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New Advanced Analytical Methodologies Developed for Quality Control of Herbal Extracts of Foeniculum Vulgare Mill Formulations

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ABSTRACT

Today Unani system of treatment practised, taught and researched under its local names in over 20 countries including Afghanistan, China, Canada, Denmark, Germany, Finland, Netherlands, Norway, Poland, Korea, Japan, Saudi Arabia, Sweden, Switzerland, Turkey, UK and USA. Unani system of medicine is unmatched in treating chronic diseases like arthritis, asthma, mental, cardiac and digestive disorders, urinary infections, and sexual diseases. Unani medicine established that disease was a natural process and that symptoms were the reactions of the body to the disease. Unani medicine plays a vital role when the individual experiences the humoral imbalance. Plan of study there is a need to establish internationally recognized methodology for quality standardization of traditional herbal medicines like multiple marker based quantification, determination of total metabolite content or application of hyphenated techniques such as LC-PDA, LC-MS or GC-MS which will give better understanding of bioactive multi component herbal formulations PLAN OF WORK Selection of formulations, Physico-chemical evaluation of formulations, Determination of contaminants, Chemical contaminants (Heavy metal, Pesticides residues), Fungal contaminants (Aflatoxins), Development of HPTLC fingerprinting, Development of fingerprint profile by GC-MS, Development of analytical methods for the quantification of marker Validation of the developed method, MATERIALS USED FOR THE STUDY Since the work carried out in present investigation was the part of an AYUSH/Govt. of India sponsored project for the development of analytical standards of some compound Unani formulations to be included in Unani Pharmacopeia/Formulary. Unani formulations were selected for analysis for General physico-chemical evaluations like organoleptic characteristics, loss on drying at 105° C, moisture content by Karl Fischer method, total ash, acid insoluble ash, water soluble ash, pH of 1 % and 10 % suspensions, petroleum ether extractive value, chloroform extractive value, acetone extractive value, methanol extractive value , total phenolic content by UV, content of sugar by Anthrone reagent method, aflatoxins (HPLC) and pesticide (GC-MS) showed absence of contaminants/ or below the acceptable limit in all batches of Itrifal-e-Badiyan, HPTLC fingerprinting of methanol, chloroform and petroleum ether extracts of Itrifal- e-Badiyan showed the presence of five, nine and nine peaks in chromatograms after visualizing with anisaldehyde sulphuric acid. A simple rapid and economic simultaneous HPLC method was developed and validated for the quantification of gallic acid, ellagic acid and ascorbic acid, in in Itrifal-e-Badiyan formulation.

Keywords: HPLC, Badiyan, Unani, physicochemical evaluation, HPTLC fingerprints

INTRODUCTION

The Unani System of Medicine, one of the oldest systems of medicine, had its origin in Greece. The great Greek Philosopher and Physician, Hippocrates (460-377 B.C) is the founder of Unani medicine later Galen and Avicenna enriched the system. Unani system of medicine was introduced in India by Arabs in 13th century. Due to its efficacy and scientific base, it was accepted by masses and the system took firm roots in India (Anonymous, 2017; Anonymous, 2016).

Today Unani system of treatment practised, taught and researched under its local names in over 20 countries including Afghanistan, China, Canada, Denmark, Germany, Finland, Netherlands, Norway, Poland, Korea, Japan, Saudi Arabia, Sweden, Switzerland, Turkey, UK and USA. Unani system of medicine is unmatched in treating chronic diseases like arthritis, asthma, mental, cardiac and digestive disorders, urinary infections, and sexual diseases. Unani medicine established that disease was a natural process and that symptoms were the reactions of the body to the disease. Unani medicine plays a vital role when the individual experiences the humoral imbalance. The correct diet and digestion can bring back the humoral imbalance. Diet therapy aims at treating certain ailments by administration of specific diets.

The Unani physician believes that the healthy state of the human body is maintained by a power known as "Tabiyat" or "Quwwat-e-Mudabbira" (medicatrix naturae), gifted to it from its creator. The concept of "Tabiyat" is much vaster than the concept of immunity system of body. It controls, regulates and restores the physiological mechanisms of the body and helps in potentiating the immunity of the body and its resistance against various ailments. Suppression of this gifted power leads to disease. Therefore, the duty of the physician is to use such methods/treatments that encourage the body's own innate healing response (Tabiyat). This can be achieved by stimulating the "Hararat-e-Ghariziya" (Vital force of body), which is decreased in a diseased person making him vulnerable to environmental and pathological challenges.

