Symposium

Quality control analysis of Khamira Abresham Hakim Arshad Wala and Khamira Marwareed

Abstract

Background: Unani system of medicine is based on the principles proposed by Galen, a Greek practitioner. Since then, many Arab and Persian scholars have contributed to the system. Among them, Ibn-e-Sina, an Arab philosopher and Physicist, who wrote "Kitab-al-shifa" are worth mentioning. This system has an extensive and inspiring record in India. Khamira Abresham Hakim Arshad Wala (KAHAW) and Khamira Marwareed (KM) are semisolid sugar-based preparations with potent cardiac tonic and well-known antioxidant properties. It has been studied that therapeutic intervention of KAHAW may be used to prevent or to decrease the deterioration of cognitive function and neurobehavioral activities, often associated with the generation of a free radical. However, no considerable attempt has been made for quality evaluation of KAHAW. **Materials and Methods:** In the present investigations, KAHAW and KM were evaluated for their phytochemical screening, physicochemical standards, presence of minerals, contaminants such as aflatoxins, pesticide residues, thin layer chromatography, and high performance thin layer chromatography (HPTLC) as per WHO and AYUSH guidelines for development of quality standards, which can be used by Unani industries. **Results:** Physicochemical parameters of the prepared compound formulation KAHAW and KM were studied such as total ash, acid insoluble ash, water soluble ash, solubility matter in alcohol and water, and loss on drying at 105°C, pH as per the methods described in WHO guidelines. The heavy metal, aflatoxins, and pesticide residues were also determined for safety evaluation. The total phenolic and flavonoid contents were estimated using catechin and quercetin as standard after preparing calibration plot, respectively. HPTLC fingerprinting of hydrolyzed chloroform extracts of KAHAW and KM was carried out to determined a number of spots present in them. **Conclusions:** This study on pharmacognostical standardization of KAHAW and KM will provide useful information for its identification and quality control and can be applied by different manufacturers of these formulations.

Key words:
Abresham, Khamira, Marwareed, quality control

Introduction

Unani system of medicine is based on the principles proposed by Galen, a Greek practitioner. Since then, many Arab and Persian scholars have contributed to the system. Among them, Ibn-e-Sina, an Arab philosopher and Physicist, who wrote "Kitab-al-shifa" are worth mentioning. This system has an extensive and inspiring record in India. Khamira Abresham Hakim Arshad Wala (KAHAW) and Khamira Marwareed (KM) are semisolid sugar-based preparations with potent cardiac tonic and well-known antioxidant and

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Access this article online	
Website: www.ddtjournal.org	Quick Response Code
DOI: 10.4103/2394-6555.180164	

How to cite this article: Khan MS, Khan W, Ahmad W, Singh M, Ahmad S. Quality control analysis of Khamira Abresham Hakim Arshad Wala and Khamira Marwareed. Drug Dev Ther 2016;7:47-50.

immuno-potentiating properties.[1-3] It has been studied that therapeutic intervention of KAHAW may be used to prevent or to decrease the deterioration of cognitive function and neurobehavioral activities, often associated with the generation of a free radical.[4,5] However, no considerable attempt has been made for quality evaluation of KAHAW and KM.

Experimental

Preparation of Khamira Abresham Hakim Arshad Wala and Khamira Marwareed

The Unani formulations KM and KAHAW were prepared as per the official method.[6]

Quality control analysis

Physicochemical characters

Physicochemical parameters of the prepared compound formulations of KAHAW and KM were studied such as total ash, acid insoluble ash, water soluble ash, pH, extractive values in alcohol, petroleum ether and water, loss on drying, aflatoxins, and pesticide residues; GBC-908 AA model Atomic Absorption Spectrophotometer was used to determine the concentration of heavy metals as per the methods described in WHO guidelines.[7]

Identification

High performance thin layer chromatography fingerprinting

Samples for analysis were applied on precoated silica plate using Linomat V sample applicator (CAMAG). For optimization of high performance thin layer chromatography (HPTLC) solvent system, a number of solvent system was tried, but the most satisfactory resolution was obtained in the solvent toluene: Ethyl acetate. The chromatograms were developed using solvent system and scanned at λ_{max} using CAMAG Scanner III.

