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ARQ ZEERA: PREPARATION, ASSESSMENT AND EVALUATION

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ABSTRACT

The study of standardization of herbal drugs depends on reliable, specific & sensitive quality control method used in a combination of classical & modern instrumental method. The present work involves standardization of Arq Zeera in order to assess the quality. Dried raw materials like, fruit, stem are used in Arq Zeera formulation. All ingredients were powdered. As a mean of quality control parameter, the plant powders used in the formulations were confirmed with the help of monographs from the ICMR for the diagnostic characters using microscopic analysis.The formulation was subjected to pharmacognostic studies, physico-chemical properties, phytochemical analysis and HPLC fingerprint profile to evaluate the quality control in herbal industries. The preliminary phytochemical analysis indicated presence of alkaloids, fats and fixed oils, essential oils, glycosides, resins and saponins. HPTLC fingerprint profileof formulations showed a characteristic pinkish red band of Thymol at Rf of 0.43.

KEYWORDS: Arq Zeera, polyherbal formulation, Unani, Standardization

INTRODUCTION

The recent emerging trends have made people more inclined towards natural way of living and holistic approaches to maintain the health. One of the oldest traditional system of medicines is Unani system which needs reconsideration over principles and preparation of standardized medicines in order to come along with modern medicines and ultimately in the benefit of human health. Though the Unani formulations are gaining global acceptance due to their clinical efficiency, their quality control and standardization is still not upto the mark. Hence, the concept of standardization is becoming essential.

Arq Zeera is a herbal formulation used extensively as treatment of obesity in Unani System of Indian Medicine.Arq can be defined as a liquid obtained by distillation of certain liquids or drugs soaked in water using distillation apparatus.Arq Zeera is prepared from four herbs Ajwain (Trachyspermum ammi), Zangabeel (Zingiber officinale), Zeera Siyah (Carum carvi), and Zeera Safaid (Cuminum cyminum) by simple distillation.It improves appetite, digestion and assimilation. It digests toxins, gives relief in gas, alleviates intestinal spasms-vomiting, and removes excess mucus. It is effective in digestive disorders such as anorexia, digestive weakness, flatulence, indigestion etc. It has carminative, digestive, antispasmodic, diuretic and galactagogue properties..

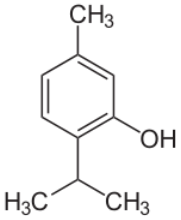
The Pharmacopeial standards in Ayurvedic, Siddha and Unani are not adequate enough to ensure the quality of formulations. Analysis of marker compounds is necessary to maintain the quality and identity of the formulations. In order to standardize, the formulation was prepared at laboratory scale as per pharmacopoeial standards. It was subjected to analysis for microscopic studies, physicochemical parameters, microbial load and high-performance thin layer chromatographic studies. No work has been carried out in the estimation of markers compounds in the Arq Zeera till now and hence the main objective was to develop identification, accurate, specific and reproducible method for the estimation of thymol in Arq-Zeera.

MATERIALS AND METHODS

1. Raw Materials,Chemicals and Reagents

Plant Raw materials used for the preparation of Arq Zeera were procured from Ratan Gandhi Shop, Pydhonie (Mumbai) with the knowledge of Unani physician. The materials were dried in an oven preset at 45°C, powdered, sieved through an 85-mesh (BSS) sieve and stored in air tight containers.

The thymol standard was procured from Himedia and Assigned purity: 98%. The vanillin reagent used for visualization was from Merck (Germany).The solvents toluene, ethyl acetate, Acetonitrile and Water were from Sigma (Aldrich).



Thymol

Raw materials complying the pharmacopoeial quality and quantity were subjected to the preparation of Arq Zeera as per the composition [Table 1]. All the ingredients were soaked in 96 ml purified water as above given ratio (Table 1). Then they were processed for distillation along with purified water at 100° C for about five and half hrs and collected the 60 ml of Araq Zeera.

2. Preparation of Arq Zeera

Table 1: Formulation composition.

S. No.	Unani name	Botanical /English name	Parts used	Quantity
1	Ajwain Desi	Trachyspermum ammi	Fruit	2 g
2	Zanjabeel	Zingiber officinale	Rhizome	1 g
3	Zeera Siyah	Carum carvi	Fruit	1 g
4	Zeera Safaid	Cuminum cyminum	Fruit	3 g
5	Aab Sadah	Purified Water		96 ml

3. Quality Evaluation of Arq Zeera

Organoleptic evaluation

The formulation was studied for its preliminary characters like colour,texture,odour and taste.

- **Preliminary Phytochemical Evaluation**

Phytochemical screening of some major secondary metabolites (flavonoids, tannins, cardiac glycosides,terpenoids,steroids and saponins) in Arq Zeera was carried out by performing preliminary coour based phytochemical tests.

- **Physical Evaluation**

The prepared formulation was subjected for physical studies like density, viscosity, pH.

