Quality Assessment of a traditional Unani Formulation Arq Zeera
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ABSTRACT
Arq Zeera a poly herbal liquid formulation official in National Formulary of Unani Medicine is used as carminative and antiobesity in Unani System of Medicine. In this present work we attempt to prepare the formulation as per National Formulary of Unani medicine (NFUM) and developed of standard operational procedure (SOP) for the quality of the formulation. The physico-chemical properties (density, viscosity, refractive index, and optical rotation), microbial load, toxic metal analysis, pesticide residue, and aflatoxins were studied to develop SOP of the formulation. Density and viscosity were found to be 0.9996 to 0.9998 g/ml and 1.02 and 1.04 cST respectively of the preparation. refractive index at 25°C and optical rotation was not more than 1.337 and + 0.95 respectively. Study revealed that microbial load was absent in the preparation. Heavy metals analysis of the formulation by ICP-OES revealed the absence of cadmium, mercury, arsenic in the formulation but lead was detected under acceptable limits at prescribed dose. Pesticide residues analysis by GCMS/MS revealed the absence of organochloride, organophosphates and pyrithins group of pesticide in the entire drug. Aflatoxins (B1, B2, G1, G2) studies were done by LCMS/MS showed the absence in the formulation. The results obtained may be helpful to the regulatory authorities, scientific organizations and manufacturers for developing standards and maintaining the quality of formulation.

INTRODUCTION
The demand of herbal products is increasing day by day due to their efficacy, less side effects in the treatment and good faith of society on herbal medicine and also their products (1). Traditional medicinal systems of many countries contain rich knowledge on phytomedicines. Several scientific reports reviewed in detail the therapeutic potentials of medicinal plants in alleviating animal diseases (2, 3). The World Health Organization (WHO) noted that majority of the world population relies on traditional medicine for primary health care. Medicinal and aromatic plants which are widely used as medicine and constituent a major source of natural organic compounds. India is one of the richest sources of medicinal and aromatic plants. Because of the rapid progress of the herbal drug industry in India for the last quarter century, an increasing need is felt to standardize the herbal products (4). It is necessary to develop the scientific protocols such as SOP and pharmacopeial standards of the poly herbal formulation. Due to this scientific awareness a scenario has created to undertake the research activities like standardization of traditional medicines and to develop the scientific methods for the manufacture of quality medicines. Arq Zeera is a liquid formulation, prepared by simple distillation i.e. it is a distillate (arq) form. In this present study we were undertaken with the intention of efficiency, quality and safety profile of arq zeera for commercial purposes.

MATERIALS AND METHODS
Materials
All the ingredients were procured from Unani raw drug dealers with the knowledge of Unani physician, identified by Dr. H. B. Singh from National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. Voucher specimen and identification certificate reference number NISCAIR/RHMD/Consult/2011-12/1753/53 was obtained and kept in the department for future reference. All the ingredients were taken of medicinal and aromatic plants which are widely used as medicine and constituent a major source of natural organic compounds. India is one of the richest sources of medicinal and aromatic plants. Because of the rapid progress of the herbal drug industry in India for the last quarter century, an increasing need is felt to standardize the herbal products (4). It is necessary to develop the scientific protocols such as SOP and pharmacopeial standards of the poly herbal formulation. Due to this scientific awareness a scenario has created to undertake the research activities like standardization of traditional medicines and to develop the scientific methods for the manufacture of quality medicines. Arq Zeera is a liquid formulation, prepared by simple distillation i.e. it is a distillate (arq) form. In this present study we were undertaken with the intention of efficiency, quality and safety profile of arq zeera for commercial purposes.

Preparation of formulation
Crushed the cleaned and dried all the four ingredients viz: Zingiber officinale rhizome, Carum carvi Cuminum cyminum and Trachyspermum ammi fruits in an iron morter to obtained coarse powder and soaked in 12 l purified water. Transferred the soaked all four ingredient to the distillation plant along with purified water. Distilled the same at 100°C for about five and half hrs and collected the 7.5 l of Arq Zeera.

Analytical parameters
The prepared three batch samples were subjected for analytical parameters such as physico-chemical studies like density, viscosity, pH, refractive index, and optical rotation were carried out (6).

Microbial evaluations (7):
Test for Contaminating fungus (yeast and mould)
1 ml of the formulation was mixed in 100 ml of pH 7.2 phosphate buffer. 1 ml of the preparation was added to 15 ml of the liquefied potato dextrose agar medium in two petri dishes at not more than 45°C and incubated at 25°C for 7 days. The dishes were observed and numbers of colonies were counted.

