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Qualitative and quantitative analysis of phytocomponents in marketed Unani formulation Habb-E-Azaraqi

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Abstract
The present study investigates the phytochemical composition of the ethanolic extract of Habb-e-Azaraqi, a traditional herbal formulation. The aim was to identify the presence of various bioactive compounds and evaluate their potential therapeutic significance. Preliminary phytochemical screening indicated the presence of alkaloids, carbohydrates, glycosides, phenols, and proteins. Fourier Transform Infrared (FTIR) spectroscopy revealed functional groups such as alcohols, carboxylic acids, amines, aldehydes, and aromatics. Atomic absorption spectrometry quantified trace amounts of heavy metals at concentrations of 0.2644 ppm, 0.3960 ppm, and 0.00113 ppm. High-Performance Liquid Chromatography (HPLC) confirmed the presence of bioactive components, while Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified 94 phytochemical compounds including 2-propanone, guanethidine, and naphthalene-sulfonic acid. These findings support the medicinal value of Habb-e-Azaraqi and provide a scientific basis for its traditional use.

Keywords: Habb-E-Azaraqi, phytochemicals, FTIR, HPLC, GC-MS

Introduction

Herbal Medicinal Products are intricate mixtures derived from one or more plants, encompassing a range of bioactive constituents extracted from plant parts or crude plant material [1]. The use of HMPs for therapeutic purposes is on the rise, particularly in rural areas, driven by the high costs and side effects associated with many conventional drugs, along with the perception that HMPs are safer and more effective. However, despite their perceived safety, HMPs may contain toxic or otherwise harmful compounds [2, 3].

Unani medicine is a traditional system that employs natural remedies derived from plants, minerals, and animal products. It includes two main types of therapeutic approaches: Mufrad (single drug) and Murakkab (compound formulations). These treatments, whether classical or pharmacopoeial, are selected based on the physician's judgment and the patient's condition, adhering to Unani's humoral theory [4].

This system is well-established in India and is considered generally safe. However, the effectiveness and safety of Unani medicines can vary depending on their method of preparation and dosage. Unani dosage forms fall into four categories: solid, semisolid, liquid, and gaseous. One such solid form is Habb (pill), known in various languages (Arabic: Habb, Hindi: Goli, Latin: Pilula), which is prepared by mixing powdered drugs into a dough and forming it into spherical shapes of different sizes and weights [5].

Habbe-e-Azaraqi is a classical Unani polyherbal pill, frequently cited in authoritative texts such as Qarabadeen Azam, Bayaz Kabeer, and the National Formulary of Unani Medicine. This formulation acts as a Muqawwi-i-Dimagh (brain tonic) and Muqawwi-i-Asab (nerve tonic), with stimulant properties beneficial for treating conditions like epilepsy (Sara), facial palsy (Laqwa), tremor (Ra'sha), gout (Niqras), and polyarthritis (Waja 'al-Mafasil) [6].

This brownish-black pill has a bitter taste and includes ingredients such as Azaraqi Mudabbir (detoxified Strychnos nux-vomica), Filfil Siyah (Piper nigrum), Filfil Daraz (Piper longum), and Arq-e-Ajwain (Trachyspermum ammi). The herbal combination contains various bioactive compounds, including alkaloids, flavonoids, and essential oils like piperine and thymol, offering immunomodulatory, anticonvulsant, neuroprotective, antidepressant, analgesic, anti-inflammatory, antioxidant, and anticancer benefits [6, 7, 8, 9].

The extraction and identification of bioactive compounds from medicinal plants play an essential role in the innovation and development of pharmaceutical products. However, isolating pharmacologically effective components from plant extracts is often time-consuming and complex. Despite its significance, this area of research remains underexplored, with limited available literature [10].

The current study aims to analyze both primary and secondary metabolites, enhancing the accuracy of analysis by employing sophisticated techniques such as High-Performance Chromatography (HPLC), Fourier Transform Infrared Spectroscopy (FTIR), Atomic Absorption Spectroscopy, and Gas Chromatography-Mass Spectrometry (GC-MS). The findings from this investigation are expected to support future research and highlight the potential of Habbe-e-Azaraqi as a natural therapeutic alternative.

