

Standardization and Phytochemical Evaluation of the Unani Formulation Dawa-e-Hilteet in Pill and Majoon Dosage Forms

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Abstract: Unani system of medicine was based on humoral theory of Buqarat. Drugs are used in single form as well as in compound form. There is a need to standardized formulation according to some parameters for providing evidence-based medicine with purity and efficacy. Dawa-e-Hilteet is a classical Unani formulation traditionally used in treating fever (Humma). This formulation had not been standardized to date. This study aims to standardize the formulation in two dosage forms-Majoon and Pills-by evaluating physicochemical properties, phytochemical composition, safety profile, and active constituents using pharmacopoeial protocols and GC-MS analysis. Dawa-e-Hilteet was a formulation of four ingredients *Ferula asafoetida* (Hilteet), *Commiphora myrrha* (Mur Makki), *Piper nigrum* (Filfil Siyah) and *Ruta graveolens* (Suddab). The results of study showed acceptable results for heavy metals and microbial contamination. GC-MS revealed 68 phytochemical constituents. The findings provide a foundational reference for further studies, promoting the evidence-based application of this Unani formulation.

Keywords: Dawa-e-Hilteet, Unani medicine, Standardization, Phytochemical analysis, GC-MS

1. Introduction

Unani system of medicine is one of the traditional medicines and has existence from decades. The therapeutic values of Unani medicine were based on both single drugs and compound formulations. Present work was based one of the classical compound drug of Unani medicine i.e. *Dawa-e-Hilteet* (DH). *Dawa-e-Hilteet* have been using by Unani physicians in Humoral fever, painful condition of liver, as Deobstruent and scorpion sting, specifically in humoral fever since long [1-9]. This study aims to establish the standardization profile of the Unani compound formulation *Dawa-e-Hilteet* through physicochemical, phytochemical, and analytical parameters in both Majoon and Pill dosage forms. This is the compound of four drugs *Ferula asafoetida*, *Commiphora myrrha*, *Piper nigrum* and *Ruta graveolens* [9]. These drugs were scientifically proven for different activities as antimalarial, anti-inflammatory, larvicidal and antimicrobial properties [10-13]. This formulation was used by most Unani Physicians in the form of *Majoon* (Majoon Form of *Dawa-e-Hilteet*: MFDH) while in *Ghana Mana* a renowned Unani book, it was used as in *Habb* (Pill) Form (Habb Form of *Dawa-e-Hilteet*: HFDH). This formulation had not been standardized to date and it was a need of the day to introduce the drug with evidence and data related to the standardization and safety profile. This study was based on the standardization of *Dawa-e-Hilteet* in two mentioned forms by using pharmacopoeial parameters and GC-MS study to provide data for chemical constituents, all the data may helpful for further studies. All procedures were carried out in the laboratories of Dept. of Pharmacology and Pharmacy of National Institute of Unani Medicine, Bangalore. Standardizing *Dawa-e-Hilteet* enhances its reliability, ensures safety and efficacy, and

supports its acceptance in evidence-based integrative medicine.

2. Materials and Methods

Reagent and instruments:- Pet. ether, Benzene, Acetone, Chloroform, Ethanol, Sodium Hydroxide, Hydrochloric acid, Sulphuric acid, Ammonia, Fehling A&B Solution, Nitric acid, Benedicts Reagent, Ethyl acetate, Molisch's reagent, Mayer's solution etc., All chemicals used in the experiments were of analytical grade. Mixer Grinder, Soxhlet apparatus and heating mantle, Hot air oven, Muffle furnace, pH meter (Eutech Instruments, pH tutor), Friability tester (Labindia Tablet Friability Tester, Model No. FT1020, Apison Labtech), Disintegration tester (Tab Machine, Model No. TD-20s Disintegration tester), Hardness tester (Monsanto Hardness tester cat no.SSI-62A, serial number-11012010), Wensler moisture analyzer, U.V visible Spectrophotometer, Precoated plates of silica gel 60 F254, U.V Chamber, Shimadzu QP2010Ultra Model and the Turbo mass ver 5.5 software, were used in the study according to requirement of test.

Plant Material: Ingredients of compound were of pharmacopoeial quality; *Piper nigrum* L. (Filfil Siyah) was collected from the NIUM Pharmacy Bangalore, *Ferula asafoetida* Regel (Hilteet) and *Commiphora myrrha* Nees (Mur Makki) were purchased from local market of Bangalore. *Ruta graveolens* L. (Suddab) was self-collected from herbal garden of NIUM Bangalore. All the drugs were identified and authenticated by Dr. S. Noorunnisa Begum, Senior Assistant Professor, Center for Repository of Medicinal Resources (CRMR) Trans Disciplinary University (TDU) Yelahanka, Bengaluru-560064, vide authentication certificate No-Drug Authentication /FRLHT Acc. No. were

5294, 5295, 5296 and 5297. The voucher specimen No-78/IA/ Res/ 2020 of the drugs were submitted in drug museum of National Institute of Unani Medicine for future referencing.

