adopted by the pharmaceutical industries for quality assessment of traditional preparations.[11] Different scientific approaches have been reported for standardization of traditional Unani preparations.[12-15] In this research work, standardization of JAS was carried out in terms of its physicochemical, phytochemical and safety profile.

Physicochemical and Phytochemical Profile of *Jawarish-e-Amla Sada*

Reducing sugar and the crude fiber content of JAS was found to be 63.72 ± 0.013% and 0.91 ± 0.033% respectively. In preliminary phytochemical evaluation; the methanolic extract of JAS showed the presence of flavonoids, tannins and glycosides while alkaloids and essential oils were not qualitatively detected. The results for resins were found to be positive in the aqueous extract of JAS [Table 2].

Chromatographic Characterization of *Jawarish-e-Amla Sada*

For chromatographic separation of gallic acid from JAS and its ingredients, we employed the mobile phase reported by our group for chromatographic characterization of some medicinally important plants.[15] This demonstrates the reproducibility and application of a validated method to other plant-based matrices and complex polyherbal formulations. Although, quantification of gallic acid from some of the plants and compound preparations using TLC technique has been reported,[15,16] this is the first attempt for quantification of gallic acid from JAS.

Briefly, the separation of gallic acid was achieved from the methanolic extract of the samples on TLC plate using toluene: ethyl acetate: formic acid (2:7:1, v/v/v) as a mobile phase and visualization of the bands was made possible at 254 nm without derivatization. Gallic acid was detected at R_f = 0.57 and its identity in the complex matrix of different

Table 2: Preliminary phytochemical evaluation of JAS

Phytochemical constituents	Tests	Observation	Inference
Flavonoids	Methanolic extract+increasing amount of NaOH	No yellow coloration	Absent
	Methanolic extract+Lead acetate	Yellow coloration	Present
Tannins	Methanolic extract+5% $FeCl_3$	Blue-black colouration	Present
	Methanolic extract+Lead acetate	No white precipitate	Absent
	Methanolic extract+$K_2Cr_2O_7$	No red precipitate	Absent
	Methanolic extract+$KMnO_4$	Decolouration with turbidity	Present
Alkaloids	Mayer's test: 2–3 ml of methanolic extract+2–3 drops of Mayer's reagent	No precipitation	Absent
	Wagner's test: 2–3 ml of methanolic extract+2–3 drops of Wagner's reagent	No reddish coloration	Absent
Glycosides	Methanolic extract+1.0 mL H_2O+NaOH	Yellow coloration	Present
Essential oils	Methanolic extract+drops of vanillin sulfuric acid	No white crystals	Absent
Resins	Boiled aqueous extract+concentrated H_2SO_4	Reddish brown color	Present

JAS – *Jawarish-e-Amla Sada*

JAS samples was putatively confirmed by comparing the R_f values and spectra of characteristic band with that of the standard gallic acid [Figure 2]. The HPTLC chromatogram of JAS and standard gallic acid has been depicted in Figure 3.

The method was validated as per ICH guidelines and was found to be rapid, specific, precise, sensitive and rugged [Table 3]. Using the regression equation obtained, the exact content of gallic acid in JAS samples and its ingredients was calculated [Table 4].

Source of gallic acid in JAS was found to be *Amla Khushk*, *Post-e-Turanj* and *Gulnar Farsi*, as the marker was detected and quantitated from these ingredients using the validated HPTLC method. The content of gallic acid in these three ingredients of JAS was in the following order: *Amla Khushk* > *Post-e-Turanj* > *Gulnar Farsi.* The bioactive marker gallic acid was not detected in other three ingredients of JAS namely *Sandal Safaid*, *Mastagi* and *Dana Heel Khurd*. The methanolic extract of *Mastagi* was not found compatible with the mobile phase used. This may be attributed to the non-volatile components of *Mastagi*, which were extracted with methanol and found immiscible with mobile phase too. Hence, spotting of this extract on HPTLC plate was not carried out.

The content of gallic acid in JAS (in-house) was found to be 2.17 ± 0.057 mg/g and it varied significantly in two other marketed formulations of JAS (M_1 and M_2). JAS – M_2 showed significantly higher content of gallic acid when compared to JAS – M_1. A possible reason for such variation may be use of the varied amount of the main ingredient of JAS (*Amla Khushk*). The content *Amla Khushk* used in the preparation of JAS – M_2 and JAS – M_1 was 8% and 2.6% respectively (as per the label claim). In the present investigation, it was found that both the marketed formulation of JAS showed significantly lower content of gallic acid compared to the JAS prepared in-house.

