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VALIDATING THE CONCEPT OF ISLAH-E-ADVIA BY PHYSICOCHEMICAL PARAMETERS WITH REFERENCE TO HALELA (*TERMINALIA CHEBULA* RETZ)

L. Banu [*1], M. I. Khan [2], A. Wadud [2] and S. N. Basar [1]

Government Unani Medical College and Hospital [1], Magadi Road, Basaweshwarnagar Bengaluru -560079, Karnataka, India.
National Institute of Unani Medicine [2], Kottigepalya, Bengaluru -560091, Karnataka, India.

Keywords:

Islah-e-Advia, Mudabbar, Terminalia chebula, Unani medicine, Bioactive constituents, Safety evaluation

Correspondence to Author:
Dr. Layeeqa Banu

Assistant Professor,
Government Unani Medical College and Hospital, Magadi Road, Basaweshwarnagar Bengaluru - 560079, Karnataka, India.

E-mail: dr.layeeqa@gmail.com

ABSTRACT: Background: Halela (*Terminalia chebula* Retz) is one of the constituents in the well-known Unani compound medicine Itriphal, which has been used for thousands of years to treat a variety of ailments. In order to improve the therapeutic activity of the drug, the present study adapted the process of Tadbeer / Mudabbar (rectification/purification) which is based on the concept of Islah-e-Advia. This process thus helps in making the drug effective, safe, and specific, or minimizing undesirable characteristics by acting on the drug's constituents without compromising its medicinal value through various methods (rectification process). **Objectives:** To further grasp the significance of the notion of Islah-e-Advia, before and after Halela Mudabbar (rectification), a comparative analysis was conducted. **Methods:** Mudabbar was done by roasting dry Halela powder with Almond oil for 7-10 minutes on a low flame on standard cooking gas. Physico-chemical and quantitative HPLC examinations of gallic acid, ellagic acid, chebulagic acid, and chebulinic acid were performed with Halela before and after the Mudabbar procedure. **Results:** Quantitative HPLC analysis of gallic acid, ellagic acid, chebulagic acid, and chebulinic acid was increased to 0.6, 5.7, 3.8, and 0.6 mg/mL in post mudabbar halela (PMH) as compared to 0.5, 4.8, 3.5, and 0.4 mg/mL in pre halela (PH), suggesting a significant increase in tannin content in PMH. **Conclusion:** The results clearly show alterations in PMH, proving the notion of Islah-e-Advia and likely enhancing the drug's activity. Standards for pre and post mudabbar Halela were established for future reference. This study may provide an incentive for proper evaluation of the plant as a medicinal agent against human diseases and also to bridge the lacunae in the existing literature and future scope, which may offer immense opportunity for researchers engaged in validating the traditional claims and development of safe and effective botanical medicine.

INTRODUCTION: Pharmaceuticals used in Unani and other traditional medical systems are thought to be harmless, although this is only partially accurate because some drugs may have detrimental side effects due to their inherent nature. As a result, Unani medications go through a purification or rectification process to improve their quality, either by strengthening desired properties or minimising unwanted ones in order to make them more specific, effective and safe. This notion is known as Islah-e-Advia and the drug rectification strategies are known as Mudabbar [1].

In Ayurveda, it is named as *Shodhana* [2]. Only a few studies have shown that the Mudabbar process improves the drug's potency and efficacy. When compared to crude form, Mudabbar khare khasak (*Tribulus terrestris* L.) demonstrated an increase in diosgenin content [3]. Vatsanabha (*Aconitum ferox*) purified by cow's urine have converted

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pseudoaconitine and aconitine into less toxic substances like veratroyl pseudo aconine and benzoyl aconine [4]. Purified Kupeelu (*Strychnosnux-vomica* L.) using Kanji and *Adraka swarasa* as media reduced the strychnine and brucine content compared to raw Kupeelu [5]. Azaraqi (*Strychnosnux-vomica* L.) when purified according to the methods described in ancient Unani texts (*i.e.*, using a combination of water and cow milk, cow milk, clarified butter, and yellow clay with milk). Compared to raw azaraqi, the results demonstrated a decrease in strychnine quantity, regardless of the purifying procedure utilized [6].