Materials and Methods

1. **Drug procurement:** The drug sample was procured from the local medical store, Hamdard Dawakhana, located in Nampally, Hyderabad, Telangana, India.

2. **Preparation of ethanolic extract:** Habbe-e-Azaraqi tablets were finely powdered using a mortar and pestle. The 100mg powder was dissolved in 100 mL of ethanol, which served as the solvent. The mixture was then sonicated for 3 minutes at 35°C- 40 °C. Following sonication, the sample was allowed to stand for 24 hours, after which it was filtered to obtain the filtrate for subsequent analysis [11, 12, 13].

3. Qualitative Analysis

Phytochemical Screening: It refers to the process of identifying and detecting the presence of various phytocomponents in a plant extract or sample [14, 15, 16, 17]. Preliminary phytochemical screening was conducted to test for the presence of alkaloids, carbohydrates, saponins, glycosides, phenols, and proteins.

Fourier Transform Infrared Radiation (FTIR)
FTIR spectroscopy was performed using an Alpha-Bruker FTIR spectrometer equipped with Opus 7.5 software. Zinc-Selenium (ZnSe) attenuated total reflectance (ATR) plates were used as the window material, with a spectral acquisition range of 4000–500 cm^{-1}. A silicon carbide (SiC) glower served as the infrared radiation source. The detector, measuring 1 × 1 mm, operated with an RMS voltage of 1.8 × 10^{-2} V. The Fourier transform (FT) size was set to 16K, and the amplifier bandwidth was optimized at 5 kHz. A total of 16 scans were recorded at a defined spectral resolution. The interferometer modulated the IR radiation before directing it to the sample compartment and subsequently to the detector, which captured the signal and generated an interferogram. This interferogram was then analyzed to identify the functional groups present in the sample [18, 19].

Atomic Absorption Spectrometry AAS
A 0.5 g portion of the sample was accurately weighed and placed in a 100 mL beaker for acid digestion. To this, 5 mL of 65% nitric acid was added, and the mixture was boiled for 30 minutes. After cooling, 70% perchloric acid was added, and the mixture was gently boiled until dense white fumes appeared. The digestion mixture was then allowed to cool. Subsequently, 10 mL of deionised water was added carefully to facilitate the release of residual fumes [10, 20].

4. Quantitative Analysis

High Performance Liquid Chromatography (HPLC)
Chromatographic separation was carried out using an Atlantis BEH C18 column (250 × 4.6 mm i.d., 5 µm particle size). The mobile phase consisted of formic acid in water and formic acid in acetonitrile in a 40:60 (v/v) ratio. The analysis was performed at ambient temperature with a flow rate of 0.2 mL/min, a UV detection wavelength of 200 - 400nm, and an injection volume of 10 µL. The total run time for the chromatographic method was 10.0 minutes [10, 21].

Gas Chromatography Mass Spectrometry (GCMS)
GC-MS analysis of the sample was carried out using an Agilent 6890 Gas Chromatograph coupled with a 5973N Mass Selective Detector (MSD), equipped with an HP-5MS capillary column (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min, with a split ratio of 10:1. The injector temperature was initially set at 40 °C and ramped up to a final temperature of 280 °C. The ionization source temperature was maintained at 230 °C. The oven temperature was programmed to increase from 150 °C to 10 °C per minute until reaching 300 °C. The scan interval was 0.5 seconds, with mass fragments ranging from 29 to 600 Daltons. The total GC runtime was 32 minutes [10, 22, 23, 24, 25].

The relative average peak areas, retention times, molecular formulas, and corresponding molecular weights of the sample components were recorded. Mass spectra were interpreted using the NIST/WILEY (1999) spectral library database. The spectra of unknown compounds were compared with those of known reference compounds stored in the database to identify the components present in the sample extract by name, molecular weight, and molecular formula.

