

QUALITATIVE AND QUANTITATIVE ANALYSIS OF PHYTOCONSTITUENTS IN THE UNANI FORMULATION HABB-E-BUKHAR

SEEMA FIRDOUSE^{1*}, SHOYEB AHMED², MOHAMMED ABDUL MUNAIM³

^{1,2}Department of Pharmaceutical Analysis, Anwarul Uloom College of Pharmacy, Hyderabad, Telangana-500001, India. ³Anwarul Uloom College of Pharmacy, Hyderabad, Telangana-500001, India

*Corresponding author: Seema Firdouse; Email: seemamiyna@gmail.com

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ABSTRACT

Objective: Habb-e-Bukhar is a specific medication regimen in the traditional unani system for treating fever caused by conditions such as elephantiasis and intestinal diseases. It acts as both an Antipyretic and Diaphoretic to address acute fever.

Methods: The current research aims to analyze the qualitative and quantitative composition of Habb-e-Bukhar to assess the primary and secondary metabolites present in the sample extract. The study utilized a range of advanced analytical methods such as FTIR, AAS, HPLC, and GC-MS analysis. The sample was prepared through the maceration method, and an initial phytochemical screening revealed the presence of Alkaloids, Carbohydrates, Volatile oils, Flavonoids, Glycosides, Steroids, and triterpenoids.

Results: The Fourier-transform infrared spectroscopy reveals the identification of functional groups at specific wavenumbers: 3960.26 for Amide, 3687.85 for Alcohol, 2765.05 for Aliphatic, and 2099.54 for Alkynes; as well as, at wavelength of 1641.55 denoting the presence of Alkenes (C=C). Moreover, findings from the Atomic Absorption spectrometer suggest that the drug contains notable levels of certain heavy metals, such as chromium at 0.00642 ppm, lead at 10.00 ppm, and argon at three ppm detected with precision in their respective wavelengths.

Conclusion: The GC-MS technique was utilized to detect and characterize the phytochemicals found in the ethanolic extract. The findings indicated the existence of seven constituents.

Keywords: Unani medicine, Habb-e-Bukhar, Phytoconstituents, FTIR, AAS, HPLC, GC-MS

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INTRODUCTION

Herbal Medicinal Products consist of complex mixtures of one or more plants that contain a variety of medically active ingredients derived from plant parts or crude plant extracts. The use of HMPs in treating certain human ailments has become widespread, particularly in rural areas [1]. This trend is due to the high costs and side effects associated with most conventional medications, as well as the perception that HMPs are effective and safer alternatives. While HMPs are generally considered safe, they may contain toxic and potentially harmful components [2].

Unani medicine is a recognized traditional medical system in India, falling under the umbrella of AYUSH, which encompasses Ayurveda, Yoga, Unani, Siddha, and Homeopathy. Habb-e-Bukhar is a polyherbal tablet formulation from the Unani system used for treating elephantiasis and malarial fever [3]. Its ingredients include Bambusa bambos Druce, Cinchona officinalis, Tinospora cordifolia, and Acacia arabica Willd. The tablet was designed to treat fevers as well as common colds and coughs according to Unani terminology; it is beneficial for various types of phlegmatic (balghami), bilious (safrawi), and compound fevers (murakkab bukhar) [4].

Kanakana (cinchona officinalis)-The active component quinine is being explored by some countries as a potential treatment or medication with promising activity against Coronavirus. Satt-e-Gilo (tinospora cardifolia), Miers is reported to have potent antiviral effects against HSV and has been suggested for immune-boosting activity [5, 6]. Tabasheer (bambusa bambosa): It is beneficial in treating involuntary muscle spasms, bronchial asthma, fever, bacterial infection, Diabetes etc. Samagh-e-Arabi-It enhances sexual desire and offers benefits for respiratory infections. Additionally, it is used as a local astringent douche or enema in gonorrhoea, cystitis, vaginitis leucorrhoea, piles etc [7].

Isolation and characterization of bioactive compounds from medicinal plants are crucial for pharmaceutical development. Analytical techniques

play a key role in the discovery, advancement, and production of pharmaceuticals. However, the process of isolating pharmacologically active elements from plant extracts is lengthy and laborious. Previous research has not thoroughly addressed this aspect, and there is insufficient available material for it [7, 8]. The present study to screen the primary and secondary metabolites and to improve analysis performance by utilising advanced methods such as High-Performance Chromatography, Fourier Transform Infrared Spectroscopy, Atomic absorption spectroscopy, and Gas Chromatography-Mass Spectrometry. The results obtained will facilitate further research opportunities and demonstrate the potential use of Habb-e-Bukhar as a natural alternative for medical treatment [9, 10].