Regulatory norms for herbal drugs

The safety problems emerging with herbal medicinal products are due to a largely unregulated growing market where there is a lack of effective quality control. Lack of strict guidelines on the assessment of safety and efficacy, quality control, safety monitoring and knowledge on traditional medicine/complementary and alternative medicine are the main aspects which are found in different regulatory systems. Under some regulatory systems plant may be defined as a food, a functional food, a dietary supplement or a herbal medicine. Some of the parameters that help in understanding the development of herbal drug regulation in a given nation are general policy structure, drug registration system, development of pharmacopoeia, national monographs, inclusion in essential medicine list and drug type (OTC or prescription).

OBJECTIVE & PLAN OF STUDY

Herbal medicines have a strong potential in the primary health care**.** Most Ayurvedic and Unani traditional products are marketed as dietary supplements worldwide. Although herbal medicinal products have been perceived by the public as relatively low risk, there has been more recognition of the potential risks associated with this type of product as the use of herbal medicines increases. Potential harm can occur via inherent toxicity of herbs, as well as from contamination, adulteration, plant misidentification, and interactions with other herbal products or pharmaceutical drugs. Quality control is crucial to ensure the safety and correct handling of herbal medicines. There have been numerous reports on the toxicity, the misidentification and substitution of plant species. Herbal medicines have been reported to contain heavy metals and synthetic prescriptions or non-prescription drugs. The prevalent use of herbal medicines due to their easy availability has raised concerns over their quality, efficacy and safety. Many herbal products are sold even without prescriptions and consist of a decoction of several herbal materials defined in a formula.

PLAN OF WORK

- Selection of formulations.
- Physico-chemical evaluation of formulations.
  - Description
  - Loss on drying
  - Moisture content determination by Karl-Fischer method
  - Total ash
  - Acid insoluble ash
  - Water soluble ash
  - pH of 1% solution
  - pH of 10% solution
  - Extractive value determination by Successive Extraction Method
  - Alcohol soluble matter
  - Water soluble matter
  - Total phenolic contents by UV spectrophotometer
  - Determination of sugar content by Anthrone Method
  - Dimensional variation
  - Disintegration test
- Determination of contaminants
  - Chemical contaminants (Heavy metal, Pesticides residues)
  - Fungal contaminants (Aflatoxins)
- Development of HPTLC fingerprinting.
- Development of fingerprint profile by GC-MS
- Development of analytical methods for the quantification of markerconstituents (HPLC/HPTLC/UPLC-MS/GC-MS).
- Validation of the developed method.

MATERIALS USED FOR THE STUDY

Since the work carried out in present investigation was the part of an AYUSH/Govt. of India sponsored project for the development of analytical standards of some compound Unani

formulations to be included in Unani Pharmacopeia/Formulary. All the components used were identified by a qualified Hakims and Botanist, which were further authenticated by Pharmacognosist. The voucher specimens of all the raw materials used and formulations have been procured in Bioactive Natural Product Laboratory for further use.

Formulations chosen for the study

Itrifal-e-Badiyan

Itrifal Badiyan composed of eight ingredients; *Terminalia chebula, Terminalia belerica, Emblica officinalis, Vitis vinifera, Rosa damascene, Zataria multiflora, Foeniculum vulgare* and *Prunus amygdalus,* which are official in National Formulary of Unani Medicines (NFUM) published by Govt. of India (NFUM) and mainly prescribed in Unani System of Medicine as brain tonic.

Table 1: Ingredients of Itrifal-e-Badiyan formulation

S.No	Unani name	Botanical Name	% w/w
1.	Post-e-Halela Zard	*Terminalia chebula*	7
2.	Post-e-Halela Kabuli	*Terminalia chebula*	7
3.	Post-e-Balela	*Terminalia bellarica*	7
4.	Aamala	*Emblica officinalis*	7
5.	Kishneez	*Vitis vinifera*	7
6.	Gul-e-Surkh	*Rosa damascene*	7
7.	Satar	*Zataria multiflora*	7
8.	Badiyan	*Foeniculum vulgare*	50

EXPERIMENTAL

ITRIFAL-E-BADIYAN

Physico-chemical standardization of Itrifal-e-Badiyan - Physico-chemical standardization of Itrifal-e-Badiyan was carried out for different parameters like organoleptic characteristics, loss on drying, moisture content by Karl Fischer method, total ash, acid insoluble ash, water soluble ash, pH of 1 and 10 % suspensions, petroleum ether, chloroform, acetone and methanol extractive values, total phenolic content by UV, content of sugar by Anthrone reagent method, water soluble matter and alcohol soluble matter as per the procedures mentioned in general experimental section Preparation of blank solution. A blank solution was prepared by adding three mL each of distilled water, sodium carbonate solution, and FC reagent in test tube.