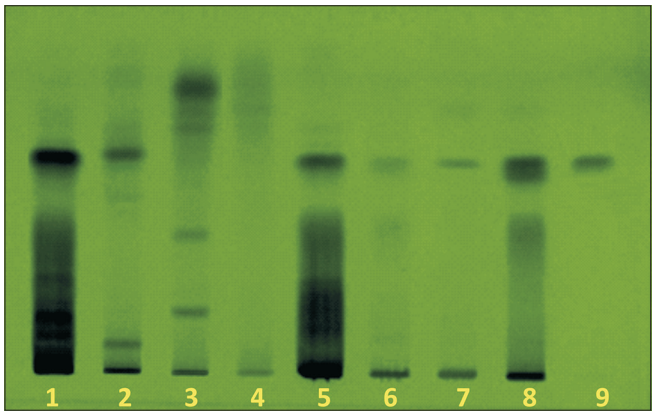

Figure 2: High performance thin layer chromatographic plate photo showing presence of gallic acid in *Jawarish-e-Amla Sada* (JAS), its ingredients and marketed formulations of JAS at 254 nm. Track details: (1) *Amla Khushk* (2) *Post-e-Turanj* (3) *Sandal Safaid* (4) *Dana Heel Khurd* (5) *Gulnar Farsi* (6) JAS – M_1 (7) JAS – M_2 (8) JAS-in-house (10) Gallic acid 100 µg/mL

Safety Profile

In acute toxicity study, oral administration of the formulation JAS (aqueous slurry) did not cause any mortality as well as no significant change in the body weight, food and water intake was observed when compared with the animals of

Table 3: Results of method validation parameters for estimation of gallic acid using validated HPTLC technique

Parameters	Results
LOD and LOQ (µg/mL)	10.0 and 20.0
Linearity (µg/mL)	20.0–150.0
Regression equation	y=56.67x – 56.74
Coefficient of determination (r^2)	0.992
Instrumental precision (% RSD), *n*=7	1.12
Repeatability (% RSD), *n*=5	1.12
Intra-day precision (% RSD)	1.37
Inter-day precision (% RSD)	1.94
Recovery (%)	98.12
Specificity	Specific
Ruggedness	Rugged

HPTLC – High-performance thin layer chromatography, LOD – Limits of detection, LOQ – Limits of quantitation, RSD – Relative standard deviation

Table 4: Gallic acid content in different samples of JAS and its ingredients

Samples	Content of gallic acid (mg/g)
Amla Khushk	1.870±0.056
Post-e-Turanj	1.260±0.040
Gulnar Farsi	1.090±0.038
JAS-in-house	2.170±0.057
JAS–M_1	0.892±0.020
JAS–M_2	1.470±0.033

Values are mean±SD, *n*=3. SD – Standard deviation, JAS – *Jawarish-e-Amla Sada*

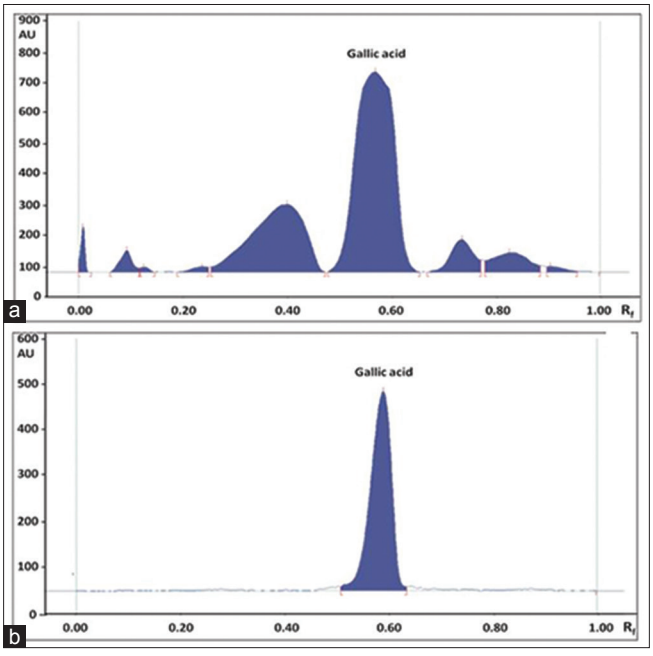

Figure 3: High-performance thin layer chromatographic chromatograms of *Jawarish-e-Amla Sada* (a) and gallic acid (b) at 254 nm

Table 5: Cage side observations for animals during acute toxicity study

Parameter	Observation (for animals of each group including control)
Subcutaneous slug and abdominal distension	
Dullness, opacity and ptosis of eyes	Nil
Discharge from the eyes	
Breathing abnormalities	
Condition of the fur and skin	
Pupil diameter	
Color and consistency of faeces	Normal
Condition of teeth	
Gait	

control group. The cage side observations also appeared normal [Table 5].