High performance liquid chromatography fingerprinting

High performance liquid chromatography (HPLC) analysis of the samples was performed using Waters HPLC system (Waters Corporation, USA) equipped Waters 2998 PDA detector. Separation was performed in a Merck C18 (250 × 4.6) 5 µm column by maintaining the isocratic flow rate (1 mL/min) of the mobile phase (Acetonitrile: Methanol) and peaks were detected at 264 nm absorbance.

Determination of total phenolic content

Total phenols were determined by Folin–Ciocalteu reagent method.[8] The standard curve was prepared using 25–300 µg/mL solutions of gallic acid in methanol.

Determination of total flavonoid content

Aluminum chloride colorimetric method was used for flavonoids determination.[8] The calibration curve was prepared by preparing rutin solutions at concentrations 10–100 µg/mL in methanol.

Determination of total carbohydrate content

Anthrone method was used for total carbohydrate determination.[9] The calibration curve of standard was prepared by preparing glucose solutions at concentrations 20–100 µg/mL in methanol.

Results and Discussion

The KAHAW formulation was semisolid, yellow colored, sweet in taste, and aromatic odor. Whereas KM was semisolid, grayish white colored, sweet in taste, and odor. The physicochemical investigation of the drugs is an important task in detecting adulteration or improper handling of drugs. The loss on drying at 105°C in the formulation was found to be 35.83 ± 0.50%w/w and 38.10 ± 0.30%w/w. Total ash value of plant material indicated the amount of minerals and earthy materials present in the plant material, presence of calcium, magnesium, and sulfate. Analytical results showed total ash value of 6.41 ± 0.67%w/w and 4.15 ± 0.28%w/w, respectively. The amount of acid-insoluble siliceous matter present in formulations was 1.72 ± 0.06% w/w and 1.68 ± 0.18% w/w. The water-soluble ash present in formulations was 0.80 ± 0.09%w/w and 1.22 ± 0.09% w/w. The water-soluble extractive value indicated the presence of sugar, acids, and inorganic compounds. Less or more extractive value indicates the addition of exhausted material, adulteration or incorrect processing during drying, or storage or formulating.[10] The water-soluble extractive value of KAHAW and KM was found to be 75.75 ± 2.37% w/w and 73.49 ± 3.92% w/w, respectively. The alcohol-soluble extractive value was found to be 40.08 ± 2.25% w/w and 35.41 ± 2.52% w/w, respectively. Test for the presence of contaminants (heavy metals, pesticides, and aflatoxins) revealed that they are under limits.

Qualitative and quantitative phytochemical investigation

Preliminary phytochemical investigation revealed the presence of plants secondary metabolites such as carbohydrates, protein, tannins, glycosides, flavonoids, and steroids. Quantitatively phenolic, flavonoids, and carbohydrates were determined and found to be 1.97% w/w, 0.41% w/w, and 73.64% w/w in KAHAW and 1.14% w/w, 0.68% w/w, and 77.71% w/w, respectively.

High performance thin layer chromatography fingerprinting

HPTLC fingerprinting is mainly used to get the chemo-chromatographic profile of the formulations, it helps in quality control and check the presence of adulterants. HPTLC fingerprinting of KAHAW and KM was carried out using various types of solvent system for separation of as many as phytochemicals. Results revealed that the presence of several constituents in the formulations. The number of