The fruit of Halela (*Terminalia chebula* Retz), is considered as the "king of medicines"[7] and is held in high regard in Unani and other systems of herbal medicines. This herb is one of the ingredients of the famous Unani herbal compound preparation *Itriphal* [8], known as Triphala in Ayurvedic medicine, consisting of Halela (*Terminalia chebula* Retz), Balela (*Terminallia bellirica* (Gaertn.) Roxb., and Amla (*Phyllanthus emblica* L.) [9] in equal proportions are essential ingredients used to cure several ailments such as the diseases of the nervous system, hepatobiliary, gastrointestinal disorders, skin diseases, urinary tract infections, and eye disorders. This herb can be used to treat chronic diarrhoea and dysentery, particularly in children. The medicine aids in the control of fluids and bleeding [1]. *Terminalia chebula* is rich in tannin-containing about 30-32%, belonging to the pyrogallol type [10]. Gallic acid, ellagic acid, chebulagic acid, chebulinic acid, corilagin, punicalagin, chebulanin and neo-chebulanin are pharmacologically active compounds that have antioxidant, antibacterial, antiviral, cytoprotective, antiproliferative, anti-arthritic, hepatoprotective, cardioprotective, anti-diabetic, hypolipidimic, anti-ulcer, immuno-modulatory activity [11].

Mainly pericarp (post) of the fruit is used as medicine [12]. It is anti-inflammatory, astringent, laxative, carminative, antipyretic, antiemetic, and do have some antibiotic property [13, 14]. It is a blood purifier, antioxidant, and radioprotector [15]. It provides strength to the heart, liver, spleen, stomach, and intestines. HalelaZard is purgative for yellow bile (Safra') and phlegm (Balgham), while HalelaSiyā and Kablī induce purgation for phlegm (Balgham) and black bile (Sawda') [16, 17]. Halela helps remove the abnormally raised humors from the body and thus treats the ailments caused by humoral imbalance. It is anti-aging, memory booster, and anti-hemorrhagic. Halela, particularly Halela Kabuli is said to have diuretic properties and can be used in urinary symptoms and is considered to be the drug of choice for diseases due to cold humors [16]. Halela inhibits cancer cell growth and urease activity of helicobacter pylori [15].

The present study aims at identifying the overall medicinal value of Halela with special cognizance in the Unani system of medicine only after the Mudabbar (rectification) process, but however, there is no scientific data to know what changes take place after this process and any changes in the phytoconstituents of the drugs. Hence the present study was done by roasting the powder of Halela with Almond oil (*Prunus amygdalus* (Rosaceae) var. dulcis (sweet almonds) as described in the Unani classical literature [1]. And the study compares the drug phytoconstituents before and after the rectification process to understand the phytochemical changes by physicochemical and instrumental analysis. Thus the present study was an attempt to establish the evaluation of Halela as a medicinal agent against various human diseases such as tumors, intermittent fever, rheumatism, paralysis, memory loss, diabetes, neurological problems, hepatomegaly, and constipation [18].

MATERIALS AND METHODS:

2. Chemicals and Reagents: All of the chemicals, such as petroleum ether, benzene, chloroform, acetone, ethanol, sodium hydroxide, hydrochloric acid, sulphuric acid, ammonia, fehling A and B solution, nitric acid, benedicts reagent, ethyl acetate, molish's reagent, mayer's solution, etc. were of analytical grade, and they were purchased from Sisco research laboratories Pvt. Ltd. Mumbai and Central drug house Pvt. Ltd New Delhi.

2.1. Purchase, Identification, and Powdering of Test Drug: Halela and Almond Oil were procured from Hamdard herbal supplier, Bengaluru, India, and Halela was authenticated by Dr. Nurunnisa, the senior research associate. A voucher specimen (FRLHT No. 3838) has been deposited in the crude drug museum of the Pharmacognosy Department of NIUM, Bengaluru, India. Halela was crushed into small pieces with the help of Iron Mortar and

pestle, and subjected to the mixer to make into fine powder. The powder was stored in airtight glass jar, for further purposes.

2.2. Method of Mudabbar (Rectification) Process1: Mudabbar of Halela was done as described in khazayinulad *via*. 100 g of *Terminalia chebula* powder was taken in a pan and 100 ml of Almond Oil was added to it. It was kept stirring for 5-8 min in cooking gas on a low flame until the drug completely absorbed the oil.