Table: 1- Formulation composition of Arq Zeera

<table>
<thead>
<tr>
<th>S.No</th>
<th>Unani name</th>
<th>Botanical name/English name</th>
<th>Parts used</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ajwain Desi</td>
<td>Trachyspermum ammi</td>
<td>Fruit</td>
<td>250 g</td>
</tr>
<tr>
<td>2</td>
<td>Zanjabeel</td>
<td>Zingiber officinale</td>
<td>Rhizome</td>
<td>125 g</td>
</tr>
<tr>
<td>3</td>
<td>Zeera Syah</td>
<td>Carum carvi</td>
<td>Fruit</td>
<td>125 g</td>
</tr>
<tr>
<td>4</td>
<td>Zeera Safaid</td>
<td>Cuminum cyminum</td>
<td>Fruit</td>
<td>375 g</td>
</tr>
<tr>
<td>5</td>
<td>Aab sada</td>
<td>Cuminum cyminum</td>
<td>Purified Water</td>
<td>12 l</td>
</tr>
</tbody>
</table>

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Total Aerobic microbial count (Total bacterial count)
1 ml of the sample was suspended in 100 ml of buffered sodium chloride-peptone solution pH 7. 0.1% w/v of polysorbate 80 was added to assist the suspension of poorly wettable substances. 1 ml of the preparation and about 15 ml of the liquefied casein soyabean digest agar was added to two petri dishes at not more than 45°C and incubated at 30°C to 35°C for 4 days. The dishes were observed and numbers of colonies were counted.

Test for Escherichia coli
1 ml of the sample was suspended in 100 ml of buffered lactose broth by shaking in a sterile screw-capped jar. 0.1% w/v of polysorbate 80 was added to assist the suspension of poorly wettable substances. 1 ml of the preparation was transferred in a sterile screw-capped container and 50 ml of nutrient broth was added. Preparation was then shaken and allowed to stand for 1 hour and shaken again. The cap was loosened and jar was incubated at 37°C for 24 hours. The dishes were tested for presence of acid and gas as per standard procedure.

Test for Salmonella
25 ml of the preparation was suspended by shaking with 100 ml of nutrient broth in a sterile screw-capped jar and allowed to stand for 4 hours and shaken again. The cap was loosened and jar was incubated at 35°C to 37°C for 24 hours. 1.0 ml of the enrichment culture was added to each of the two tubes containing . 10 ml of selenite F broth and tetrathionate bile-brilliant green broth respectively and incubated at 36ºC to 38ºC for 48 hours. Each of these two cultures, were subcultured on bismuth sulphite agar and brilliant green agar. Plates were incubated at 36°C to 38°C for 18 to 24 hours and observed for the presence of black-green or pink colony respectively.

Test for Staphylococcus
1 ml of the sample was suspended by shaking with 100 ml of nutrient broth in a sterile screw-capped jar and allowed to stand for 4 hours and shaken again. The cap was loosened and jar was incubated at 35°C to 37°C for 24 hours. 1.0 ml of the enrichment culture was added to soyabean-casein digest medium. Medium was examined for the presence of growth. A portion of medium was streaked on the surface of Vogel-Johnson agar and Mannitol-salt agar medium. Plates were incubated at 36°C to 38°C for 18 to 24 hours and observed for the presence of black and yellow colonies surrounded with yellow zones.

Inductive couple plasma analysis:
Determination of these heavy metals was performed according to Ph. Eur. chapter 2.2.58(8).

A Perkin Elmer Elan 6000 ICP-OES equipped with an As-91 auto sampler was used. Instrument was calibrated using reference standards of 1ppm and 10ppm. Approximately 0.1 ml of sample was accurately weighed into a metal free container and dissolved in 1 ml of Aquaregia and heated on a hot plate to extract the metal. Then solution was filtered in a volumetric flask and washing of deionized water was added to it and volume made up to 10 ml. The solution was used for analysis.