Procedure

Three mL of each standard and sample solutions were taken in a 10 mL test tube and to this added 3.0 mL of FC reagent and 3 mL of sodium carbonate solution. Kept the solution in dark for 30 minutes for colour development. Absorbance was taken at 765 nm against blank solution and plotted the standard calibration curve and calculated the amount of phenolics in the samples.

Determination of total carbohydrate by anthrone method

Carbohydrates are first hydrolyzed into simple sugars using dilute hydrochloric acid. In hot acidic medium, glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green coloured product with an absorption maximum at 630 nm.

MATERIALS

- 2.5 N HCl
- Anthrone reagent: Dissolved 200 mg anthrone in 100 mL of ice-cold 95%H_2SO_4. Prepared fresh before use.
- Standard glucose: Dissolved 100 mg in 100 mL water.

Working standard: 10 mL of stock diluted to 100 mL with distilled water.

Procedure

Weighed 100 mg of the sample into a boiling tube. Hydrolyzed by keeping it in a boiling water bath for three hours with 5.0 mL of 2.5 N HCl and cooled to room temperature. Neutralised it with solid sodium carbonate until the effervescence ceases. Made up the volume to 100 mL and centrifuged. Collected the supernatant and took 0.5 and 1.0 mL aliquots for analysis. Prepared the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1.0 mL of the working standard. '0' serves as blank. Made up the volume to
1.0 mL in all the tubes including the sample tubes by adding distilled water. Then added 4.0 mL of anthrone reagent. Heated for eight minutes in a boiling water bath. Cooled rapidly and read the green to dark green colour at 630 nm. Draw a standard graph by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis. From the graph calculated the amount of carbohydrate present in the sample tube.

Dimensional variation

Length, breadth and thickness of the tablets (n=20) were measured with a calibrated vernier caliper.

Disintegration test

This test determines whether the tablets disintegrate within a prescribed time when placed in a liquid medium under the prescribed experimental conditions. For the purpose of this test, disintegration does not imply complete solution of the dosage unit or even of its active constituent. Disintegration is defined

as that state in which no residue of the unit under test remains on the screen of the apparatus or, if a residue remains, it consists of fragments of disintegrated parts of tablets component.

METHOD

The disintegration test for the tablets has been performed as per the method prescribed in Indian Pharmacopoeia. The apparatus used for the experiment was as per the requirement of Indian Pharmacopoeia. The apparatus consists of a beaker-rack assembly, a liter beaker, a thermostatic arrangement for heating the fluid and a mechanical device for rising and lowering the basket in the immersion fluid at constant frequency rate. The assembly was suspended in the liquid medium in a suitable vessel, preferably a one litre beaker. The volume of the liquid was such that the wire mesh at its highest point was at least 25 mm below the surface of the liquid, and at its lower point was at least 25 mm above the bottom of the beaker. At no time should the top of the basket-rack assembly become submerged. There was a thermostatic arrangement for heating the liquid and maintaining the temperature at 37°± 2°C. Introduced one tablet into each tube and, if directed in the appropriate general monograph, added a disc to each tube. Suspended the assembly in the beaker containing the specified liquid and operate the apparatus for the specified time.

Determination of alcohol soluble matter

Macerated 5.0 g of the air dried drug, coarsely powdered, with 100 mL of alcohol of the specified strength in a closed flask for twenty-four hours, shakes frequently during six hours and allowing standing for eighteen hours. Filtered rapidly, taking precautions against loss of solvent, evaporated 25 mL of the filtrate to dryness in a tared flat bottomed shallow dish, and dried at 105° C, to constant weight and weighed.
Calculated the percentage of alcohol-soluble extractive with reference to the air-drieddrug.

Determination of water soluble matter

Proceed as directed for the determination of alcohol-soluble extractive, using waterinstead of ethanol.