Preparation of Dawa-e-Hilteet: All four ingredients were separately powdered, *Mur Makki* & *Hilteet* were passed through 60 number sieve and *Filfil Siyah* & *Suddab* through 80 number sieve. All ingredients were in equal quantity. Majoon form of *Dawa-e-Hilteet* was form by mixing in *Qiwam* of two fold honey. *Habb* form of *Dawa-e-Hilteet* was prepared using the same ingredients and added the water as binder. (Fig: 1) Other binders were also used as *Luab-e-Isapghol*, *Samagh-e-Arabi* and *Kateera* to find out the suitable binder.

Methodology: *Dawa-e-Hilteet* was standardized on various parameters from physico-chemical, analytical and biological parameters. The parameters included in physico- chemical standardization were organoleptic characters, Ash values, Extractive values carried out by successive, non successive (hot & cold) ^[14-15] extraction methods in different solvents as Distilled water, chloroform, petroleum ether, acetone, ethanol, hydro alcohol, benzene by adopting standard procedures, pH,^[16] and specific parameters to *Habb* (Pills), like disintegration in distilled water & in gastric solution, hardness, uniformity and friability ^[17-18]. Preliminary phyto chemical test of Alkaloids (Dragendroff's test, Mayer's test, Wagner's test) ^[16], Carbohydrates (Fehling's test, Benedict's test, Molisch's test) ^[16], Cardiac glycosides (kellar killiani test),^[19] Anthraquinones Glycosides (Borntrager's test) ^[19], phytosterols/Terpenes (Salkowski's test, Hosse's test, Leibermann Burchard's test,) ^[19], Tannins (Ferric chloride test, Lead acetate) ^[16], Fixed oils (Filter paper test/ spot test)^[19] Flavonoids (Ammonia test, Alkaline reagent test, Lead acetate test) ^[19, 21], phenols (Ferric chloride test, Lead acetate test, Leibermann)^[16], Saponins (Foam test)^[21], amino acid and protein (Ninhydrin test,^[15] Biurett's test, ^[16]Millions test,^[16] Xanthoprotein test,^[19] Test for protein contains sulphur ^[20], Diterpenes (Copper acetate test)^[15], Quinine,^[21] Anthraquinones,^[21] Coumarins,^[21] Resin,^[16]; Tests for Inorganic Constituents^[22]; Quantitative estimation of Alkaloids,^[23] Flavonoids, ^[24] Tannins,^[24] Phenolics,^[25] Reducing^[26] and Non reducing sugar.^[27]GC- MS and TLC were used as analytical methods. GC-MS (Gas-Chromatography-Mass Spectroscopy) The GC-MS was performed in 50% Hydroalcoholic extract of DH by using Simadzu QP2010Ultra Model and the software used is Turbo mass ver 5.5. Silica column was packed with Elite - 5MS. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1 ml/min. Total GC running time was 60 minutes^[28]. TLC was performed by using precoated plates of silica gel 60 F₂₅₄ used as stationary phase and Benzene: Ethyl acetate (95:5), Pet ether: Diethyl ether (4:3) as mobile phase. Hydroalcoholic extract (50%) of DH was used as sample. Standard pharmacopoeial procedure was carried out. Rf value was calculated with the help of formula {Rf= Distance Travelled by Solute/ Distance Travelled by Solvent system}. ^[14, 16, 29] Heavy metal analysis and microbial load were also performed for purity standards. ^[30, 31].