Special care was taken to avoid the burning of the drug. It was then collected in an airtight container and used for further tests (**Fig 1-3.**

FIG. 1: HALELA (*TERMINALIA CHEBULA* RETZ) FRUIT

FIG. 2: POWDERED HALELA

FIG. 3: POST MUDABBAR HALELA

2.3. Successive Extraction [19]: To determine successive extractive values, extractions were carried out in Soxhlet apparatus using different solvents to increase the order of polarity successively, *viz.* Petroleum Ether, Chloroform, Acetone, Ethanol, Benzene, and Distilled water. 25

g of the powdered drug was subjected to extraction with each solvent successively till the solvent became colorless. The drug solvent ratio was taken as 1:10. The extracts so obtained were subjected to a rotary evaporator and subsequently concentrated under reduced pressure (vacuum at 40° C).

The extractive values were determined concerning the weight of the drug (w/w). The mean extractive value was calculated after repeating the process three times. The above procedures were carried out in both pre and post mudabbarHalela. All the filter-sterilized test samples were stored at -20°C in the freezer in an airtight sterile Container for further use.

2.4. Physicochemical and Phytochemical Studies [20]: Organoleptic properties (*i.e*.) Appearance, color, taste, and smell of powders were evaluated. Physicochemical tests were carried out in both the samples: total ash, acid, insoluble ash, and Water soluble ash, loss of weight on drying at 1050 C, and solubility in water.

Preliminary Phytochemical analysis was carried out on extracted samples like Alkaloids, Glycosides, Carbohydrates, Phenolic compounds, Tannins, Phytosterols, Fixed oils, Coumarins, Diterpenes, Flavonoids, Proteins and Amino acids.

3. High-performance Liquid Chromatography (HPLC) Analysis: It was carried out by Natural Remedies Pvt Ltd, Bengaluru, to estimate the content of gallic acid, ellagic acid, chebulinic acid, and chebulagic acid in PH and PMH.

Equipment: LC 2010CHT – From Shimadzu, quaternary pump, column oven, autosampler, and PDA detector.

Standard Solution A: 1.0 mg/mL of USP Chebulagic Acid RS in boiling water.

Standard Solution B: Dissolve 0.1 g of USP Terminalia chebula Fruit Dry Extract RS in 100 mL of boiling water, sonicate and pass through a membrane filter of 0.45-µm pore size.

Sample Solution: Transfer 0.1 g of Terminalia chebula Fruit Powder, accurately weighed, to a 100-mL volumetric flask. Add 50 mL of boiling water and sonicate for 10 min. Dilute with water to 100 mL, mix well and pass through a membrane filter of 0.45-µm pore size.

Chromatographic System Detector: UV 270 nm.

Column: 4.6-mm × 25-cm; 5-µm packing L1 (similar to Merck kGaAPurospher Star LP HPLC Column, RP-18).

Flow Rate: 1.5 mL/min Injection volume: 20 µL System suitability.

Samples: Standard solution A and Standard solution B. Suitability requirements.

Chromatogram Similarity: The chromatogram of Standard solution B is similar to the reference chromatogram provided with the lot of USP *Terminalia chebula* Fruit Dry Extract RS being used. Resolution: NLT 11.4 between chebulagic acid and chebulinic acid peaks, Standard solution B Tailing factor: NMT 1.5, Standard solution A.

Relative Standard Deviation: NMT 2.5%, Standard solution A.

Analysis:
Samples: Standard solution A, Standard solution B and Sample solution. Using the chromatograms of Standard solution A, Standard solution B, and the reference chromatogram provided with the lot of USP *Terminalia chebula* Fruit Dry Extract RS being used, identify the retention times of the peaks corresponding to chebulagic acid and chebulinic acid. The approximate relative retention times of chebulagic acid and chebulinic acid peaks are 1.0 and 1.15, respectively.

Separately calculate the percentages of chebulagic acid and chebulinic acid in the portion of *Terminalia chebula* Fruit Powder taken:

$$\text{Result} = (ru/rs) \times Cs \times (V/W) \times F \times 100$$

ru = peak area of the relevant analyte from the Sample solution rs = peak area of the relevant analyte from Standard solution A. Cs = concentration of the relevant analyte in Standard solution A (mg/mL) V = volume of the Sample solution (mL). W = weight of *Terminalia chebula* Fruit Powder taken to prepare the Sample solution (mg). F = conversion factor for the analytes (1 for chebulagic acid and 0.79 for chebulinic acid) Add

the chebulagic acid and chebulinic acid percentages.