Results

A Preliminary Phytochemical Screening

Table 1: Qualitative Phytochemical Screening of Habbe-e-Azaraqi Extract

S. No	Chemical test	Results
01	Alkaloids	+
02	Carbohydrates	+
03	Saponins	-
04	Glycosides	+
05	Phenols	+
06	Proteins	+

Note: + indicates = Present, - indicate = Absent

FTIR analysis

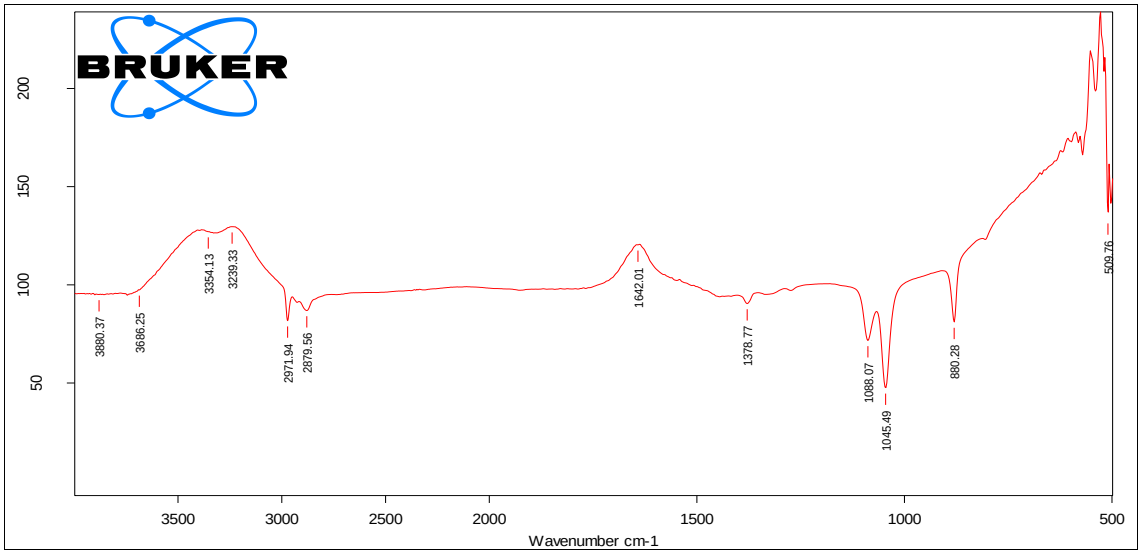


Fig 1: Interferogram of Habbe-e-Azaraqi Extract

AAS analysis

Table 2: Heavy metal analysis by AAS of Habbe-e-Azaraqi Extract

S. No	Heavy Metal	Wave length	Value	Permissible limit
01	Chromium	357.9nm	0.2644 (ppm)	1.3
02	Lead	283.3nm	0.3960 (ppm)	2
03	Arsenic	350nm	0.0011 (ppm)	3