MATERIALS AND METHODS

Drug procurement

The drug sample was procured from local medical Hamdard Dawakana, Nampally, Hyderabad, Telangana, India.

Preparation of ethanolic extract

Habb-e-Bukhar tablets should be ground into a fine powder using a mortar and pestle. The powdered drug was dissolved in 100 ml of ethanol as the solvent, followed by sonication for 3 min at 40 °C. Subsequently, the sample is kept for 24h, and then it should be filtered to collect the filtrate for subsequent analysis [9-11].

Phytochemical screening

The ethanol-based extract was utilized to conduct phytochemical screening tests in order to assess the characteristics of the active components present [12-14, 19, 20].

Fourier Transform Infrared Radiation (FTIR)

Zinc-selenium ATR plates served as the window material in the Alpha-Bruker FTIR equipped with Opus-7.5 version, covering a spectral range

of 4000-500 cm^{-1} . The light source was a SiC glower and employed an RMS voltage of 1.8×10^{-2} for the 1x1 mm-sized detector element. With an FT size of 16K and optimized operating bandwidth at 5 kHz using the amplifier, a total of 16 scans were performed at resolution settings. The interferometer modulated the infrared light from the radiation source before it reached the sample compartment and then to the detector, which captured signals to produce an interferogram for evaluating functional groups present in the sample [15, 16].

Atomic Absorption Spectrometry AAS

The sample was weighed 0.5g and placed in a 100 ml beaker and subjected to acid digestion. To the sample, add 5 ml of 65% HNO_3 and the mixtures were boiled for 30 min and then after cooling add 70% HClO_4 , and the mixtures was gently boiled until dense fumes appeared. later the mixture was allowed to cool. Add 10 ml of deionised water until the fumes are released [17].

High-Performance Liquid Chromatography (HPLC)

Atlantis BEH C18 (250 X 4.6 mm i. d, 5m particle size) is utilized for chromatographic separation. At ambient temperature, a mobile phase combination of formic acid in water and formic acid in acetonitrile at a ratio of 50:50% by volume is employed, with a flow rate of 0.3 ml/min, UV detection at 229 nm, an injection volume of 5 μl , and a total run time of 15.0 min [18, 22].

Gas Chromatography-Mass Spectrometry (GCMS)

The Agilent 6890GC with 5973N MSD and an HP 5MS column (30 mm x 0.25 mm ID x 0.25 thickness) was used for GC-MS analysis of

the sample. Helium served as the carrier gas at a flow rate of 1 ml/min with a split ratio of 10:1. The injection temperature started at 40 °C, ramping up to a final temperature of 280 °C, while the ionization source remained constant at 230o. The oven temperature increased from 150 °C to 300 °C at a rate of 10 °C per min. A scan interval of 0.5 sec covered fragments ranging from 29 to 600Daltons with in a total running time of 32 min. The NIST database helped interpret the obtained GC-Mass spectrum by comparing the sample's unknown components to known components in the database library, determining their names, molecular weights, and molecular formulas, and retention time [19, 21, 23-25].

RESULTS

Phytochemical screening

The ethanolic extract was subjected to preliminary phytochemical screening, shows the presence of Alkaloids, carbohydrates, volatile oil, flavonoids, glycosides Steroids and triterpenoids as shown in table 1.

FTIR analysis

FTIR analysis confirms the presence of Amide, Alcohols, Aliphatic hydrocarbons, S-OR, and Esters.

HPLC analysis

HPLC confirms the presence of bioactive components at various retention time 2.66, 2.82, 3.14, 5.67,6.09,7.81,7.99 and 8.92. This analytical technique is useful for both qualitative and quantitative estimation.