Pesticide residues and Aflatoxins analysis
Pesticide residues were carried out by standard methods AOAC 970.33 and aflatoxins by AOAC 990.33 (9). For pesticide residues analysis, Thermo Finnigan GCMS-MS was used with DB-5 fused silica capillary column (30m X 0.25 mm i. d., 0.25 µm film thicknesses). Helium was used as a carrier gas at a flow rate of 1.0 ml/min. Mass Spectrometer Ion Trap detector Type was used. The injection port was maintained at 250°C, and the split ratio was 40:1. Oven temperature programming was done from 60°C hold 1.5 min, 60 to 120 @15°C/min, 120 to 220 @8°C/min, 220 to 280 @5°C/min, Hold 5 min. Interface temperature was kept at 250°C. Ionization source temperature was at 230°C, and 70 eV electron impact modes were employed. Ionization mode was electron Impact ionization and the scanning range was from 40 amu to 400 amu. Pesticide residues standards (organochlorides, organophosphates and pyrethins groups) were procured from sigma (Aldrich). For aflatoxins analysis, Agilents LCMSMS (Model: 6410B) was used with RRLC Column: C18, 50mmX2.1mm, 1.8um particle size and maintained 40°C. Mobile Phase was used as 0.1% formic acid + 5 mM ammonium acetate in water and Methanol at a flow rate of 0.2ml/min. Mass Spectrometer QQQ detector type was used. Aflatoxins standards solutions were obtained from sigma (Aldrich) and kept in at 20°C in a colored amber vial.

RESULT AND DISCUSSION
Arg zeera prepared by simple distillation as given in unani formulary is a colourless liquid preparation with aromatic odour. The results of physical evaluation of arg zeera are as shown in table 2.

Table 2: Physical parameters of the Arq Zeera

<table>
<thead>
<tr>
<th>S No.</th>
<th>Parameters</th>
<th>Batch I</th>
<th>Batch II</th>
<th>Batch III</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Density</td>
<td>0.9996 g/ml</td>
<td>0.9996 g/ml</td>
<td>0.9998 g/ml</td>
</tr>
<tr>
<td>2</td>
<td>Refractive index</td>
<td>1.337</td>
<td>1.337</td>
<td>1.337</td>
</tr>
<tr>
<td>3</td>
<td>Viscosity</td>
<td>1.02 CS</td>
<td>1.02 CS</td>
<td>1.02 CS</td>
</tr>
<tr>
<td>4</td>
<td>Optical rotation</td>
<td>+ 0.90</td>
<td>+ 0.91</td>
<td>+0.95</td>
</tr>
<tr>
<td>5</td>
<td>pH</td>
<td>6.7</td>
<td>6.8</td>
<td>6.8</td>
</tr>
</tbody>
</table>

Microbial contamination
Study revealed that the total bacterial count, Enterobacteriaceae, Salmonella, Staphylococcus aureus, and total fungal count were not found in the preparation as shown in table 3.

Table 3: Microbial load of Arg Zeera

<table>
<thead>
<tr>
<th>S No.</th>
<th>Parameter Analyzed</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total Bacterial Count, cfu/ml</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>2</td>
<td>Total Fungal Count, cfu/ml</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>3</td>
<td>Enterobacteriaceae, cfu/ml</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>4</td>
<td>Salmonella/25 ml</td>
<td>Absent</td>
</tr>
<tr>
<td>5</td>
<td>Staphylococcus aureus, cfu/ml</td>
<td>&lt;1.0</td>
</tr>
</tbody>
</table>

Heavy metals analysis.
Toxic heavy metal like arsenic, cadmium and mercury were not detected in the drug. Lead was detected under limit permissible shown in table 4.

Table 4: Result of Heavy Metal analysis

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cadmium (Cd)</td>
<td>Not Detected</td>
<td>0.3 mg/kg</td>
<td>0.5 mg/kg</td>
<td>0.5 mg/kg</td>
<td>4 mg/kg</td>
</tr>
<tr>
<td>2</td>
<td>Lead (Pb)</td>
<td>0.3 mg/kg</td>
<td>10 mg/kg</td>
<td>5 mg/kg</td>
<td>10 mg/kg</td>
<td>5 mg/kg</td>
</tr>
<tr>
<td>3</td>
<td>Arsenic (As)</td>
<td>Not Detected</td>
<td>-</td>
<td>-</td>
<td>90 mg/kg</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Mercury(Hg)</td>
<td>Not Detected</td>
<td>0.1 mg/kg</td>
<td>-</td>
<td>-</td>
<td>0.1 mg/kg</td>
</tr>
</tbody>
</table>

Pesticide residue analysis
Pesticide such as organochlorides groups (o,p-DDD, p, p-DDD, o, p-DDE, p, p-DDE, o, p-DDT, p, p-DDT, α-HCH, β-HCH, γ- HCH, δ-HCH, Endosulfan ) organophosphates (Diazinon, Malathion etc) and pyrethine were absent in all the drug. Retention time of sharp peak of the formulation was started from 5 to 9.09 shown as in figure 1. In the standards group organochlorines, retention time of sharp peak was from 14.72 to 25.09, while in organophosphate group from 8.21 to 20.26 shown as in fig.3.-4. RT of the sharp peak of pyrethines were started from 16.22 to 33.49 shown as figure 5.