3. Results and Observation

All tests were carried in triplicate and mean with SD was recorded were ever applicable- Results obtained were described in tabulated form in respective tables, Organoleptic description of *Dawa-e-Hilteet* (Table: 1), Moisture content, Ph of 1%, Ph of 10%, Total Ash, Acid Insoluble ash, and water soluble ash in MFDH are 7.46±0.02, 6.47±0.06, 5.82±0.02, 2.92±0.04, 0.87±0.05, 2.33±0.04 accordingly. Moisture content, pH of 1%, pH of 10%, Total Ash, Acid Insoluble ash, and water soluble ash in HFDH are 4.58±0.36, 5.38±0.20, 5.32±0.10, 5.87±0.05, 1.32±0.13, 4.81±0.07 accordingly. Extractive Values of MFDH (Table: 2), Specific Tests of HFDH friability, hardness and disintegration (Table: 3). Disintegration time of HFDH in Distilled water and Gastric solution (pH approx. 1.2) is 56.04 minutes and 48.50 minutes accordingly. Weight variation found in only two pills out of 20 pills having average weight of 538 mg. The phytochemicals estimated quantitatively as non reducing sugar 0.5289mg/ml at 490nm absorbance, reducing sugar 12mg/ml at 540nm absorbance, total phenolic 10.610 mg/ml at 765 nm absorbance, total alkaloids 8.8 mg CE at 470 nm absorbance, total flavonoids 54.21 µg/ml of QE/g at absorbance 510 and total tannins 18.095 µg/ml at absorbance 700nm. Heavy Metals result (Table: 4), Rf values in hydroalcoholic extract in different solvent system (Table: 5, Fig: 2), Microbial load of *Dawa-e-Hilteet* (HFDH) (Table: 6) and in GC-MS total 68 components were isolated, some of major components mentioned in table (Table: 7, Graph: 1).

Preliminary Phytochemicals- The preliminary phytochemical screening of 50% hydroalcoholic extract of DH was found positive for Alkaloids, Carbohydrate, Cardiac glycosides, Terpenes, Phenols, Fixed oils, Flavonoids, Tannins, Diterpenes, Saponins, Amino acids, Coumarins, Resins while Quinines, Starch and Anthraquinones were absent.

Inorganic phytochemical tests- Iron and Chloride were only found to be positive in inorganic phytochemical tests.

4. Discussion

Natural resources are considered safe from unwanted and unusual affects. Several Unani books deal with the drugs as in single and compound form. Atibba also insist to use the single drug rather than compound formulation. But as the need of compound formulation came due to; diseases were having complex nature, need of more than two drugs as well as to increase the activity, to normalize the drug action, for preservation and palatability they set certain rules and regulations. They provided the guidelines in *Qarabadeen*, *Bayaz* and *Kunnash* such as particle size of *Sufoof* for inhalation, *Itrifal* and *Jawarish* according to absorption. Today risk of adulteration has increased due to lack of knowledge to identify the drug in crude form, increased rate of adulteration for both money purposes, handling by layman and increased in demand. W.H.O and many nations established some pharmacopoeial guideline to evaluate the single and compound formulations. Main aim of these rules and regulations are to provide safe, pure and effective drugs.

There is some confusion regarding the safety of herbal medicines that all are completely free from side effects.^[32] For fulfillment all these requirements pharmacognostical study, phytochemistry and development of monographs are basic steps. All these steps are related to the standardization process of single drugs and compound formulations.

Standardization of the studying compound formulation DH was carried out in two forms as it used by *Atibba*; in *Majoon* form mostly and *Habb* form described in Ghana Mana. Standardization of the compound provides some guidelines about this particular compound according to their ingredients. It started from the identification of crude drug. The organoleptic characters of the compound such as taste, colour and odour were found, bitter pungent, aromatic and greenish brown respectively. The taste of Majoon form was pungent and producing burning sensation in chest (tested by the author and evaluated by four other individuals). Pill form was pungent and more bitter but easy to take in with water as it does not come in contact with esophagus for long duration.

The mean percentage of loss in weight on drying of DH was found to be $7.46 \pm 0.02\%$ in MFDH and $4.58 \pm 0.36\%$ in HFDH. Less moisture content in the drug will avoid the contamination. In MFDH moisture content was found less in comparison to other semi solid forms, may be due to the absence of water in the qiwam preparation, use of the honey in two folds of drug or due to the excessive amount of volatile components.^[33]

Acidic and basic nature of drug is responsible for biopharmaceutical properties, potency, and pharmacokinetics. Studies were shown that acidic ionization constant (pKa values) below 3 and bases values above 8 absorbed poorly. Gleeson's in his study mentioned that oral bioavailability higher in acids and it affects its solubility. Another study carried out by GlaxoSmithKline was reported that basic compounds distributed in all over the body.^[34] In the present study pH values in 1% and 10% solution of MFDH were found to be 6.47 ± 0.06 and 5.82 ± 0.02 respectively. The mean value of pH in 1% and 10% solution of HFDH were found 5.38 ± 0.20 and 5.32 ± 0.10 . Values of 1% and 10% solution of both forms were between the 3 and 8. It may affect its absorption. Another thing was its acidic nature; support its better oral bioavailability.