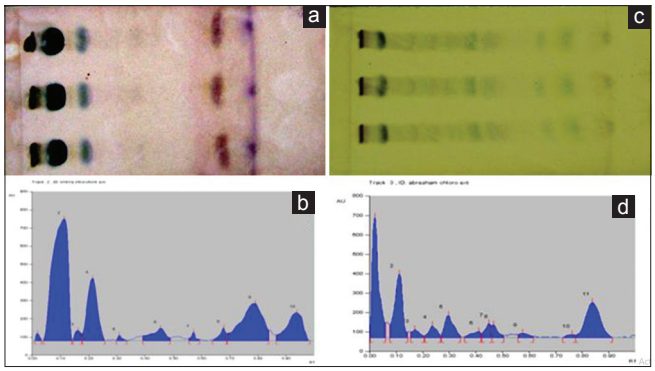

Figure 1: Developed thin layer chromatography plate and high performance thin layer chromatography of Khamira Marwareed (a); high performance thin layer chromatography of Khamira Marwareed at 410 nm (b); developed thin layer chromatography plate of Khamira Abresham Hakim Arshad Wala (c); high performance thin layer chromatogram of Khamira Abresham Hakim Arshad Wala at 450 nm (d)

Table 1: High performance thin layer chromatography and high performance liquid chromatography fingerprinting data of Khamira Abresham Hakim Arshad Wala and Khamira Marwareed

HPTLC/ HPLC	Khamira Marwareed	khamira Abresham Hakim Arshad wala
HPTLC fingerprints	Number of spots and R_f (9) 0.11, 0.19, 0.22, 0.25, 0.30, 0.50, 0.68, 0.77, 0.85	Number of spots and R_f (10) 0.11, 0.17, 0.24, 0.30, 0.41, 0.45, 0.47, 0.58, 0.76, 0.84
HPLC fingerprints	Number of peaks at 280 nm (7) at RT 1.698, 2.082, 2.323, 2.997, 3.917, 4.860, 5.858	Number of peaks at 262 nm (7) at RT 1.605, 2.136, 2.347, 2.965, 3.155, 3.868, 4.026

HPTLC – High performance thin layer chromatography; HPLC – High performance liquid chromatography; RT – Retention time

constituent in the extract and their retention factor (Rf) are summarized in Table 1 and chromatographic profile is shown in Figure 1.

High performance liquid chromatography fingerprinting

HPLC fingerprinting is more accurate and precise, and it helps in checking the standard of formulations. HPLC fingerprinting of KAHAW and KM was carried out and the results revealed that the presence of several constituents in the formulations. The number of constituents in KAHAW and KM with their retention time (Rf) are summarized in Table 1 and chromatographic profile is shown in Figure 2.

HPTLC and HPLC results indicate the number of constituents and further facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds.

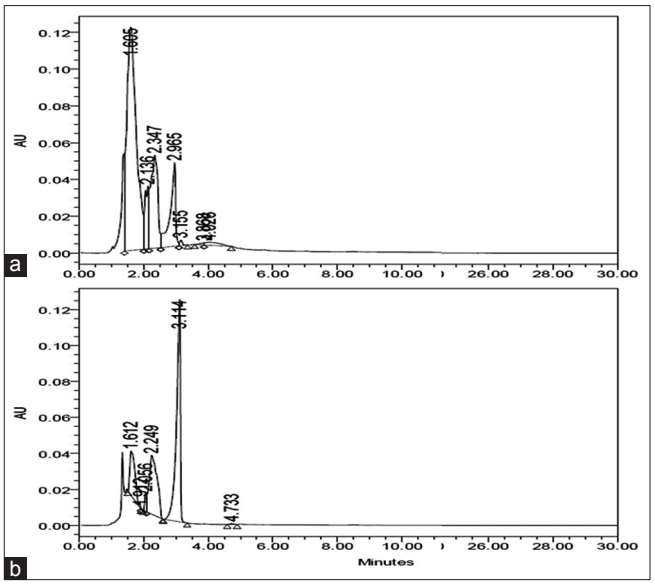

Figure 2: High performance liquid chromatography chromatogram of Khamira Abresham Hakim Arshad Wala at 262 nm (a); high performance liquid chromatography chromatogram of Khamira Marwareed at 262 nm (b)

Conclusion

This study on quality control of KAHAW and KM will provide useful information for its identification and can be applied as a reference for setting limits for the reference standards for quality control and quality assurance by different manufacturers of these formulations.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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