Analysis of contaminants

Determination of heavy metals by atomic absorption spectroscopy

Analysis done by atomic absorption spectrophotometer for the determination of contamination of medicinal plant materials either accidently or intentionally with heavy metals such as arsenic, lead, cadmium, copper etc. can be attributed to many causes including environmental pollution and traces of pesticides. These may prove to be very dangerous for human health, even present in traces amounts.

Preparation of samples for the analysis

Three replicates of each sample were taken for the study. Samples (5-10 g) were taken in a silica crucible. Put it on the hot plate at 100°C for two hours. This process is called charring. After charring put it into muffle furnace at 600°C for four hours to make it complete ash so that no traces of carbonaceous substance was present. Taken it out from the furnace and cooled in a decicator. Then added five mL of concentrated nitric acid into it and put it in hot plate in fuming hood for slow heating upto dryness. Now put it again in the muffle. The pressure was kept at 17 L/min and 2.0 L/min for air and acetylene furnace for one hour. Then taken it out of the furnace and again cooled it into decicator. Then added 5 % of nitric acid into it. Boiled the mixture, cooled and then filtered with the help of whatmann's filter paper and made the volume upto 10 mL in volumetric flask. The prepared samples analyzed by atomic absorption spectroscopy.

Quantification of Aflatoxins (B1, B2, G1, G2) by HPLC-PDA

Aflatoxins are highly toxic secondary metabolites. They are produced by many ubiquitous fungi, mainly, *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins B1, B2, G1 and G2 occur the most frequently and can be found in a wide range of food- and feedstuffs, especially cereals (wheat, barley, sorghum and so on), dried fruits (pistachios and nuts) and spices.

Extraction method for aflatoxins

Fifteen gram of herbal drug was taken in 250 mL beaker, containing 100 mL methnol and 0.1 N HCl 25 mL, mixture was shaked for one hour using magnetic stirrer and filtered through Buchner funnel under vaccum in a two litre conical flask .Then poured filtrate into one litre separating funnel-1(SF-1) and added 100 mL of saturated sodium chloride solution (10% approx.) and 100 mL of hexane and shaked vigorously for five to ten minutes and allowed settling down the layers. The lower layer (aqueous layer) was transferred into another one litre separating funnel-2 (SF-2), whereas upper layer of hexane was discarded. Hundred mL of sodium chloride solution and 150 mL of dichloromethane (DCM) was added in separating funnel -2 (SF-2) and shaked vigorously for five to ten minute. Allowed to settle down the layers and collected DCM layer (lower layer) in another separating funnel-3(SF3) containing saturated sodium chloride 50 mL. Repeated the same process three times (with 100- 150 mL of sodium chloride and DCM) in SF2 and each time collected the DCM layer in SF3. Gently shaked SF3 and allowed to settle down DCM layer (lower). Passed the DCM layer through funnel containing muffled sodium sulphate (one drop/5sec.) filled with glass wool and collected lower layer of DCM in 250 mL separating flask and evaporated using rota vapour (upto 5-10 mL) and used for cleanup process.

Chromatographic conditions

The HPLC-PDA method for the determination of Alfa toxins was carried out on a Waters Alliance e2695 separating module (Waters Co., MA, USA) using photo diode array detector (waters 2998) with auto sampler and column oven. The instrument was controlled by use of Empower software installed with equipment for data collection and acquisition.

Compounds were separated on a C18 reverse phase column (25 x 4.6 mm, particle size 5 µm, Merck, Germany) maintained at room temperature. The mobile phase consisted of acetonitrile and water in the ratio of 1:1 v/v. The flow rate was 1.0 mL/min; the column was maintained at room temperature. The analysis was performed at the wavelength 360 nm.

Determination of pesticides by GC-MS

Sample preparation by QuEChERS method

Ten g of slurry previously homogenized were weighed in a 50 mL centrifuge tube. Ten milliliters of acetonitrile, containing 1% (v/v) of acetic acid, were then added to the sample, and the mixture was hand-shaken for one min. afterwards, three g of
magnesium sulphate was added and the tube was hand-shaken immediately for 20 s. Later, 1.7 g of sodium acetate and one g of sodium citrate were added and the tube was hand-shaken for another one min to provide well-defined phase separation after eight min of centrifugation at 4000 rpm. During the clean-up step, four mL aliquot of the upper layer was transferred to a centrifuge tube (15 mL) containing 0.6 g $MgSO_4$ and 0.5 g C18. The tube was hand-shaken for one min and centrifuged at 4000 rpm for eight min. An aliquot of the supernatant was transferred into a auto sampler vial to its injection into the GC–MS system.

