Research Article

Quality control analysis of Safoof-e-Pathar phori: Antiurolithiatic formulation

Abstract

Aim: Safoof-e-Pathar phori (SPP), a Unani polyherbomineral formulation used for antilithiatic activity. The present study involves standardization of SPP to assess the quality. SPP were subjected to pharmacognostic studies, physiochemical properties, phytochemical analysis, high-performance thin layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), and gas chromatography-mass spectrometry (GC-MS) fingerprint profile to set the standards, which can be used as reference of quality by herbal industries for its preparation and human use. **Materials and Methods:** The quality control of SPP has been carried out as per the AYUSH and WHO guidelines. The HPTLC fingerprinting has been done using chloroform: ethyl acetate (9:2, v/v) for petroleum ether extract, chloroform: toluene: ethyl acetate (7:2:4, v/v/v) for chloroform extract and for methanol extract petroleum ether: ethyl acetate (9:2, v/v) was used as mobile phase. HPLC was carried out using mobile phase consisted of acetonitrile and water in the ratio of 50:50 (v/v) for the methanolic extract. GC-MS fingerprinting profile has been carried out using hexane extract. **Result:** SPP was subjected to qualitative estimation of phytochemicals using standard methods, which revealed the presence of various bioactive components such as anthraquinone glycosides, carbohydrates, resins, proteins, flavonoids, phenolics, tannins, and terpenoids. The quantitative estimation of total phenolics and flavonoid content showed 0.44 mg/g and 1.02 mg/g, respectively. The HPTLC fingerprint showed presence of number of compounds for extracts at different R_f values. However, HPLC fingerprinting showed presence of 23 well-separated compounds and GC-MS showed presence of 22 compounds. **Conclusion:** The quality control parameters in present study reveal complete standardization profile of SPP for the 1st time, which would be of immense value in checking quality of developed formulation for human use.

Key words:
Gas chromatography-mass spectrometry, high-performance liquid chromatography, pharmacognostic evaluation, Safoof-e-Pathar phori

Introduction

In Unani system of medicine, powdered drugs are called as Safoof. Safoof is an important class of Unani medicinal preparation obtained by powdering and mixing of herbal, metal, mineral, and animal drugs.[1] Safoof-e-Pathar phori (SPP) is an Unani polyherbo-mineral formulation and has been used in Unani system of medicine for its anti-urolithiatic activity based on Unani pharmacopeia.[2] It is a powdered formulation, which contains six different plant/mineral constituents: Pathar phori (*Didymocarpous*

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pedicellata),[3] kulthi (*Dolichous biflorus*),[4] revand chini (*Rheum emodi*),[5] namak turb (*Raphanus sativus*), jawakhar (potassium carbo-nate), and shora qalmi (potassium nitrate).[6] The present study deals with standardization of SPP based on pharmacognostic studies, physicochemical properties, phytochemical analysis and high-performance thin layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), and gas chromatography-mass spectrometry (GC-MS) fingerprinting.

Materials and Methods

Collection and authentication of drugs

The formulation was prepared using authenticated constituents as per the standard traditional protocol by a Unani physician in Department of Surgery, Faculty of Medicine, Jamia Hamdard, and supplied for analysis. Voucher specimen was also deposited in laboratory for future reference. It contained three crude drugs, namely, *D. pedicellata, D. biflorus*, and *R. emodi* which were collected from Khari Baoli, local drug market, New Delhi and were authenticated by Dr. H. B. Singh, Ref. NISCAIR/RHMD/1327/129, New Delhi.

Physicochemical studies

The organoleptic characters of the powder formulation were observed. Phytochemical constituents such as alkaloids, tannins, glycosides, resins, terpenes, flavonoids, carbohydrate, and saponins were tested using standard chemical reagent.[7,8] Other parameters determined were total ash, acid insoluble ash, water soluble ash, extractive values in ether, alcohol and water, successive extractives in petroleum ether, chloroform, acetone, methanol and water, loss on drying at 105°C, PH of filtrate of 1% and 10% w/v aqueous solutions as per the AYUSH protocol.[9-11]

Total flavonoid content

Aluminum chloride colorimetric method was used for flavonoids determination.[12] The calibration curve of rutin was prepared by preparing different dilutions in the concentrations range of 10–100 μg/mL in methanol.