Table 1: Phytochemical screening

S. No.	Phytochemical test	Results
1	Alkaloid	+
2	Amino acids	-
3	Carbohydrates	+
4	Volatile oils	+
5	Flavonoids	+
6	Glycosides	+
7	Tannins	-
8	Steroids	+
9	Triterpenoids	+
10	Proteins	-

Note: +indicates = present, -indicate = absent

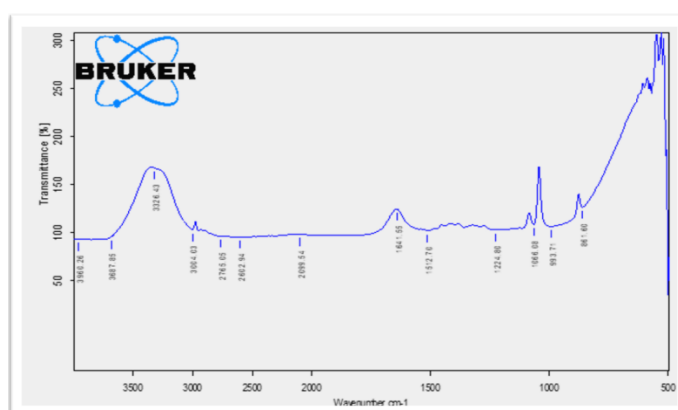


Fig. 1: Interferogram of ethanolic extract of Habb-e-Bukhar

Table 2: Heavy metal analysis by AAS

S. No.	Heavy metal	Wavelength	Valve
1	Chromium (Cr)	357.9 nm	0.0064
2	Lead (Pb)	283.3 nm	0.0005
3	Arsenic (As)	350 nm	0.001