GC-MS chromatographic conditions

GC-MS system (Agilent 7890 A coupled with 75C EI/CI mass detector, USA) attached with CTC PAL. The analysis was conducted on a 30.0 m x 250 µm HP-5MS- coated fused silica capillary column with 0.25 mm film thickness. Analysis of pesticides as done with an auto-sampler CTC Combi-PAL (Zwinger, Sweden) coupled to a GC-MS system (Agilent 7890 A coupled with 75C EI/CI mass detector, USA), equipped with a split/split less injector in the split less mode and at 280°C during the chromatographic run. The analysis was conducted on a 30.0 m x 250 µm HP-5MS-coated fused silica capillary column with 0.25 mm film thickness was employed in the separation of pesticides, using helium 99.99% as carrier gas at a 1.0 mL min^{-1} flow rate. The oven temperature was as followed: 60°C (1.0 min); then 170°C at 25°C min^{-1}; then 290°C at 6°C min^{-1} and hold at 290°C for 1.0 min. The mass detector conditions were: transfer line temperature 250°C; ion source temperature 230°C; ionization mode – electron impact at 70eV. The optimization of the retention times and chromatographic resolution were done in the SCAN mode and with a one µg mL^{-1} standard. In order to quantify the pesticides in water, SIM (Selected ion monitoring) mode was then chosen and three specific ions were selected for each analysis.

Determination of contaminants in Itrifal-e-Badiyan

The Itrifal-e-Badiyan formulation has been analyzed for the content of heavy metals,aflatoxins and pesticide as per the procedure mentioned in study.

HPTLC finger printing of Itrifal-e-Badiyan

High Performance Thin Layer Chromatography was performed to develop fingerprint profiles of Itrifal-e-Badiyan formulation. Methanol, petroleum ether and chloroform extracts were used for the fingerprint development. The methanol (160 mg/mL), chloroform (160 mg/mL) and petroleum ether (200 mg/mL) extracts were prepared by sonicating 0.8, 0.8 and 1.0 g of Itrifal-e-Badiyan in 20 mL of respective solvent for 30 min, followed by centrifugation to get the supernatant, which was concentrated under the flow of nitrogen and the final volume was adjusted to five mL using respective solvents.

HPTLC instrumentation and sample application

The HPTLC fingerprints of the different extracts of all drugs were established by developing the solvent systems for their separation by thin layer chromatography. The solvent system in which maximum and well resolved spots were found, selected for HPTLC. The samples were applied in triplicate (8.0 µL), each the width of the band was kept to 5.0 mm and distance between tracks was 13 mm on precoated silica gel 60 F_{254} plates (E. Merck, 0.20 mm thickness) using Linomat V (HPTLC sample applicator). After sample application, the plates were developed up to 80 mm in
development chamber saturated with the respective solvent system. The chromatograms were scanned at 254 and 366 nm wavelength followed by spectral analysis. Reprostar was used for taking photographs of the HPTLC plates. Plates were also scanned at visual range after spraying with visualizing reagent.

RESULTS AND DISCUSSION

Physico-chemical standardization of Itrifal-e-Badiyan

Physico-chemical evaluations like organoleptic characteristics, loss on drying, ash content and extractive value etc. were carried and the results are tabulated with standard deviation in Table 28. All the findings are based on the analysis of three batches.

Table 02: Physico-chemical parameters of the Itrifal-e-Badiyan

S. No.	Parameters	Observations (n=9)	Limits (Lower-Upper)
1.	Colour of the formulation	Dark brown	
2.	Odour	Characteristic	
3.	Taste	Sweet	
4.	Consistency	Semi solid	
5.	Loss on drying at 105° C (%w/w)	19.4 ± 0.4	18.5-20.0
6.	Moisture content by Karl Fischer	19.2 ± 0.65%	18.8-20.0