The mean percentage values of the total ash, acid insoluble ash and water-soluble ash of the MFDH were found to be $2.92 \pm 0.04\%$, $0.87 \pm 0.05\%$ and $2.33 \pm 0.04\%$ respectively. The mean percentage values of the total ash, acid insoluble ash and water-soluble ash of the HFDH were found to be $5.87 \pm 0.05\%$, $1.32 \pm 0.13\%$ and $4.81 \pm 0.07\%$, respectively. Less value of acid insoluble ash is providing a data that the silica or sand like substances, carbonates and phosphates were in less amount in both types of formulation of DH.^[35]

Weight variation in pills was found in only two pills out of batch of 20 pills. Weight variation of pill was used for the determination of ingredients present for therapeutic purpose. According to USP weight variation for the pill weighing 324mg is 5% of the average of 20 pills weight. Variation

should not be more than 2 pills. It affects the therapeutic activity.^[18]

Pills hardness value by mean was $3.5 \pm 0.28\text{kg}$ in water as binder. As it was described in Ghana Mana to form the pill with honey as binder, the pill bind with honey was too soft even breakable to hold in between the fingers. In place of honey; water, *Samagh-e-Arabi*, *Kateera* and *Luab Isapghol* were used as binder. Hardness in *Samagh-e-Arabi*, *Kateera* and *Luab Isapghol* was $2.66 \pm 0.33\text{Kg}$, $2 \pm 0.00\text{Kg}$ & $2.16 \pm 0.16\text{Kg}$, respectively. In all of these binders the hardness value of water was acceptable. Hardness is important quality of a pill as the harder pill takes time to disintegrate and soft pills cannot be handled easily. The hardness of 4kg is considered suitable.^[18]

Friability is also an important point to rule out how much of the pill losses during packaging. As the friability of pill having water as binder was obtained $0.26 \pm 0.02\%$ and indicating negligible loss during packaging as the limit of friability should not be more than 0.8%.^[18]

Disintegration of the pills was more than 30 minutes and reached up to 56.04 ± 2.09 minutes in water as binder. It was approximately same in all binders with a slight difference. Increase in disintegration time was might due to particle size of powdered drug or may due to *Mur Makki* which is an ingredient of pill. *Mur Makki* and *Muqil* is oleo gum resins belongs to the same family, the API notes that pills coated with *Gugglu* exhibit prolonged disintegration time.^[31] Increase in time of disintegration may be helpful for the purpose of treatment as the drug remain in blood for long period and act up to second dose have been taken. It provides a base for further study and exploration to make the pill by adding other excipients. These excipients may enhance disintegration to less than 53 minutes.

Extractive values of DH in different solvent system were higher in aqueous medium followed by ethanol. Maximum yield percentage in aqueous medium indicates the more polar substances.^[35] Water extract was higher may be due to the presence of abundant constituents such as tannins, saponins, starch and terpenoids. In the same manner good yield percentage in ethanol may due to flavonoids, Alkaloids, Terpenoids, Tannins and Polyphenols etc^[15].

According to previous data yield percentage of *Hilteet* and *Mur Makki* was higher in aqueous medium as 57.87%, 47.78% respectively. This suggests that the crude drugs were intact and free from adulteration.^[36, 37] Aqueous extractive values were $62.53 \pm 0.03\%$, $54.23 \pm 0.9\%$ and $29.54 \pm 0.07\%$ in hot, cold and successive methods respectively, followed by the extractive values in ethanolic medium. Yield values in other solvents were in lesser amount.

Alkaloids, Carbohydrate, Cardiac glycosides, Terpenes, Phenols, Fixed oils, Flavonoids, Tannins, Diterpenes, Saponins, Amino acids and Coumarins were present in preliminary phytochemical study of 50% hydroalcoholic extract of DH. Effect of DH may be due to the presence of these phytochemicals, as the alkaloids present in plants acts as chemotherapeutic agent and activity against microbes. Tannins present in plant material possess anti-inflammatory

antioxidant, antifungal, antibacterial, anti-parasitic, antiviral and cytotoxic activities. Coumarins present in plant material act as anti-inflammatory, anti-endemic and antimicrobial properties.^[38] In inorganic phytochemical test of ash showed presence of Iron and Chloride which indicates organic nature of ash.

Total phenolics, total alkaloids, reducing sugar, non-reducing sugar, flavonoids and tannins in hydroalcoholic extract were found 10.61µg/ml of GAE/g, 8.8mg CE, 12mg/ml, 0.5289mg/ml, 54.21µg/ml of QE and 18.09µg/ml, respectively.

T.L.C of Hydroalcoholic extract: in T.L.C of hydroalcoholic extract in solvent system Benzene: Ethyl acetate (95:5) & Pet ether: Diethyl ether (4:3) Rf value were found as 0.1, 0.16, 0.25, 0.43, 0.68 and 0.24, 0.32, 0.42 respectively. Spots were appeared in blue colour under the U.V chamber. It is useful for the purity of compound and its ingredient. As the reference TLC of the compound were not available, these Rf values may use as standard for the further references.