Total phenolic content

Total phenols were determined by Folin Ciocalteu method.[12] The standard curve was prepared using 25, 50, 100, 150, 200, 250, and 300 μg/mL solutions of gallic acid in methanol.

Fingerprinting profile by high-performance thin layer chromatography, high-performance liquid chromatography and gas chromatography-mass spectrometry

Sample preparation

For HPTLC and HPLC analysis, the powder of SPP (1.0 g) was extracted separately with 15 mL of methanol by sonication for 30 min at 45°C. The process was repeated twice to ensure complete extraction. Before use, all samples were filtered through a 0.45 μm nylon membrane filter. For HPTLC fingerprinting profile the chloroform and petroleum ether extracts of SPP powder (1.0 g) was extracted separately with 15 mL of chloroform and petroleum ether, respectively by sonication for 30 min at 45°C. The solutions were made separately.

The powdered SPP (1.0 g) was extracted separately, with 20 mL of hexane by sonication for 30 min at room temperature. The process was repeated twice to ensure complete extraction. The extracts obtained were pooled and dried under reduced pressure. The residue obtained from each extract was redissolved separately in 25 mL of HPLC grade hexane and subjected to GC-MS analysis for quanlitative analysis. Before use, all samples were filtered through a 0.45 μm membrane filter.

High-performance thin layer chromatography instrumentation and chromatographic conditions

HPTLC was carried out on 5 × 10 cm aluminum plates coated with 0.2 μm layers of silica gel $60F_{254}$.(E-Merck) using chloroform: Ethyl acetate (9:2, v/v) for petroleum ether extract, chloroform: Toluene: Ethyl acetate (7:2:4, v/v) for chloroform extract and petroleum ether: Ethyl acetate (9:2, v/v) for methanol extract as mobile phase. Densitometric scanning was performed for petroleum ether, chloroform and methanol extracts at 400, 530, and 254 nm, respectively, using CAMAG TLC scanner III operated by winCats software (CAMAG, Switzerland).

High-performance liquid chromatography instrumentation and chromatographic conditions

HPLC was carried out on a Waters Alliance e2695 separating module (Waters Co., MA, USA) using photodiode array detector (Waters 2998) with autosampler and column oven. Compounds were separated on a C18 reverse phase column (25 × 4.6 mm, particle size 5.0 μm, Merck, Germany) maintained at room temperature. The mobile phase composed of acetonitrile and water in the ratio of 50:50 (v/v). The flow rate was 1.0 mL/min; and column was maintained at room temperature. Analysis was performed at a wavelength of 296 nm using 10 μL of injection volume.

Gas chromatography-mass spectrometry instrumentation and chromatographic conditions

GC analysis was carried out using Agilent gas chromatograph equipped with a split/splitless injector (230°C) and a mass spectrometer detector (230°C). The carrier gas as helium (1.0 mL/min), and HP-5MS 5% phenyl methyl silox (325°C: 30 m × 250 μm × 0.25 μm) was used as capillary column. About 2 μL of samples was injected in splitless mode and programmed as follows: 170°C for one min, 8°C min^{-1} up to 250°C hold for two min, finally

3°C min^{-1} up to 310°C hold for two min. The multi spark discharge was operated under 70 eV, with a scan range of 70–600 amu. Total run time was 63 min. Identification of individual components was achieved using the Wiley and NIST Library.

Determination of contaminants

The official method of Association of Official Analytical Chemists (AOAC) for analysis was followed for the determination of aflatoxins (AOAC official method 991.31 and 970.52).[13] The methanolic acidic extract was taken for the analysis. Dried extract was taken in 200 µL of hexane and 50 µL of trifluoroacetic acid. This solution was then vortexed in a vortex mixture exactly for 30 s and allowed to stand for 5 min (exact). Finally, 1.95 mL mixture of water and acetonitrile (9:1, v/v) was added to this solution. Known concentration (20 ppb, 40 ppb, and 80 ppb) of standard aflatoxin B1, G1, B2, and G2 were taken and derivatized in the same manner as for sample.