	method (%w/w)		
7.	Total ash (%w/w)	1.03 ± 0.05	0.90-1.2
8.	Acid insoluble ash (%w/w)	0.36 ± 0.08	0.30-0.4
9.	Water soluble ash (%w/w)	0.32 ± 0.03	0.28-0.35
10.	pH of 1 % suspension	4.71 ± 0.06	4-5
11.	pH of 10 % suspension	4.18 ± 0.07	4-5
12.	Petroleum ether extractive value (%w/w)	0.44 ± 0.01	0.35-0.45
13.	Chloroform extractive value (%w/w)	0.42 ± 0.01	0.40-0.47
14.	Acetone extractive value (%w/w)	0.27 ± 0.009	0.20-0.3
15.	Methanol extractive value (%)	25.95 ± 0.40	24-27
16.	Total phenolic content by UV (%w/w)	2.66 ± 0.05	2-3
17.	Content of sugar by Anthrone reagent method (%w/w)	60.43 ± 2.11	60-65
18.	Water soluble matter (%w/w)	51.54±1.3	50-53
19.	Alcohol soluble matter (%w/w)	30.55±1.1	28-32

HPTLC finger printing for Itrifal-e-Badiyan

The HPTLC fingerprint analysis of methanol, chloroform and petroleum ether extracts of Itrifal-e-Badiyan was carried out.

Analysis of contaminants in Itrifal-e-Badiyan

Heavy metal analysis of Itrifal-e-Badiyan

Heavy metal analysis of arsenic, lead, mercury and cadmium was performed for all the test samples by using the atomic absorption spectrophometer as per the procedure mentioned in the experiment section 5.2.1. Results of the heavy metal analysis performed for all batches of Itrifal-e-Badiyan found to be satisfactory with the given limits (Table 03).

Table 3: Results of heavy metal analysis in Itrifal-e-Badiyan

Batch	Arsenic (ppm)	Mercury (ppm)	Lead (ppm)	Cadmium (ppm)
Limits	NMT 3	NMT 1	NMT 10	NMT0.3
1	<0.1	<0.1	<1	<0.1
2	<0.1	<0.1	<1	<0.1
3	<0.1	<0.1	<1	<0.1

Quantification of Aflatoxins by HPLC-PDA of Itrifal-e-Badiyan

By using the developed and validated extraction and HPLC-PDA method, as discussed in experiment section 5.2.2., the content of aflatoxins (B1, B2, G1 and G2) of three different batches of Itrifal-e-Badiyan were investigated. All the samples were successfully analyzed for the content of aflatoxin and found that the samples were free from aflatoxins and safe for the use.

Badiyan

Using newly developed and validated HPLC coupled with photo diode array (PDA) method, six bioactive compounds; gallic acid, ellagic acid, chebulinic acid, tannic acid, quercetin and ascorbic acid were quantified in Itrifal-e-Badiyan Unani polyherbal formulation.

Method validation

The method was validated as per the ICH guidelines for different parameters like linearity, specificity, accuracy, LOD and LOQ.

Sample analysis

The Itrifal-e-Badiyan was found to have 6 marker components, which were in quantifiable amounts. Each marker components were identified by comparing the R_t and checked for the similarity of spectra The peak purity measurements are calculated in order to find any overlapping of impurities.

Simultaneous estimation of gallic acid, ellagic acid and ascorbic acid in Itrifal-e- Badiyan

A simple rapid and economic simultaneous HPLC method was developed and validated for the quantification of gallic acid, ellagic acid and ascorbic acid, in poly herbal Unani formulations, containing aamla as an ingredient. The HPLC experimental method has been carried as per the procedure mentioned in the experimental section B 5.4.2.

Sample analysis

The amount of gallic, ellagic and ascorbic acids in Itrifal-e-Badiyan Unani formulation was analyzed using developed and validated chromatographic method. The samples were injected in triplicates in HPLC column and peak area of all the triplicate samples were used for analysis of content by regression equation. Thedeveloped mobile phase gave optimal separation, with well defined and well resolved sharp peaks in both standard and samples (Fig. 06) at R_t 2.2 ± 0.1 min for ascorbic acid, 3.58 ± 0.3 min for gallic acid and 7.67 ± 0.3 min for ellagic acid. It was found to contain 0.06, 0.09 and

0.12% w/w of gallic, ellgic and ascorbic acids, respectively in Itrifal-e-Badiyan polyherbal formulation.

SUMMARY AND CONCLUSION

Traditional systems of medicine have been in vogue for centuries and use of plant- based medicine has been increasing all over the world especially for conditions like cancer, high blood pressure, allergies, and for general well being. Commercialization and manufacture of these medicines to meet this increasing demand has resulted in a decline in their quality, primarily due to a lack of adequate regulations pertaining to this sector of medicine. Hence it is necessary to come up with a systematic approach to develop well-designed methodologies for the quality control of polyherbal formulations. By considering these facts, the aim of the present research work was to develop high standard quality parameters for some polyherbal formulations which are frequently used in Unani system of medicine. The work has given emphasis on the importance of the qualitative and quantitative methods for characterizing the samples, quantification of the biomarkers and/ or chemical markers and the fingerprint profiles along with the conventional parameters followed for the standardization of polyherbal Unani herbal formulations.