4. Statistical Analysis: Results obtained were mentioned in mean ± SEM. The results were analyzed by one-way ANOVA post-test with Tukey's Kramer Multiple Comparisons test. $p<0.5$ was considered as significant.

RESULTS: Organoleptic properties of pre-Halela powder were fine powder, yellow in colour with a blackish tint. Agreeable odour, bitter and astringent in taste and smooth texture. Whereas post mudabbar Halela appearance was blackish oily powder, with agreeable odour, bitter in taste, with rough and oily texture **Table 1** loss on drying method (10.36 and 3.27), Total ash (3.72 and 3.88), Acid insoluble ash (0.46 and 0.56), Water soluble ash (1.18 and 2.73), solubility in water (50.28 and 46.90), and successive extractive values in Petroleum Ether (0.22 and 42.15), Benzene (0.21 and 0.092), chloroform (0.39 and 0.32), Acetone (57.21 and 33.68), Ethanol (17.10 and 7.23) and Water (15.60 and 10.23) were found respectively in pre and post Mudabbar Halela **Table 2** & **3.**

TABLE 1: ORGANOLEPTIC PROPERTIES OF PRE-HALELA AND POST-MUDABBAR HALELA

S. no.	Organoleptic properties	Pre Halela	Post-Mudabbar Halela
1	Appearance	Fine powder	Blackish and oily powder
2	Colour	Yellow with a blackish tint	Blackish
3	Smell	Unpleasant	Unpleasant and oily odour
4	Taste	Bitter, Astringent	Bitter
5	Texture	Smooth	Rough and Oily

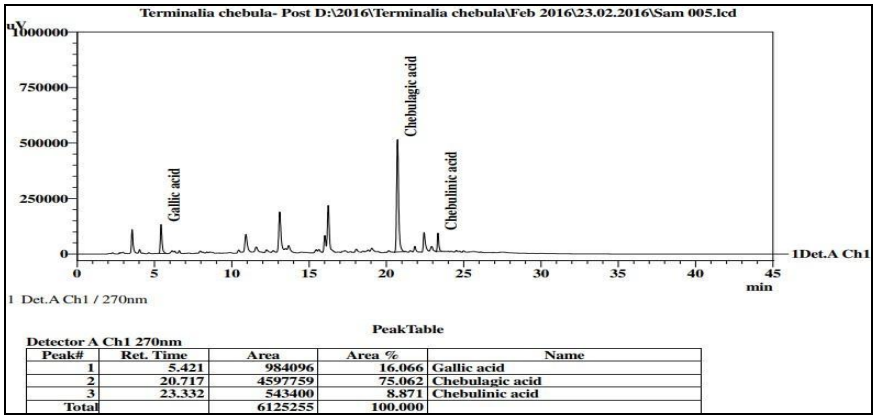

FIG. 4: PREMUDABBARHALELA SAMPLE

TABLE 2: PHYSICO-CHEMICAL PARAMETERS OF PRE AND POST-MUDABBARHALELA

Name of the ingredients	Results expressed as % w/w (n = 3)				
	LOD	TA	AIA	WSA	WSE
Pre Halela	10.36±0.04	3.72±0.33	0.46±0.05	1.18±0.56	50.28±0.25
Post Mudabbarhalela	3.27±0.06	3.88±0.44	0.56±0.11	2.73±0.39	46.90±0.44

AIA = acid insoluble ash; LOD = loss on drying at 105°C; TA = total ash; WSA = water soluble ash; WSE = water soluble extractive.