In the heavy metal analysis of compound DH estimated values of lead, mercury, cadmium and arsenic were <0.15ppm, <0.1ppm, BDL and <0.1ppm respectively. All these values were in within the limits set by W.H.O as for lead, mercury, cadmium and arsenic 10ppm, 1.0ppm, 0.3ppm and 10ppm respectively. It is a necessary part to safe use of drug. Presence of excessive amount of heavy metals in herbal drugs may responsible for encephalopathy, peripheral neuropathy, anemia, chronic nephritis, headache, convulsions, brain damage and central nervous system disorders. Result was supporting that the D.H is safe to use for therapeutic purposes^[39, 40].

According to W.H.O there are some limits for microbial load in herbal drugs. Total bacterial count 10⁵ CFU, total fungal count 10³ CFU, enterobacteriaceae 10³ CFU, Salmonella species and Staphylococcus aureus should be absent.^[40] DH fulfilled the safety criteria of compound and having non contaminated ingredients.

GC-MS of the compound formulation was providing several peaks, there were 68 components found. In all these components formic acid, acetic acid, D-galactose and 3-methoxy-10(15)-dihydrofuranodien-6-one were previously evaluated in other studies.^[41] Furo [2, 3-B] Quinoline, 4, 6, 7-Trimethoxy also known as skimmianine. The skimmianine was isolated from *Ruta graveolens* L and shown significant anti-inflammatory activity by decreasing the level of PGE₂, TNF-α and IL-6 & COX-2 activity.^[42] Another constituents 8-methoxypsoralen and 1-methyl-2-[6'-(3' ', 4' '-methylenedioxyphenyl) hexyl]-4-quinolone were found in *Ruta graveolens* were shown activity against fungi.^[43]

There are several points that need more exploration as use of binder or excipients to make the pill to disintegrate in less time. DH was used by most Atibba in the form Majoon and whole of the process of standardization supports that Majoon form is more suitable for the treatment purpose due to its compatible taste and easy dissolving nature to fulfill the criteria of bioavailability of drug for action.

5. Conclusion

The present study successfully standardized the Unani formulation *Dawa-e-Hilteet* in Majoon and Pill forms by evaluating its physicochemical, phytochemical, and safety parameters. GC-MS analysis confirmed the presence of 68 bioactive components. Both dosage forms met pharmacopoeial standards, with Majoon form showing better compliance in terms of taste and bioavailability. This standardization offers foundational data for future pharmacological and clinical research, promoting the rational use of traditional medicine.

Acknowledgement

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Conflict of interest

There is no conflict of interest.

Table 1: Organoleptic description of *Dawaa-e-Hilteet*

	Appearance	Colour	Smell	Taste
MFDH	Semi-solid	Greenish brown	Strong	Bitter and pungent
HFDH	Solid Rounded	Greenish Black	Strong	Bitter and pungent

Table 2: Extractive Values of MFDH

Solvents	Pet. Ether (%)	Chloroform (%)	Acetone (%)	Ethanol (%)	Benzene (%)	Aqueous (%)	Hydroalcoholic (%)
Methods of extraction							
Successive extractive values	0.17±0.03	0.08±0.008	1.44±0.18	16.83±0.05	0.24±0.01	29.54±0.07	—
Cold extractive values	0.032±0.01	0.18±0.07	0.89±0.04	10.96±0.09	0.10±0.006	54.23±0.9	—
Hot extractive values	0.25±0.04	0.42±0.08	1.34±0.03	23.60±1.62	0.65±0.01	62.53±0.03	32.24±0.19

Table 3: Specific Tests of HFDH

Tests	Friability	Hardness	Disintegration
Binder			
Water	0.26±0.02 %	3.5±0.28 kg	56.04±2.09min
Samagh-e-Arabi	0.03±0.01%	2.66±0.33 kg	52.70±1.34min
Kateera	0.11±0.02%	2±0.00 kg	52.48±2.57min
Luab Isapghol	0.05±0.00%	2.16±0.16 kg	56.78±1.69min

Table 4: Heavy Metals result

S. No	Heavy metals	Results	Protocol
1	Lead	<0.15ppm	ICP-OES
2	Cadmium	BDL	ICP-OES
3	Mercury	<0.1ppm	ICP-OES
4	Arsenic	<0.1ppm	ICP-OES

(BDL= below detection limit)