HPLC analysis

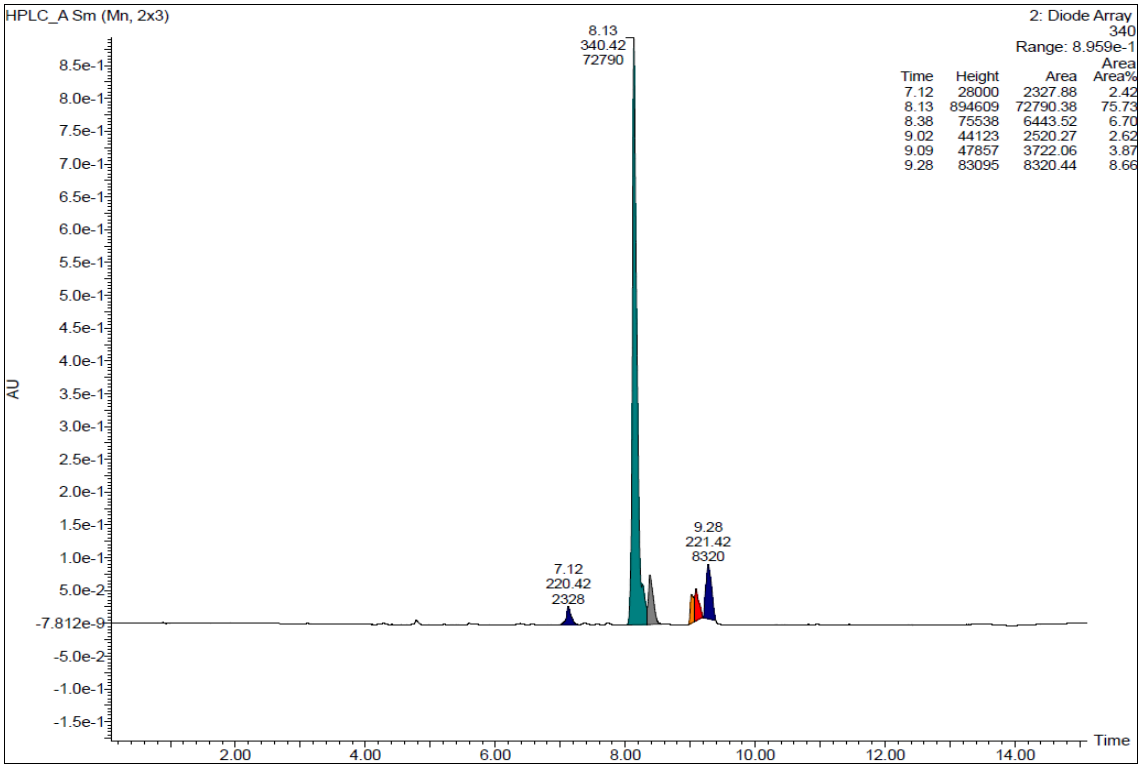
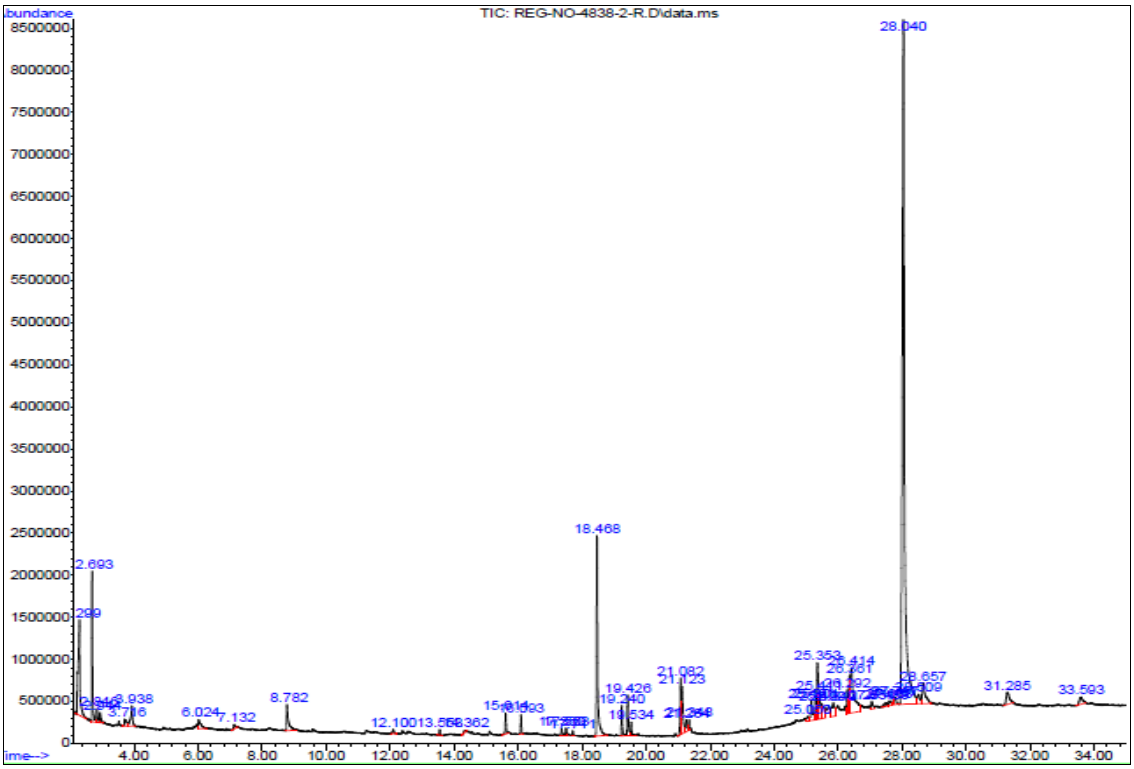