CONCLUSION

Considering the importance of a Unani formulation JAS as a potential medicine, an attempt has been made to establish the physicochemical, phytochemical and safety profile of the formulation. These values may be useful as reference standards to be followed in the traditional preparation of JAS in order to have a batch to batch consistency. The developed HPTLC method for the quantification of gallic acid can be applied to various polyherbal formulations and plant raw materials containing gallic acid as a marker. Findings of the toxicity study may ensure an adequate safety margin of JAS for its intended oral use. Standardization of a Unani medicine using such routinely practiced scientific techniques may help in building confidence for their possible use as a therapeutic medicine and their global acceptance.

ACKNOWLEDGMENT

Part financial assistance from DBT Sponsored Star College Scheme (Sanction No. BT/HRD/11/02/2011) and a part of the project work done by UG students of Ramnarain Ruia College is highly acknowledged.

REFERENCES

1. Shailajan S, Yeragi M, Purohit A. Optimized separation and quantification of eugenol from a traditional Unani medicine *Jawarish-e-Bisbasa* using HPTLC. Int J Pharm Sci Rev Res 2011;9:146-51.
2. Anonymous National Formulary of Unani Medicine. 1st ed. New Delhi: Ministry of Health and Family Welfare, Government of India, Controller of Publications 1983. p. 97-8.
3. Ahmad F, Rashid H, Bhatia K, Rehman H, Kaur M, Anjum S, *et al*. Modulatory effect of a unani formulation (*Jawarish Amla Sada*) on cyclophosphamide induced toxicity in tumour bearing mice. Br J Med Med Res 2012;2:454-68.
4. Sherwani AM, Zulkifle M, Rehmatulla. A pilot trial of *Jawarish Amla* as adjuvant to anti-tubercular treatment drugs for control of adverse reactions in dots regime in pulmonary TB. JIMA 2012;44.
5. Chia YC, Rajbanshi R, Calhoun C, Chiu RH. Anti-neoplastic effects of gallic acid, a major component of *Toona sinensis* leaf extract, on oral squamous carcinoma cells. Molecules 2010;15:8377-89.
6. Kokate CK. Practical Pharmacognosy. 4th ed. New Delhi: Vallabh Prakashan; 2003. p. 122-6.
7. Lindsay H. A colorimetric estimation of reducing sugars in potatoes using 3, 5-dinitro salicylic acid. Potato Res 1973;16:176-9.
8. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments. 19th ed. Pune: Nirali Prakashan Publishers; 2008.
9. Shailajan S, Menon S, Sayed N, Tiwari B. Simultaneous estimation of three triterpenoids from *Carissa carandas* using validated high performance liquid chromatography. Int J Green Pharm 2012;6:241-7.
10. Organization for Economic Cooperation and Development. Paris, France: Guidelines for Testing of Chemicals. No. 420. Acute Oral Toxicity-Fixed Dose Procedure; 2001. p. 13.
11. Shailajan S, Singh A, Tiwari B. Quality control and standardization of an *Ayurvedic Taila* formulation. Int J Biomed Res Anal 2010;1:78-81.
12. Afzal M, Khan NA, Ghufran A, Iqbal A, Inamuddin M. Diuretic and nephroprotective effect of *Jawarish Zarooni Sada* – a polyherbal unani formulation. J Ethnopharmacol 2004;91:219-23.
13. Dubey N, Dubey N, Mehta RS, Saluja AK, Jain DK. Quality assessment of *Kushta-e-Gaodanti*: A traditional Unani medicine. Asian J Res Chem 2008;1:46-50.
14. Meena R, Meena AK, Khan SA, Mageswari S. Standardization of Unani poly herbal drug-*Jawarish-e-Darchini*. J Pharm Res 2010;3:1673-6.
15. Sharma A, Shailajan S. Simultaneous quantitation of gallic acid from fruits of *Phyllanthus emblica* Linn., *Terminalia bellirica* (Gaertn.) Roxb. and *Terminalia chebula* Retz. Asian J Chem 2009;21:7111-6.
16. Shailajan S, Menon S, Trivedi M, Tiwari B. Quality assessment of a traditional oil based ayurvedic formulation: *Yashtimadhuka Taila*. J Pharm Res 2012;5:1112-5.

How to cite this article: Shailajan S, Swar G, Matani A, Tiwari B. Quality evaluation and standardization of a traditional Unani formulation *Jawarish-e-Amla Sada*. Int J Green Pharm 2015;9:21-5.

Source of Support: Nil, **Conflict of Interest:** None declared.