Table 5: Rf values in hydroalcoholic extract in different solvent system

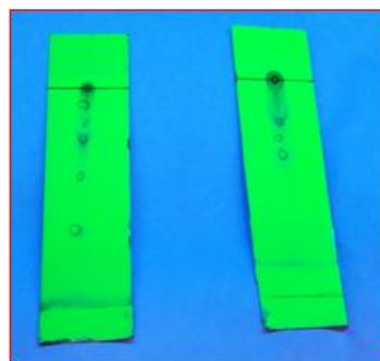
S. No	Solvent system	Solvent front	Spot distance in cm	Rf value	Colour of spots under UV Chamber
1	Pet ether: Diethyl ether	7	1.7, 2.3, 3	0.24, 0.32, 0.42	Blue
2	Benzene: Ethyl acetate	8	0.8, 1.3, 2, 3.5, 5.5	0.1, 0.16, 0.25, 0.43, 0.68	Blue

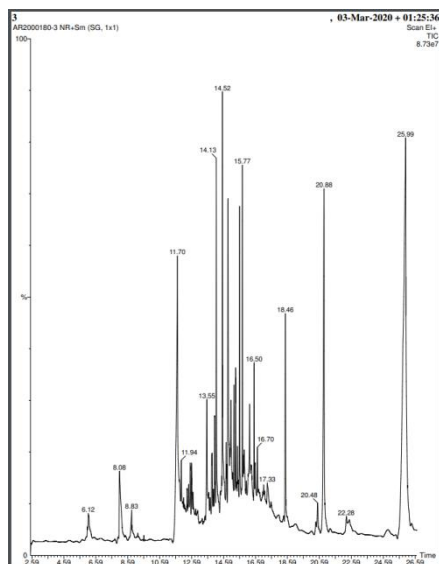
Table 6: Microbial load of *Dawaa-e-Hilteet* (HFDH)

S. No	Micro-organism	Result
Microbial contamination		
1.	Total Bacterial Count	<10 CfU/g
2.	Total Yeast and Mould count	<10Cfu/g
Test for Specific Pathogen		
1.	<i>Pseudomonas aeruginosa</i>	Absent
2.	<i>Staphylococcus aureus</i>	Absent
3.	<i>E.coli</i>	Absent
4.	<i>Salmonella</i> sps	Absent

Table 7: GC-MS components – major components (Total 68 components)

Retention time	Name of the Constituent	Molecular weight	Formula	Area	height	Area%
15.5	Beta. (3, 4-Methylene Dioxyphenyl)Propionic Acid	194	C ₁₀ H ₁₀ O ₄	362451	7751264	6.2
11.6	(S)-(+)-2-Amino-3-Methyl-1-Butanol	103	C ₅ H ₁₃ ON	760427	5179886	13.1
11.9	Isoamyl Nitrite	117	C ₅ H ₁₁ O ₂ N	67305	1436494	1.2
15.7	2H-Furo [2, 3-H]-1-Benzopyran-2-One, 5-Methoxy	216	C ₁₂ H ₈ O ₄	255096	5183324	4.4
13.5	Succinic Acid, 2-Methylpent-3-Yl Trans-Hex-3-En-1-Yl Ester	284	C ₁₆ H ₂₈ O ₄	152642	1713983	2.6
16.7	1(2H)-Chrysenone, 3,4,4A,5,6,11,12,12A-Octahydro-8-Methoxy-, (4AS-Cis)-	282	C ₁₉ H ₂₂ O ₂	35174	662945	0.6
16.5	Benzoic Acid,2-(2,4-Dinitrophenylthio)-, Methyl ester	334	C ₁₄ H ₁₀ O ₆ N ₂ S	44097	986729	0.8
16.2	3,6-Dimethoxy-4-Phenanthrol	254	C ₁₆ H ₁₄ O ₃	98075	1340245	1.7
14.1	Ficusin	186	C ₁₁ H ₆ O ₃	352952	7036638	6.1
18.4	Furo [2, 3-B] Quinoline, 4, 6, 7-Trimethoxy-	259	C ₁₄ H ₁₃ O ₄ N	221580	3844441	3.8
14.1	Isopsoralen	186	C ₁₁ H ₆ O ₃	352952	7036638	6.1
20.8	Chalepin	314	C ₁₉ H ₂₂ O ₄	246731	2684032	4.2
25.9	8-Amino-1H, 2H-Pyrazolo [4, 3-C] Quinolin-3-One	200	C ₁₀ H ₈ ON ₄	2101344	9387111	36.1
15.5	O-Anisic Acid, 2,6-Dimethylnon-1-EN-3-YN-5-YL Ester	300	C ₁₉ H ₂₄ O ₃	362451	7751264	6.2
14.5	5-Isopropyl-2-Methylphenyl 2-Methylbut-2-Enoate	232	C ₁₅ H ₂₀ O ₂	238055	4576835	4.1