Fig 2: HPLC chromatogram of Habbe-e-Azaraqi Extract

GC-MS analysis



Figu 3: GC-MS chromatogram of Habbe-E-Azaraqi Extract

Table 3: Different Phytochemical compounds detected in Habbe-e-Azaraqi Extract

S. No	Name	Molecular Formula	Molecular Weight
1.	Acetic acid	CH_3COOH	60.05 g/mol
2.	2-Propanone, 1-hydroxy-	C_3H_6	74.07
3.	Silanediol, dimethyl	$C_2H_8O_2Si$	176.24
4.	Trimethylsilyl trifluoroacetate	$C_5H_9F_3O_2Si$	186.20
5.	1-Triazene, 3,3-dimethyl-1-phenyl-	$C_8H_{11}N_3$	149.19
6.	Ethane, 1,1-diethoxy-	$C_6H_{14}O_2$	118.17
7.	(Methoxymethyl)trimethylsilane	CH_3OCH_2Si (CH_30_3	118.25
8.	1,3-Dioxan-5-ol	$C_4H_8O_3$	104.10
9.	1,6;3,4-Dianhydro-2-O-acetyl	$C_8H_{10}O_5$	186.16
10.	Carbamic acid	CH_3NO_2	61.04
11.	Di-n-propyl ether	$C_6H_{14}O$	102.17
12.	Propanoic acid, 2-oxo-	C_4H_6O	102.08
13.	1,2-Epoxy-3-propyl acetate	$C_5H_8O_3$	116.15
14.	Cholestan-5-en-3-ol piperidinome	$C_{27}H_{46}O$	386.6
15.	3-Piperidino-1,2-propanediol	$C_8H_{17}NO_2$	159.22
16.	2-Cyclopenten-1-one, 2-hydroxy	C_5H_6O	98.0
17.	Benzo[b]thiophen-2-carbonitrile	C_9H_5NS	159.21
18.	Formic acid, 2-propenyl ester	C_4H_6O	86.08
19.	Dihydroxymaleic acid	$C_4H_4O_6$	148.07
20.	Oxirane, (butoxymethyl)-	$C_7H_{14}O_2$	130.18
21.	2-Heptanamine, 5-methyl-	$C_8H_{19}N$	129.24
22.	Propanamide	C_3H_7NO	73.09
23.	Octodrine	$C_8H_{19}N$	129.24
24.	Cyclopropyl carbinol	C_4H_8O	72.10
25.	Guanethidine	$C_{10}H_{22}N_4$	198.31
26.	l-Alanine, N-cyclobutylcarbonyl-	$C_7H_{22}N_4$	198.31
27.	Benzene, 1-fluoro-4-methoxy-	C_7H_7FO	126.12
28.	3H-Naphtho[2,3-b] furan-2-one	$C_{25}H_{32}N_2O_2$	392.5
29.	1,2,4-trimethylcyclohexane	C_9H_{18}	126.23
30.	1,3,5-trimethylcyclohexane	C_9H_{18}	126.24
31.	Caryophyllene	$C_{15}H_{24}$	204.36
32.	Bicyclo [7.2.0] undec -4-ene	$C_{15}H_{24}$	204.35
33.	1,6-Anhydro-beta-D-glucopyrano	$C_6H_{10}O_5$	162.14
34.	Formic acid hydrazide	CH_4N_2O	60.05
35.	beta-D-Glucopyranose, 1,6-anhy	$C_6H_{12}O$	180.15
36.	Urea, N-methyl-N-nitroso-	$C_2H_5N_30$	103.08
37.	Diethyl Phthalate	$C_{12}H_{14}O_4$	222.24
38.	1,2-Benzenedicarboxylic acid	C_8H_6O	166.13
39.	1H-Cycloprop[e]azulen-7-ol, deca	$C_{11}H_8O$	156.18
40.	1,7,7-Trimethyl-2-vinylbicyclo	$C_{12}H_{18}$	162.27
41.	Isospathulenol	$C_{15}H_{24}O$	220.35
42.	6-Methyl-2-(4-methylcyclohex-3-e	$C_{15}H_{26}O_2$	238.37
43.	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37
44	Undecanoic acid	$C_{11}H_{22}O_2$	186.29
45	4-Hydrazono-5-hydroxyimino-4,5,6	$C_6H_7N_5$	181.15
46	Benzene, 1-fluoro-4-(2-phenyleth	$C_{14}H_{13}F$	200.25
47	3,6,8-Trimethylazulene-1-carbald	$C_{15}H_{16}O$	212.29
48	3,4,6-Trimethylazulene-1-carbald	$C_{13}H_{14}$	170.25
49	Diampromide	$C_{21}H_{28}N_2O$	324.5
50	Methylenedioxyamphetamine acetate	$C_{12}H_{15}NO$	221.25
51	Caffeine	$C_8H_{10}N_4O_2$	194.19
52	3,4-Dimethoxyphenethyl isothiocy	$C_{11}H_{13}NO_2S$	223.29
53	1,4 Benzodioxan-6-amine	$C_8H_9NO_2$	151.16
54	1,2,4-Triazolo[4,3-a] pyridine-3(	$C_6H_5N_3$	119.12
55	2,4-Decadienamide, N-isobutyl-	$C_{14}H_{25}NO$	223.35
56	(2E,4E)-N-Isobutylundeca-2,4-die	$C_{15}H_{27}NO$	237.3
57	2-Hydroxy-4-methoxybenzaldehyde	$HOC_6H_3(OCH_3)$ CHO	152.1
58	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4
59	1,2-Benzenedicarboxylic acid	C_8H_6O	166.13
60	9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$	280.4
61	Oleic Acid	$C_{18}H_{34}O_2$	282.4
62	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5
63	Anthracene, tetradecahydro-	$C_{14}H_{24}$	192.34
64	(2E,4E)-1-(Piperidin-1-yl) deca-2	$C_{15}H_{25}NO$	235.36
65	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308.49