The analysis was carried out on a Waters Alliance e2695 separating module (Waters, USA). The derivatized samples (Both extract and standards) were injected into HPLC column (C18; 15 cm × 4.6 mm) and analyzed using fluorescent detector. The peaks of aflatoxin in drug samples were compared with peak of standards (B1, G1, B2 and G2).

Pesticide determination was done (Agilent 7890A GC system, USA) using established method (AOAC official method 991.31 and 970.52). The 10.0 g of sample was dissolved into methanol and added 1.0 g of sodium oxalate in addition to diethyl ether and petroleum ether 50 mL each. It was shaken for 1.0 min. Organic layer was transferred into separating funnel and added 600 mL of water with saturated solution of sodium chloride solution. Aqueous layer was discarded and the process was repeated for 2–3 times. Organic layer was then passed through sodium sulphate solution and evaporated up to 2–5 mL. This concentrated solution was again mixed with acetonitrile (30 mL) and petroleum ether (30 mL) and eluted with diethyl ether by passing through the column. The solution was concentrated up to 5.0 mL using rotavapor (Buchi, R-215, Switzerland) and analyzed in GC-MS.

Results and Discussion

Phytochemical screening

The SPP was subjected to qualitative estimation of phytochemicals using standard methods, which revealed the presence of various bioactive components such as glycosides, carbohydrates, resins, proteins, flavonoids, phenolics, tannins, and terpenoids. All the physicochemical parameters were carried out in triplicate using standards methods of WHO and AYUSH [Table 1].

Assay of total phenolics and flavonoids

The quantitative estimation of total phenolic and flavonoid content showed 1.29% w/w and 0.629% w/w, respectively. These are the important antioxidant constituents of plants. Phenolics are the most widespread secondary metabolite in plant kingdom. It has been reported that the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers.[14] It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process.[15,16]

High-performance thin layer chromatography, high-performance liquid chromatography and gas chromatography-mass spectrometry fingerprinting profile

The HPTLC fingerprint of extracts showed presence of nine, seven and six compounds for petroleum ether, chloroform and methanol extracts, respectively, at different R_f values [Table 2 and Figure 1], However, HPLC fingerprinting profile showed presence of 23 well-separated compounds [Figure 2]. Three to four major compounds were detected in each extract by HPLC and HPTLC finger printing The GC-MS fingerprinting of SPP hexane extract lead to separation and identification of 22 components showed in chromatogram [Figure 3], which were identified as per the NIST and Wiley library using m/z [Table 2]. The respective concentration of 3-furanocarboxylic acid was highest (43.78%) followed by 3-phenyl 1–5, 6 dimethyl benzofuran (20.30%), 12-cyano-8,10-dimethyl-5,11-dihydr-oindolo [1, 2b] isoquinolin-5,11-dione (3.91%), oleic acid (3.36%), and 1,8-dihydroxy-3-methyl (2.55%).

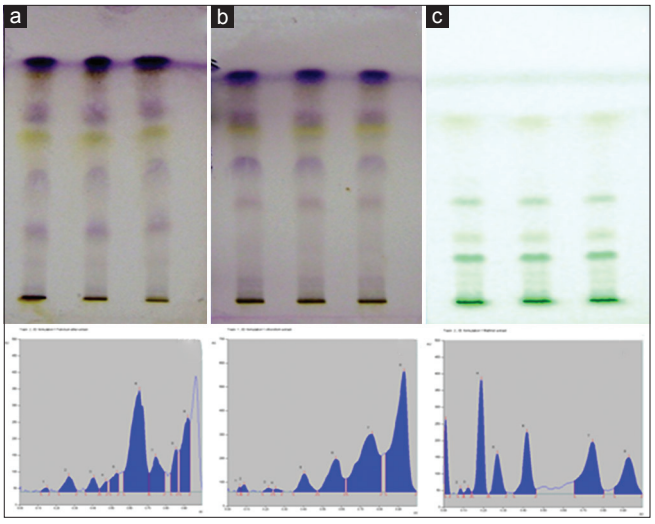

Figure 1: High-performance thin layer chromatography plate and chromatogram of Safoof-e-Pathar phori (a) petroleum ether extract showing nine spots at 400 nm (b) chloroform extract showing seven spots at 530 nm (c) methanolic extract showing 6 spots at 254 nm