**Figure 1:** Habb Form of *Dawaa-e-Hilteet***Figure 2:** TLC plates
Benzene: Ethyl acetate Pet ether: Diethyl ether



Graph 1: GC-MS of Hydroalcoholic extract

References

- [1] Ali HA, *Qarabadeen-e -Ahsani*, (CCRUM, New Delhi), 2006, 57.
- [2] Arzani MA, *Qarabadeen-e-Qadri* (Urdu Translation) (CCRUM, New Delhi), 2009, 641.
- [3] Jeelani G, *Kitab ul Murakkabat Al Maroof Mukhzin ul Murakkabat*, (Ejaz Publishing House, New Delhi), 1995, 114, 421, 332,333.
- [4] Kabiruddin HM, *Al Qarabadeen*, 2nd Edition, (CCRUM, New Delhi), 2006, 390,391.
- [5] Razi ZA, *Kitab Al Mansoori* (Urdu translation), (CCRUM, New Delhi), 1991, 417,415.
- [6] Sina I, *Al Qanoon Fil Tibb* (Urdu Translation), 5th Vol, Translated by Hakeem Ghulam Husnain Kantoori (Ejaz Publishing House, New Delhi), 2010, 1477.
- [7] Sharif HM, *Bayaz-e-Khas Al Maroof Ilaaj ul Amraz*, Translated by Allama Kabiruddin (Ejaz Publishing House, New Delhi), 2006, 237, 763.
- [8] Qamri MH, *Ghana Mana: Minhaaj-ul-Ilaaj* (Urdu Translation) (CCRUM, New Delhi), 2008, 571, 572, 584.
- [9] Jurjani AH, *Zakheera Khwarizm Shahi* (Urdu Translation), 10th Vol, by Hakeem Hadi Hussein Khan (Idara Kitab Ul Shifa, New Delhi), 2010, 11, 92.
- [10] Ghiwarei NB, Nesari TM and Gond NY, Clinical Validation of *Piper nigrum* and *Nyctanthes arbortristis* Formulation for Antimalarial Activity, *J. Res. Educ. Indian Med*, 2007, **13** (1), 33-38.
- [11] Guddeti V, Kumar JNS, Rani DN, Reddy PI and Likhitha KVL *et al*, Evaluation of Anti-Inflammatory Activity of *Ferula asafoetida* Linn Extract in Albino Rats, *Asian Journal of Research in Pharmaceutical Sciences and Biotechnology*, 2018, **6**(2), 29-34.
- [12] Chacko A, Christy PH and Kavya KS, Study on larvicidal activity of crude extracts of *Ruta graveolens* against *Aedes aegypti* and *Anopheles stephensi*, *International Journal of Mosquito Research*, 2015, **2**(4), 01-06.
- [13] Melnyk MV, Vodoslavskyi VM, Stasiv TG and Zarichanska OV, The study of microbiological activity of medicinal plants of flora of Prycarpathia, *International Journal of Green Pharmacy*, 2018, **12** (3), 158-162.
- [14] Anonymous, *Unani Pharmacopoeia of India*, Part I, Vol I, (CCRUM, New Delhi), 2007, 8, 38, 40, 85, 36, 37.
- [15] Pandey A and Tripathi S, Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug, *Journal of pharmacognosy and phytochemistry*, 2014, **2**(5), 115-119.
- [16] Anonymous, *Physicochemical standards of Unani formulations*, Part III, (CCRUM, New Delhi), 1991, 123, 124.
- [17] Ahmed S, Idris M and Adnan M, Standardization of Habb-e-Asgand: A Unani Pharmacopoeial Formulation, *Indo American Journal of Pharmaceutical Sciences*, 2015, **2**(4), 833-836.
- [18] Williams L, Wilkins. Remington, the Science and Practice of Pharmacy, Vol I, 21sted, (Wolters Kluwer Pvt Ltd, New Delhi), 2011, 917.
- [19] Rao KNV. Banji D, Sandhya S. Phytochemical and Pharmacognostical Studies on *Rumex vesicarius* Linn. *Asian Journal of Plant Science and Research*, 2011, **1** (1):102-115.
- [20] Sheikh R J, Patil MK. Qualitative Tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*; 2020, **8**(2): 603-608.
- [21] Renugadevi V and Meerabai RS, Phytochemical Analysis and Antimicrobial Activity of Leaf Extract of *Ruta graveolens* L, *Journal of International Academic Research for Multidisciplinary*, 2016, **4**(3), 57-65.
- [22] Khandelwal KR, *Practical Pharmacognosy*, 2nd ed. (Nirali Prakashan, Pune), 2013.
- [23] John B, Sulaiman CT, George S and Reddy VRK, Spectrophotometric Estimation of Total Alkaloids in Selected *Justica* Species, *International Journal of Pharmacy and Pharmaceutical Sciences*, 2014, **6**(5), 647-648.
- [24] Kavitha CCI and Indira G, Quantitative estimation of total phenolic, flavonoids, tannin and chlorophyll content of leaves of *Strobilanthes Kunthiana* (Neelakurinji), *Journal of Medicinal Plants Studies*, 2016, **4**(4), 282-286.
- [25] Kamtekar S, Keer V and Patil V, Estimation of Phenolic content, Flavonoid content, Antioxidant and Alpha amylase Inhibitory Activity of Marketed Polyherbal Formulation, *Journal of Applied Pharmaceutical Science*, 2014, **4** (09), 061-065.
- [26] Garriga M, Almaraz M and Marchiaro A, Determination of reducing sugars in extracts of *Undaria pinnatifida* (Harvey) algae by UV-visible spectrophotometry (DNS method), *Actas de Ingenieria*, 2017, **3**, 173-179.
- [27] Agrawal N, Minj DK and Rani K, Estimation of Total Carbohydrate Present In Dry Fruits, *IOSR Journal of Environmental Science, Toxicology and Food Technology*, 2015, **1**(6), 24-27.
- [28] Painuli S, Rai N and Kumar N, Gas Chromatography and Mass Spectrometry analysis of Methanolic Extract of Leaves of *Rhododendron arboreum*, *Asian Journal of Pharmaceutical and Clinical Research*, 2016, **9**(1), 101-104.
- [29] Jenkins GL, Knevel AM, Digangi FE. Quantitative Pharmaceutical Chemistry, 6thed. (CBS Publishers &