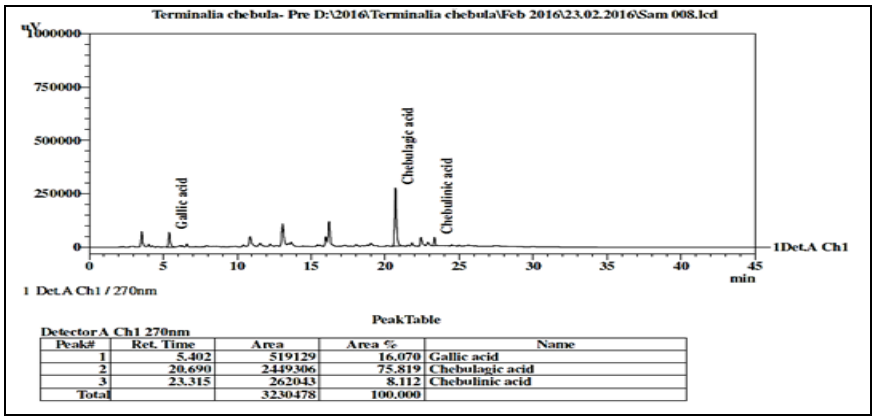

FIG. 5: POST-MUDABBARHALELA SAMPLE

TABLE 3: EXTRACTIVE VALUES OF PRE HALELA AND POST-MUDABBARHALELA

Pet. Ether		Benzene		Chloroform		Acetone		Ethanol		Water	
PH	**PMH**	**PH**	**PMH**	**PH**	**PMH**	**PH**	**PMH**	**PH**	**PMH**	**PH**	**PMH**
0.22± 0.01	42.15± 2.16	0.21± 0.04	0.09± 0.00	0.39± 0.06	0.32± 0.08	57.21± 1.95	33.68± 0.22	17.10± 3.68	7.23± 1.22	15.60± 0.28	10.23± 1.10

PH= Pre Halela; PMH=Post Mudabbarhalela.

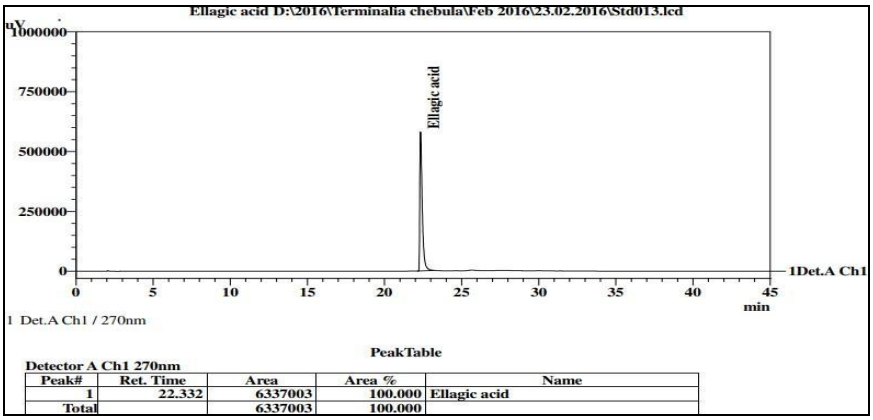

FIG. 6: STANDARD ELLAGIC ACID

Phytochemical Analysis: Phytochemical analysis revealed the presence of Alkaloids, Carbohydrates, Glycosides, Terpenes, Phenols, Flavonoids, Tannins, Anthraquinones, Saponins and proteins, and amino acids in acetone, ethanol and Water extract **Table 4.** Inorganic substances like Sulphate, Iron, Phosphate, Chloride and Nitrates were found in both the pre and post-MudabbarHalela extract.