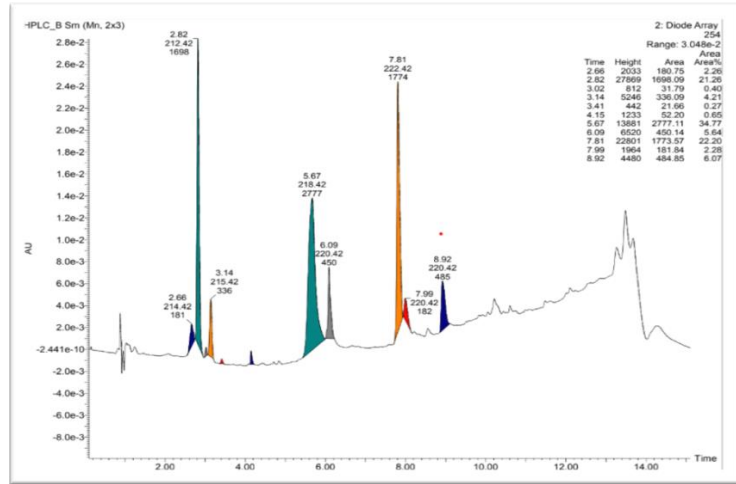


Fig. 2: HPLC chromatogram of ethanolic extract of habb-e-bukhar

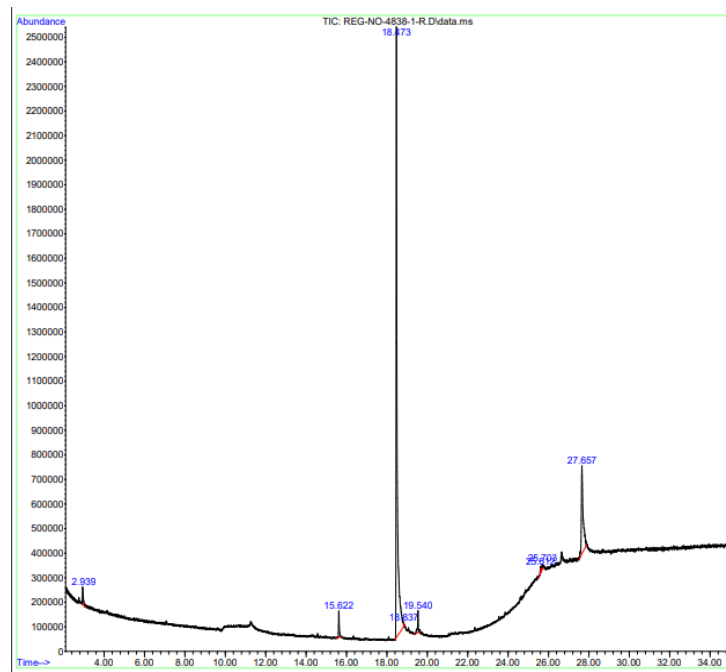


Fig. 3: GC-MS chromatogram of ethanolic extract of habb-e-bukhar

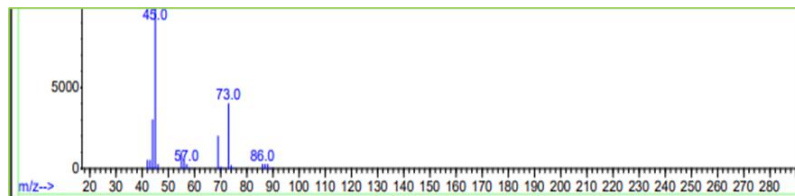


Fig. 3a: 1,1-Diethoxyethane

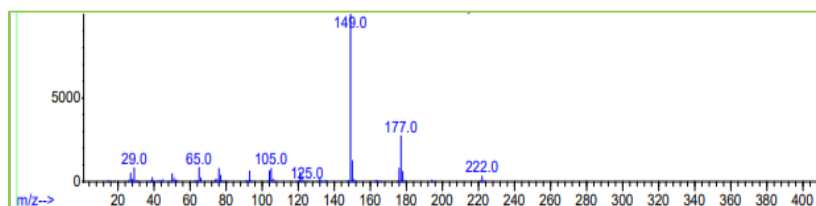


Fig. 3b: Diethyl phthalate

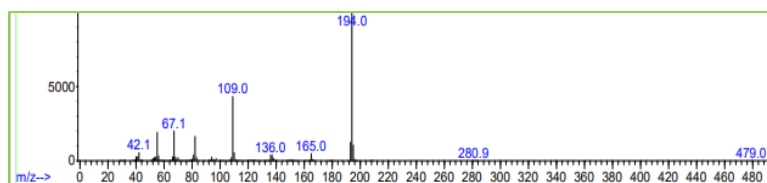


Fig. 3c: Caffeine

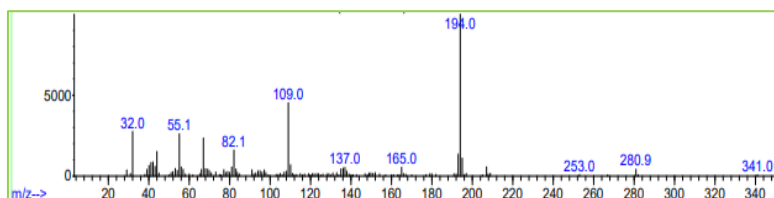


Fig. 3d: 1H-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl-caffeine

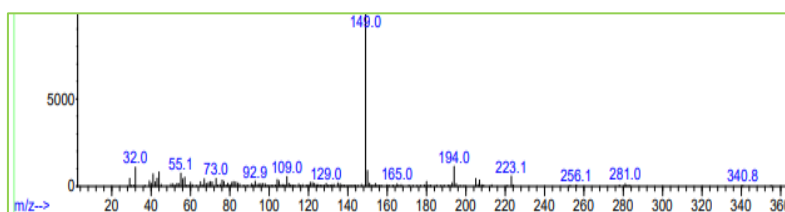


Fig. 3e: Dibutyl phthalate

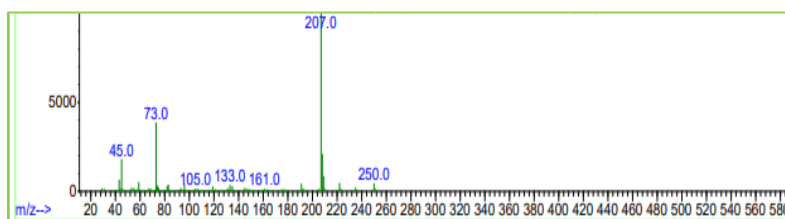


Fig. 3f: 2-methyl-5H-dibenzo[b,f]azepine

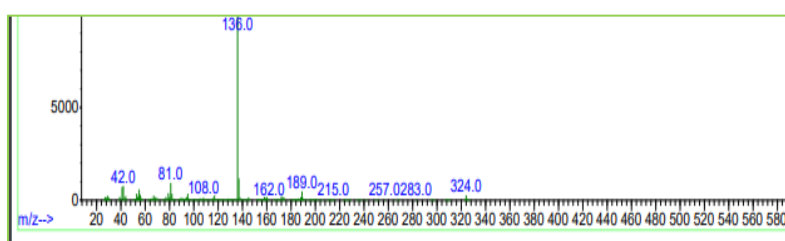


Fig. 3g: Quinine

Table 4: Different phytoconstituents detected in ethanolic extract of habb-e-bukhar