66	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	222.4
67	Silane, trimethyl [5-methyl-2-(1-.	$C_{13}H_{22}OSi$	222.4
68	Methyltris(trimethylsiloxy)silane	$C_{10}H_{30}O_3Si_4$	310.68
69	Tris(tert-butyldimethylsilyloxy)	$C_{18}H_{45}ASO_3Si_3$	468.7
70	Benzo[h]quinoline, 2,4-dimethyl-	$C_{15}H_{13}N$	207.27
71	2-Naphthalene-sulfonic acid	$C_{10}H_8O_3S$	208.23
72	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	222.46
73	1H-Indole, 1-methyl-2-phenyl-	$C_{15}H_{13}N$	207.27
74	4-Bromo-3,5-di-t-butylbenzamide	$C_{11}H_{14}BrNO$	256.14
75	Succinic acid, 2-fluorophenyl 3-	$C_{10}H_9FO_4$	212.17
76	5,8-Epoxy-15-nor-labdane	$C_{19}H_{34}O$	278.5
77	1,2-Benzisothiazole-3-propanoic	$C_{10}H_9NO_2S$	207.03
78	4-Methyl-2-(phenylacetyl)phenol	$C_{15}H_{14}O_2$	226.27
79	(E)-5-(Benzo[d][1,3] dioxol-5-yl)	$C_{17}H_{21}NO_3$	287.35
80	1-BENZO [1,3] DIOXOL-5-YLMETHYL-5	$C_{12}H_9NO_4$	231.20
81	Silicic acid, diethyl bis (trimet	$C_{10}H_{28}O_9Si_3$	296.58
82	1,4-Phthalazinedione, 2,3-dihydr	$C_9H_8N_2O_2$	176.17
83	1,4-Bis (trimethylsilyl) benzene	$C_{12}H_{22}Si_2$	222.47
84	Piperine	$C_{17}H_{19}NO_3$	285.34
85	Cyclobarbital	$C_{12}H_{16}N_2O_3$	236.27
86	2-(Acetoxymethyl)-3- (methoxycarb	$C_{17}H_{14}O_4$	282.2
87	2,4,6 (1H,3H,5H) -Pyrimidinetrione	$C_{13}H_{13}N_3O_3$	259.26
88	2,4,6-Cycloheptatrien-1-one, 3,5	$C_{13}H_{22}OSi_2$	250.48
89	Tetrasiloxane, decamethyl-	$C_{10}H_{30}O_3Si_4$	310.68
90	Silane, 1,4-phenylenebis [trimethyl-	$C_{14}H_{26}Si_2$	250.52
91	2-Naphthalene-sulfonic acid	$C_{10}H_8O_3S$	208.24
92	1,3-Bis(trimethylsilyl)benzene	$C_{12}H_{22}Si_2$	222.47
93	2-Propenoic acid, 3-phenyl-, 1,7	$C_{19}H_{24}O_2$	284.4
94	Retrofractamide-A	$C_{20}H_{25}NO_3$	327.4