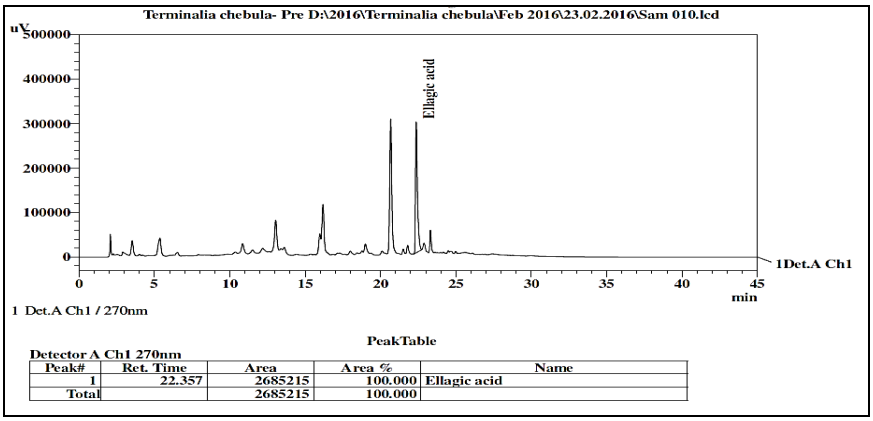

FIG. 7: ELLAGIC ACID: PRE HALELA SAMPLE

TABLE 4: PHYTOCHEMICAL ANALYSIS FOR ORGANIC CONSTITUENTS OF PRE AND POST MUDABBAR HALELA

Phytochemical tests for Organic constituents	Pet. Ether		Benzene		Chloroform		Acetone		Ethanol		Water	
	PH	**PMH**	**PH**	**PMH**	**PH**	**PMH**	**PH**	**PMH**	**PH**	**PMH**	**PH**	**PMH**
Alkaloids (Dragendorff's test)	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve
Carbohydrates (Benedicts test)	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve
Glycosides (Borntrager's test)	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve
Terpenes (leibermann Test)	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Phenols (Ferric Chloride Test)	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve
Flavonoids (Lead Acetate Test)	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve
Tannins (Ferric Chloride Test)	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve
Diterpenes (Copper Acetate Test)	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve
Quinones	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve
Anthraquinones	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve

Proteins andAmino Acids (Biurett's test)	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve
Coumarins	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve

PH= Pre Halela; PMH=Post MudabbarHalela

TABLE 5: HPLC ANALYSIS PRE AND POST-MUDABBARHALELA EXTRACT

S. no.	Assay (%w/w)	Pre-Halela	Post MudabbarHalela
1	Gallic acid	0.5	0.6
2	Chebulagic acid	4.8	5.7
3	Ellagic acid	3.5	3.8
4	Chebulinic acid	0.4	0.6

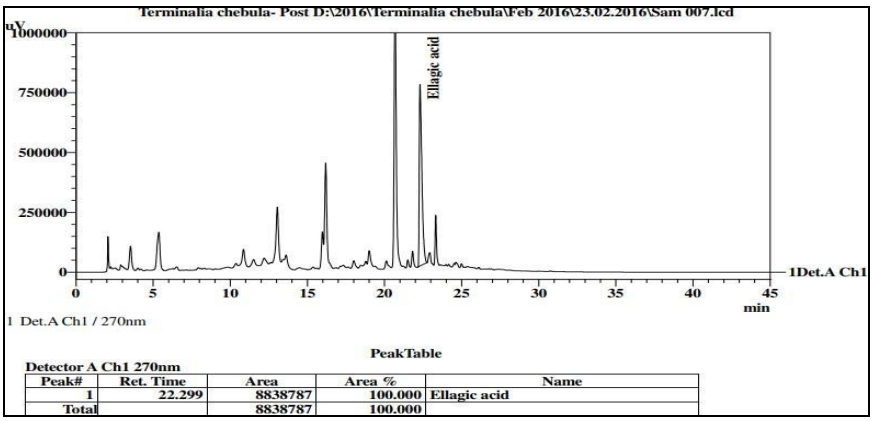

FIG. 8: ELLAGIC ACID POST-MUDABBARHALELA SAMPLE

DISCUSSION: In the Unani system of medicine, drugs of plant, animal, and mineral origins are used. To gain a desirable effect and minimize the unwanted effect of a drug, the Unani scholars have adopted certain strategies for a particular drug called Islah-e-Advia. The process is known as *Mudabbar* / *Tadbeer* (Rectification / Purification). Corrective procedures are done on the drugs before therapeutic use in order to make it more effective, safe and specific [1]. It is done to increase its rate of absorption, efficacy, and potency. *Mudabbar* is the processing method wherein alterations in physicochemical and structural characteristics of the drugs take place for newer actions, increase in efficacy and site-selective activity etc have been reported [2, 3].

Halela is an important drug of Unani medicine, being used for centuries to treat several diseases of the human body. It is an important part of many formulations and is also used in different dosage forms. Unani physicians used to rectify them through the process of Taqliya (Oil roasting) [8] to increase its efficacy and potency as it is rich in saponins, tannins, flavonoids, terpenoids, and phenolic acids. Among these compounds, ellagitannins and tannin-related compounds are considered the major constituents [13].