The Unani System of Medicine include a large number of traditional formulation and used since long time in India and abroad. This system consists of different types of formulations like Itrifal, Jawarish, Majoon, Qurs and Habbs and has been ignored for scientific validation of these formulations as well as for the quality control using modern analytical techniques.

The seven formulations have been selected (randomly) for the development of modern quality control standards with conventional parameters. The common and conventional quality control parameters such as organoleptic evaluations like colour, odour, taste and consistency; physico-chemical evaluation like loss on drying, disintegration time for tablets, friability for tablets, moisture content by Karl Fischer method, total ash, acid insoluble ash, water soluble ash, pH of 1 % solution, pH of 10% solution, extractive values, water soluble matter, alcohol soluble matter, total phenolic/ flavanoid content etc. have been carried out in triplicate of three batches of each formulations.

The evaluation for contaminants like heavy metal content by atomic absorption specrophotometer, determination of aflatoxins by HPLC and pesticide residues, by GC-MS have been carried out in each formulation.

High Performance Thin Layer Chromatography (HPTLC) was performed to develop fingerprint profiles of the formulations. Methanol, petroleum ether and chloroform extracts were used for the fingerprint development.

The plants are composed of complex mixture of primary and secondary metabolites, which are responsible for the bioactivity known as marker compounds and hence multiple-marker based analysis has recently been gaining importance. Multiple- marker-based standardization strategy adopted to minimize batch-to-batch variation and to maintain quality and ensure safety and efficacy. More over gives a rough idea about the therapeutic efficacy of each formulation (Rajani and Kanaki, 2008). To achieve the goal different analytical methods have been developed for the quantification multiple markers present in each formulations. Chromatographic methods developed for the multiple marker analysis include HPLC/UV/PDA detectors, HPTLC and GC-MS methods. All the developed methods were validated as per the ICH guidelines for various parameters like linearity, accuracy, precision, robustness, limit of detection and limit of quantification. Summary of work done in each formulation have been mentioned separately.

Itrifal Badiyan used as brain tonic, composed of the following ingredients; *Terminalia chebula* Retz. (fruit), *Terminalia belerica* Roxb. (fruit), *Emblica officinalis* Gaertn. (fruit), *Vitis vinifera* Linn. (fruit), *Rosa damascene* Linn. (flower), *Zataria multiflora* Boiss. (leaf), *Foeniculum vulgare* Linn. (seed) and *Prunus amygdalus* Batsch. (seed).

General physico-chemical evaluations like organoleptic characteristics, loss on drying at 105° C, moisture content by Karl Fischer method, total ash, acid insoluble ash, water soluble ash, pH of 1 % and 10 % suspensions, petroleum ether extractive value, chloroform extractive value, acetone extractive value, methanol extractive value , total phenolic content by UV, content of sugar by Anthrone reagent method, water soluble matter and alcohol soluble matter were carried out in three batches of Itrifal-e- Badiyan samples (in triplicate) to set the pharmacopoeial limits as given in Table 111. Analysis of heavy metals (AAS), aflatoxins (HPLC) and pesticide (GC-MS) showed absence of contaminants/ or below the acceptable limit in all batches of Itrifal-e- Badiyan.

HPTLC fingerprinting of methanol, chloroform and petroleum ether extracts of Itrifal- e-Badiyan showed the presence of five, nine and nine peaks in chromatograms after visualizing with anisaldehyde sulphuric acid, respectively.

Two new multiple marker based analytical HPLC methods as developed and validated for quality control of Itrifal-e-Aftimoon were applied in Itrifal-e-Badiyan also as given above.

a) HPLC-PDA method has been developed for the simultaneous quantitative determination of six bioactive compounds, gallic acid, ellagic acid, chebulinic acid, tannic acid, ascorbic acid, quercetin in Itrifal-e-Badiyan formulation.
b) A simple rapid and economic simultaneous HPLC method was developed and validated for the quantification of gallic acid, ellagic acid and ascorbic acid, in in Itrifal-e-Badiyan formulation.

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