Discussion

Plants are a rich source of diverse medicinal compounds due to their production of various bioactive molecules. Researchers employ a range of strategies to investigate these compounds, including bioassays for chemical screening and evaluation for biological activities.

The purpose of this research was to determine the phytoconstituents found within the ethanolic extract of Habb-e-Azaraqi. Phytochemical screening is the qualitative identification of various chemical compounds occurring naturally in plant extracts. And the sample showed the presence of alkaloids, carbohydrates, glycosides, phenols, and proteins as shown in Table 1.

Fourier Transform Infrared spectroscopy of the Habb-e-Azaraqi ethanolic extract revealed several functional groups, as evidenced by characteristic peaks. These peaks were observed at the following wavenumbers: 3880.37, 3239.33, 2879.56, 1642.01, 1378.77, 1088.07, 880.28, 509.76, 1045.49, and 1915.17 cm^{-1}, corresponding to alcohol, carboxylic acid, amine salt, alkene, aldehyde, secondary alcohol, halo compound, and aromatic compound functional groups.

Atomic absorption spectrometry was employed to quantify the presence of heavy metals in the drug product. The analysis revealed concentrations of 0.2644 ppm, 0.3960 ppm, and 0.00113 ppm for specific heavy metals, as measured at their respective characteristic wavelengths

High-performance liquid chromatography analysis confirms the presence of bioactive components, evidenced by retention times of 7.12, 8.13, 8.38, 9.02, 9.09, and 9.28 minutes. This analytical technique is useful for qualitative and quantitative determination of these compounds.

Gas chromatography-mass spectrometry analysis of the ethanolic extract of Habb-e-Azaraqi identified 94 phytochemical constituents, including compounds such as 2-Propanone, 1-hydroxy-, 9-Octadecenoic acid, 4,6-Pyrimidinetrione, 2-Naphthalene-sulfonic acid, Cyclopropyl carbinol, and Guanethidine. The retention time, molecular formula, and molecular weight for each constituent are presented in Table No.3, which are matched with the NIST library of IICT.

Conclusion

This study concludes that the ethanolic extract of Habb-e-Azaraqi was found to contain a diverse range of phytochemical constituents, as revealed through comprehensive analytical techniques including FTIR, atomic absorption spectrometry, high-performance liquid chromatography, and GC-MS. These analyses confirmed the presence of various bioactive compounds such as alkaloids, carbohydrates, glycosides, phenols, and proteins, along with numerous functional groups and trace amounts of heavy metals. The identification of 94 distinct phytochemicals further emphasizes the therapeutic potential of the extract. Overall, this study highlights the value of Habb-e-Azaraqi as a promising source of biologically active compounds, supporting its traditional medicinal use and encouraging further pharmacological investigations.

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Conflict of Interest Statement

The authors declare no conflict of interest.

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