Table 1: Summary of physicochemical parameters of Safoof-e-Pathar phori (*n*=3)

Parameter	Mean±SD	
LOD (% w/w)	5.04±0.08	
Ash value (% w/w)		
Total ash	30.06±0.74	
Acid insoluble ash	06.48±0.19	
Water soluble ash	19.18±0.17	
pH		
10% solution	6.2±0.17	
1% solution	6.5±0.08	
Successive extraction values (% w/w)		
Petroleum ether	09.66±0.52	
Chloroform	08.56±0.39	
Acetone	03.77±0.31	
Methanol	29.41±0.45	
Water	39.29±1.08	
Extractive values (% w/w)		
Ether soluble extractives	05.77±0.11	
Alcohol soluble extractives	45.70±0.73	
Water soluble extractives	47.16±0.45	
Total phenolic and flavonoid (% w/w)		
Phenolic content	1.29±0.02	
Flavonoid content	0.629±0.01	
HPTLC fingerprinting	Number of spots with R_f	
	254 nm	After spray
Methanol extract (petroleum-ether: ethyl acetate, 9:2) (v/v)	(6) 0.12, 0.19, 0.27, 0.42, 0.75, 0.93	
Chloroform extract (chloroform: toluene: ethyl acetate, 7:2:4) (v/v/v)		(7) 0.09, 0.21, 0.25, 0.40, 0.57, 0.76, 0.93
Petroleum ether extract (chloroform: ethyl acetate, 9:2) (v/v)		(9) 0.15, 0.27, 0.41, 0.47, 0.53, 0.66, 0.74, 0.86, 0.92
HPLC fingerprinting (296 nm)	R_f	
Methanol extract (water: acetonitrile 50:50, v/v)	(23) 1.6, 1.9, 2.1, 2.3, 2.5, 2.8, 3.0, 3.4, 4.7, 5.9, 6.7, 7.8, 8.6, 9.7, 10.0, 10.9, 13.1, 14.2, 15.6, 16.2, 18.1, 23.2, 28.8	

SD – Standard deviation; HPTLC – High-performance thin layer chromatography; HPLC – High-performance liquid chromatography; LOD – Loss on drying

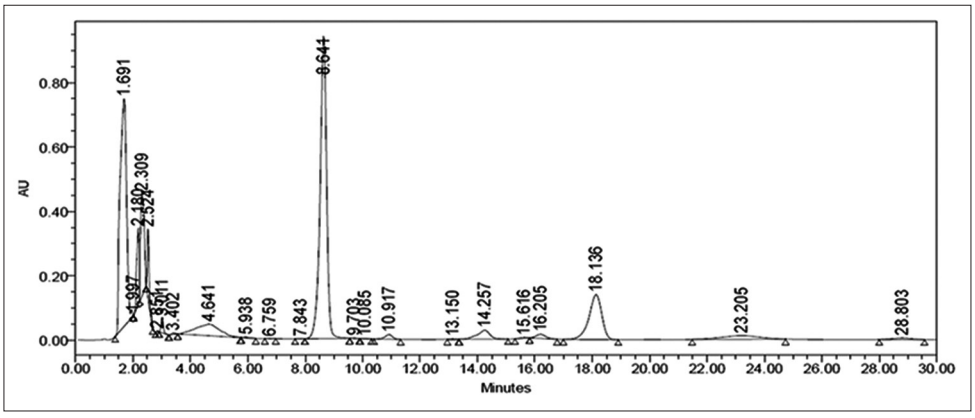

Figure 2: High-performance liquid chromatography chromatogram of Safoof-e-Pathar phori methanol extract showing 23 peaks at 296 nm

Determination of contaminants

After the comparison of GC-MS chromatograms of sample 31 standard pesticides, this was observed that SPP was not containing any pesticide. The aflatoxins (B1, B2, G1, and G2) analysis of SPP was also carried out by HPLC method and found free from any type of aflatoxins (Limit NMT 4.0 ppb for all B1, B2, G1, and G2).