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Fig: 1- GCMS chromatogram of formulation (AZ)

Fig: 2- MS spectra of the formulation (AZ)

Fig: 3- GCMS chromatogram of standards organ chlorides (OC)

Fig: 4- GCMS chromatogram of standards organ phosphates (OP)

Fig: 5- GCMS chromatogram of standards pyrethines (OC)
Aflatoxins residues analysis

Aflatoxins were also not detected in the formulation. There was no any peak obtained between retention times 6.779 to 10.986 min from the chromatogram of the formulation, while the aflatoxins standards peaks were obtained from retention time 6.779 to 10.986 min (figure 5). The sharp peaks of the formulation were started from retention time about 17min. Thus aflatoxins were not present in the preparation shown as in figure 7.

DISCUSSION

The observed physical properties clearly showed the good quality of Arq Zeera. The physical properties of Arq Zeera except Optical rotation are almost similar to water. Quantitative standards revealed that the density was 0.9996 to 0.9996g/ml and 1.333 of Refractive index was detected in the formulation. The Optical rotation was found to be +0.90 to +0.95. Microbial load is one of important parameter which mentioned in WHO to determine the quality of the formulation for medication. Microbial load of the preparation was found negative for the presence of Escherichia coli, Salmonella and Staphylococcus aureus and fungal indicate the good quality of the product. It is may be due to presence of essential oil. Because Arq Zeera is a distillate product that means it contains volatile compound. Volatile oils of many plants are known to have antimicrobial activity (14). Heavy metals such as lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) are natural constituents of the environment like air, water and soil. Furthermore, they are produced by technical and industrial processes and thus have gained importance as contaminants. Medicinal plants growing in nature can accumulate heavy metals to a certain extent depending on their individual properties and the concentration of heavy metals in soil, air and water As heavy metals pose a hazard to human and animal health, their content in plants used for consumption or medicinal purposes must be limited [15-19]. For this reason limits for heavy metals have been set for foodstuffs and medicinal products by health authorities shown as in table 4. The toxic heavy metal like cadmium, arsenic and mercury were not detected in the drug and lead under acceptable limit also indicates the maintenances of quality of the formulation. More than 700 pesticides are registered for use in the world (18). Recently, concern has increased that certain pesticides and other synthetic chemicals may be acting as pseudohormones which disrupt the normal function of the endocrine system in wildlife and humans. Birth defects, behavioral changes, breast cancer, lowered sperm counts, and reduced intelligence are among the many disorders that have been blamed on these “endocrine disrupting” compounds, though much research must be done to verify these assertions. Pesticide residues study revealed the absence of organophosphates, organochlorides and pyrethines class of pesticides in the preparation and it also help in maintaining of the quality of the product. Aflatoxins are toxic mycotoxins produced by several species of Aspergillus moulds. Four compounds produced by these moulds are aflatoxins B, B, G, and G. Of these, aflatoxin B is the most carcinogenic and the most commonly occurring variety. Aflatoxins are a group of highly oxygenated heterocyclic compounds with closely related structures. Monitoring of a variety of foods is necessary to ensure consumer safety. Scientists identified at least four related compounds that caused acute toxicity and liver carcinogenicity in duckling feeding trials. Aflatoxins were characterized as B (blue fluorescence) and G (green fluorescence). Four aflatoxins, B, B, G, and G, are synthesized by A. flavus. In cases of contamination, aflatoxin B1, the most toxic and most carcinogenic, is almost always present (20). Thus this formulation may be protected from acute toxicity and liver carcinogenicity due to lack of aflatoxins toxicity.

Hence, the physical parameters, contamination with microorganisms, heavy metals, pesticides and aflatoxins profiles together may be used for quality evaluation and the standardization of the compound formulation Arq Zeera. Thus the data generated in this analysis will help in setting up regulatory limit, to ensure the quality of in Indian medicine. A routine use of such scientific techniques will lead to standardization of the product to a certain extent and would definitely help in building confidence in use of such products for medication.

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REFERENCES


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