S. No.	Retention time (Rt)	Compound name	Molecular formula	Molecular weight (g/mol)	Percentage area	Pub chem (CID)
1	2.942	1,1-Diethoxyethane	C ₆ H ₁₄ O ₂	118.174	1.83	19991827
2	15.62	Diethyl Phthalate	C ₁ H ₁₄ O ₄	222.24	2.16	6781
3	18.47	Caffeine	C ₈ H ₁₀ N ₄ O ₂	194.19	74.84	2519
4	19.54	Dibutyl phthalate	C ₁₆ H ₂₂ O ₄	278.34	1.87	3026
5	25.613	1H-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl-Caffeine	C ₈ H ₁₁ ClN ₄ O ₂	230.65	0.48	22181
6	25.704	2-methyl-5H-dibenzo[b,f]azepine	C ₁₅ H ₁₃ N	207.27	0.75	12498159
7	27.65	Quinine	C ₂₀ H ₂₄ N ₂ O ₂	324.4	17.89	3034034

DISCUSSION

Plants produce a variety of bioactive molecules making them a wealthy supply of various forms of medicines. Various strategies are hired for his or her investigation which incorporates bioassays for chemical screening and their assessment for the presence of biological activities.

This study was done to identify the phytoconstituents present in the ethanolic extract of Habb-e-Bukhar by utilizing the GC-MS Examination. The ethanolic extract was prepared by maceration technique. The preliminary phytochemical screening tests revealed the presence of Alkaloids, carbohydrates, Glycosides, Flavonoids, Volatile Oil, Steroids, and Terpenoids.

FTIR analysis of ethanolic extract of Habb-e-Bukhar resulted in the identification of different functional groups present determined from the peak obtained. The peaks values were 3960.26, 3687.85, 3326.43, 3004.03, 2765.05, 2602.94, 2099.54, 1641.55, 1512.70, 1224.80, 1066.08, 993.71, 861.60; which constitutes for the following functional groups such as Amide(N-H), Alcohol (O-H), Amide (N-H), Aliphatic (C-H), Alkynes, Alkenes (C=C), Ester (C=O), Alkanes (C-C) and S-OR as listed in table 2.

Heavy metal analysis by using Atomic Absorption Spectrometer shows the quantity of heavy metals present in the drug (Cr) 0.00642 ppm, (Pb) 10.00 ppm (Ar) 3 ppm at particular wavelengths.

HPLC confirms the presence of bioactive components at various retention time 2.66, 2.82, 3.14, 5.67, 6.09, 7.81, 7.99 and 8.92. This analytical technique is useful for both qualitative and quantitative estimation. GC-MS analysis of ethanolic extract of Habb-e-Bukhar found seven phytochemical constituents such as 1,1-Diethoxyethane, Diethyl Phthalate, Caffeine, Dibutyl phthalate, 2-methyl-5H-dibenzo[b,f]azepine, Quinine, and 1-hexyl-4-nitro benzene. The aftereffect of GC-MS examination affirmed the presence of 7 mixtures. The retention time, % peak area, molecular formula and molecular weight as shown in table 4.

CONCLUSION

This study has been conducted due to increased awareness of lifestyle diseases and unhealthy eating habits. Herbal medicinal products are gaining popularity among people all over the world. 'Habb-e-Bukhar' a medication detailing in Unani arrangement of medication. It is an antipyretic. Initially phytochemical screening tests were done to decipher the nature of the components present in the ethanolic sample. Further separation, determination of functional groups and identification of phytoconstituents were done using techniques such as FTIR, AAS, HPLC and GC-MS; all these techniques were appropriately giving benignant results. The conformation of bioactive components obtained was matched with NIST library of IICT. This thorough research work contributes for beneficial usage of Habb-e-Bukhar in herbal medicine as well as grants scope for further study and investigation to be undertaker to explore vast knowledge regarding the plant, its constituents, their uses, and its pharmacological effects. This research assess us that Habb-e-Bukhar is used multipurpose category for various diseases.

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Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally

CONFLICT OF INTERESTS

The authors declare no conflict of interest

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