- Distributors Private Limited, New Delhi), 2008, 432, 433.
- [30] Okem A, Southway C, Ndhlala AR and Staden JV, Determination of Total and Bioavailable Heavy Metals and Trace Metals in South African Commercial herbal concoctions using ICP-OES, *South African Journal of Botany*, 2012, **82**, 75-82.
- [31] Ayurvedic Pharmacopoeia of India, Part II. Vol.III, (Ministry of AYUSH, New Delhi), 2001.
- [32] Sahoo N, Manchikanti P and Dey S, Herbal Drugs: Standards & Regulation, *Fitoterapia*, 2010, **81**, 462-471.
- [33] The Unani Pharmacopoeia of India, Part II. Vol III, (CCRUM, New Delhi), 2016, 67, 70.
- [34] Manallack DT, Prankerd RJ, Yuriev E, Oprea TI and Chalmers DK, The Significance of Acid/Base Properties in Drug Discovery, *Chem Soc Rev*, 2013, **42**(2), 485-496.
- [35] Nazish I, Kaskoos RA, Mir SR, Amin S and Ali M, Preliminary Pharmacognostical Standardisation of *Ruta graveolens* L. Aerial Parts, *Research Journal of Medicinal plant*, 2009, **3**(2), 41-44.
- [36] Standardization of Single of Unani Medicine, Part I, (CCRUM, New Delhi), 1987, 60-65, 104-108.
- [37] The Unani Pharmacopoeia of India, Part I. Vol II, (CCRUM, New Delhi), 2007, 77, 78.
- [38] Wink M, Modes of Actions of Herbal Medicines and Plant Secondary Metabolites, *Medicines*, 2015, **2**, 251-286.
- [39] Meena RP, Verma SC, Khan AS, Ansari SA and Arifi S, Standardization of Majoon-e-Azaraqi Formulation Used in Facial Paralysis, *World Journal of Pharmaceutical Research*, 2015, **4**(12), 1657-1671.
- [40] Behera B and Bhattacharya S, The importance of assessing heavy metals in medicinal herbs: a quantitative study, *TANG* 2016, **6** (1), e3.
- [41] Hanus LO, Rezanka T, Dembitsky VM and Moussaieff A, Myrrh-Commiphora Chemistry, *Biomed Papers*, 2005, **149**(1), 3-28.
- [42] Ratheesh M, Sindhu G and Helen A, Anti-inflammatory effect of Quinoline alkaloid skimmianine isolated from *Ruta graveolens* L, *Inflammation Research*, 2013, **62**, 367-376.
- [43] Olive A, Meepagala MK, Wedge ED, Harries D and Hale LA *et al*, Natural fungicides from *Ruta graveolens* L. leaves, including a new quinolone alkaloid, *J Agri Food Chem*, 2003, **51**(4), 890-896.