Tannins are compounds that are soluble in water and of molecular mass of 1000-5000 [21]. The application of tannins in medicine is based on their astringent, anti-bacterial, and fungicidal action. The antiseptic effect of tannins is due to their poly-phenol character. Recent researches indicate that tannins possess anti-herpes and cytotoxic effect in vitro on carcinoma cells of uterus and nasal pharynx and have shown to have anti-oxidation effect [22]. Total Ash values of pre-halela (PH) and post mudabbarhalela (PMH) were similar and within the range of standards [23]. The water-soluble ash of PMH was more as compared to PH. But it is within the standard range [23].

The moisture content of PH and PMH were 10.36 ± 0.048 and 3.275 ± 0.062, respectively. This difference may be due to roasting of Halela in almond oil which could have decreased the value of moisture content of PMH. PH and PMH extractive values in petroleum ether extract were 0.227 ± 0.015 and 42.15 ± 2.163, respectively. This difference in extractive value maybe because of the presence of almond oil in PMH. Petroleum ether is a good solvent for extracting oils, fatty acids, and coumarins [24]. So, the constituents of almond oil have also been extracted in petroleum ether solvent.

The extractive values in benzene and chloroform were similar in both the samples, whereas the extractive values of PMH in Acetone, Ethanol, and Water were less compared to PH. All these differences in the extractive value may be due to the roasting of Halela powder in Almond oil, as the presence of Almond oil could have altered the solubility in a different solvent system.

The presence or absence of phytochemical constituents in different samples through the phytochemical tests for organic and inorganic depends on the extraction procedures, the degree of processing, the moisture content, type of extraction, time of extraction, temperature, and nature of solvent, *etc.* [24]. HPLC studies of PMH have shown an increase in gallic acid, ellagic acid, chebulinic acid, and chebulagic acid compared to PH. This may be due to the presence of gallotannins, ellagitannins, and phlrotannins present in almond oil with ranges of 20 to 34 mg/100g [25].

Pharmacological studies have indicated that gallic acid, ellagic acid, chebulinic, and chebulagic acid have antioxidant, anti-carcinogenic, anti-microbial, antiangiogenic, and antiinflammatory agents. It is known to reduce cholesterol, free fatty acids, triglycerides, and phospholipids, besides treating critical diseases like depression, cancer *etc* [26, 27]. Studies done by Hassan El-sayed, have shown that oil roasting was effective in improving IVPD (in-vitro protein digestibility) [28]. The pharmacological studies of Almond oil have proved it to be a good hepato-protective and anti-oxidant agent. It reduces irritable bowel syndrome symptoms and helps in relieving spasm and constipation. It acts as a mild laxative [29].

These studies validate the Mudabbar process mentioned in classical Unani literature that the process increases the efficacy and safety of a drug. These processes are responsible for inducing changes in the Physico-chemical characteristic of the crude drugs and thereby rendering them the desirable degree of efficacy and safety without compromising on its useful pharmacological effect. The scientific reports available on such procedure and the likely changes produced in crude drugs also suggests that, the different procedures of *Tadbeer* as described in Unani literature are reasonable. The study aimed to determine the efficacy of Halela as a treatment for a variety of human ailments. It is said to have antioxidant, antidiabetic, antibacterial, anti-inflammatory, antimutagenic, antiproliferative, cardioprotective, antiarthritic, hepatoprotective, gastrointestinal motility, and wound healing activities [11].

CONCLUSION: Halela (*Terminalia chebula)* is one of the most versatile plants having a wide spectrum of pharmacological and medicinal activities. This versatile medicinal plant is the unique source of various types of compounds having diverse chemical structure. Though it has a number of pharmacological activities due to the presence of various types of bioactive compounds, very little work has been done on the plausible medicinal applications of this plant against the diseases. Hence the present study was an attempt to highlight the importance of Halela as a medicinal agent against various human diseases.

Thus, the present study's findings revealed that changes occur not only in the physical characteristics of the drug but also in chemical constituents within the lesser toxic range. Therefore, the study validates the concept of Islah-e-Advia postulated by Unani scholars. This study was performed to ensure that the drug is more efficacious and free from undesired effects without compromising its medicinal values. However, as the global scenario is now changing towards the use of nontoxic plant products having traditional medicinal use, a drug development program should be undertaken to develop modern drugs with the compounds isolated from *Terminalia chebula* effective against different types of diseases and also overcome the problem of drug resistance after extensive investigation of its bioactivity, mechanism of action, Pharmacotherapeutics, toxicity and after proper standardization and clinical trials.

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