It can be concluded from present investigation that the SPP was evaluated and standardized on the basis of

Table 2: Result of gas chromatography mass spectrometry finger print profile of hexane extract of Safoof-e-Pathar phori

Compound	Retention time	Percentage of components
Alpha humulene	31.840	1.13
3,7-cycloundecadien-1-ol	37.645	3.25
3-cyclohexen-1-carboxaldehyde	38.676	0.73
Eudesma-4 (14)	39.373	0.90
n-hexadecanoic acid	46.455	2.74
Colloidal sulfur	47.543	0.60
Octadecanoic acid	48.550	0.45
Vanicol	49.247	3.67
Linoleic acid	49.370	2.82
Oleic acid	49.450	3.36
1,8-dihydroxy-3-methyl	52.350	2.55
Benzofuran	53.019	1.16
6,7-bishydroxymethyl-11-hydroxy-2,3-dimethoxy	54.060	1.17
N-(4-benzyloxy-phenyl)-acetamide	54.535	0.67
Oxacyclododecan-2-one	54.643	0.92
Ethanone	55.063	1.52
3-phenyl -5,6 dimethylbenzo[b] furan	55.157	20.30
12-cyano-8,10-dimethyl-5,11-dihydroindolo[1,2 b] isoquinolin-5,11-dione	55.821	3.91
2,3-dicyano-5,8-dimethoxyquinoxaline	55.976	2.43
5(10H)-pyrido[3,4-b] quinolone, 7-methoxy	56.042	0.83
3-furancarboxylic acid	56.442	43.78
Cyanazine	57.294	1.13
Total		100.00

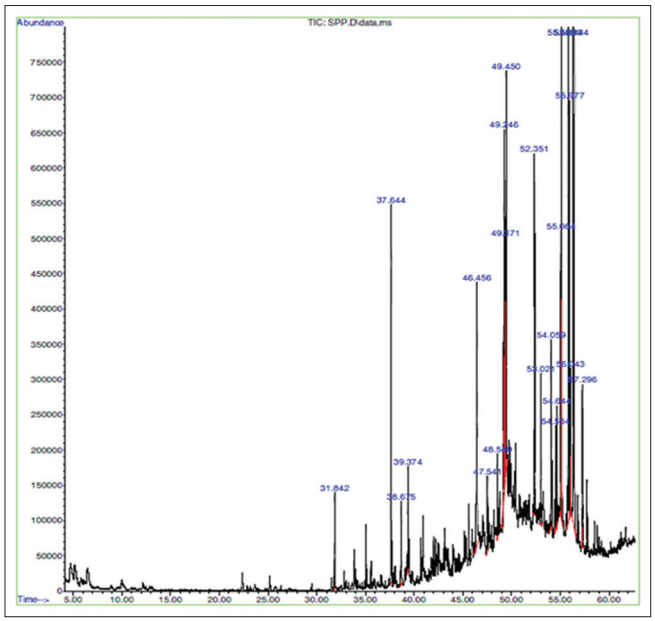

Figure 3: Gas chromatography-mass spectrometry chromatogram of hexane extract of Safoof-e-Pathar phori showed 22 compounds

physicochemical parameters, qualitative and quantitative analysis, HPTLC, HPLC, and GC-MS fingerprinting together with determination of contaminants such as aflatoxin and pesticides.

The SPP contains three plants, namely, *D. pedicellata*, *R. emodi*, and *D. biflorus*, of this *D. pedicellata* is the major ingredients of SPP. Alpha humulene also known as didymocarpene is the chief constituents of *D. pedicellata*, quantitative analysis has been done using GC-MS in hexane extracts of *D. pedicellata* 7.627 g/kg and SPP 3.34 g/kg.

Emodin and chrysophanic acid are the major anthraquinones in the *R. emodi*. Simultaneous estimation of emodin and chrysophanic acid has been done in SPP and *R. emodi* using HPTLC, HPLC, ultra-performance liquid chromatography (UPLC) photodiode array, and UPLC-quadrupole time-of-flight-MS.

Since this type of extensive quality control studies on SPP has been carried out for the 1st time, this may prove as a benchmark in quality control and standardization of SPP for its use in Unani and Ayurvedic system of medicine and may also be the part of Pharmacopeias.

Conclusion

The quality control studies carried out on SPP including physicochemical, phytochemical, fingerprinting, and marker analysis can be used for determination of its quality, as well as to check its identity/authenticity and determination of adulterants in this traditional formulation.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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