- **Chromatographic Evaluation**

Extraction of Thymol

Extraction of marker compound thymol from Arq Zeera was carried out. For this 30 ml of chloroform was added to formulation Arq Zeera and frequently shaken for 1 hour and allowed to stand for 24 hrs. Transferred the content in separating funnel and mixed thoroughly. The chloroform layer was separated and rest of the solution was again extracted with more quantities of chloroform at least three times. Combined chloroform extract was evaporated by rotatry evaporator and residue was redissolved in 30 ml methanol. The resulting solution (233 mg/ml) was filtered by Whatman filter paper and volume was reduced to 10 ml in volumetric flask.

Preparation of Standard

Thymol standard was prepared in methanol with initial concentration of 1000 ppm.Further dilution of 100 ppm was prepared using mobilke phases.

- **High Perfiormance Thin Layer Chromatography (HPTLC) evaluation**

10 µl of the filtered solution of formulation extract and standard was applied on the TLC plate as per condtions mentioned in table 1a followed by development, derivatizing with vanillin sulphuric acid agent and scanning at 513 nm.

Table 1a: Chromatographic Conditions for HPTLC.

Stationary Phase	HPTLC plates silica gel 60 F 254
Plate size	10.0x10.0 cm
Mobile Phase	Toluene : Ethyl Acetate :: 9 : 3(v/v)
Saturation Time	20 min.
Standard Used	Thymol
Spot Volume	10 µl
Band Length	8.0mm
Solvent Front	80mm
Wavelength and Lamp	366nm & Mercury lamp
Sample Applicator	CAMAG Linomat 5
Sample Detection	CAMAG Visualizer : 200480
Number of Tracks	3

- **High Performance Liquid Chromatography (HPLC) evaluation.**

HPLC was also performed to find out the thymol content in prepared formulation as per conditions mentioned in table 1b.

Table 1b :Chromatographic Conditions for HPLC.

Mobile phase	ACN: Water - 100: 100
Stationary Phase	C_{18} (4.6 × 250 mm, 5 µm).
Flow rate	1 ml/min
Injection volume	20 µl
Detection	UV at 274nm

RESULTS AND DISCUSSION

Arq zeera was prepared by simple distillation as given in unani formulary. The observed physical properties clearly showed the good quality of Arq Zeera. The organoleptic characters (table 2) yield important characteristics. These characteristics might be useful for distinguishing it from its substitutes and adulterants. Studies on physical constants can serve as a valuable source of information and provide suitable standards to determine the quality of the plant.The results of physical evaluation of arq zeera are as shown in table 3. Phytochemical evaluation helped to understand the presence of various therapeutically active constituents.It was found that tannins,flavanoids,saponins,glycosides,terpenoids were present. Steroids were absent in the formulation (table 4). The composition of these phytoconstituents in that particular formulation depends upon its nature,raw materiuals used and process applied for preparation. These chemical tests can be the means of providing preliminary information on the quality of a particular sample According to Mohan et al. different chemical compounds detected in whole plant extracts could make the plant useful for treating different ailments as having a potential of providing useful drugs of human use.

The prepared formulation was then assesed for its quality by checkiong the presence of marker compound thymol by hyphenated techniques like HPTLC.For monitoring quality, HPTLC fingerprinting is ideal which involves comparison between standard and formulations. Using HPTLC one can visualize the presence of various plant chemical constituents, out of these a marker compound can serve as a characteristic fingerprint for that formulation (Fig 1). It was found that thymol was present in the final product which can also be used for shelf life studies.

Table 2: Organoleptic Characters.

Sr. No.	Characters	Arq Zeera
1	Colour	Colourless
2	Taste	Bitter pungent
3	Texture	Liquid
4	Odour	Aromatic

Table 3: Physical evaluation.

Sr. No.	Parameters	Arq Zeera
1	Density	0.9996
2	Viscosity	1.02 CS
3	pH	6.8

Table 4: Phytochemical Evaluation.

Sr. No.	Phytoconstituent	Test	Observation	Arq Zeera
1	Tannins	0.5gm sample + few drops of 0.1% ferric chloride solution	brownish green colouration	+
2	Saponins	2gm sample was boiled in water bath and filtered. The boiled sample was 1ml of filtrate was mixed vigoursly to form a stable persistent froth. A formation of emulsion was observed after mixing the froth with about 3 drops of olive oil to indicate presence of sapponin.	Persistent froth	+
3	Cardiac Glycosides	5ml of each aq.extract was treated with 2ml of glacial acetic acid which contain one drop of ferric chloride chloride solution.1ml of sulphuric acid was added.	Formation of brown ring.	+
4	Terpenoids	5ml of each aq. Extract was mixed with 2ml of chloform and 3ml of conc. sulphuric acid.	Reddish brown coloration	+
5	Flavonoids	5ml of dil ammonia solution were added to a portion of aq.filtrate of each plant extract followed by the addition of concentrated sulphuric acid.	Yellow colour disappeared	+
6	Steroids	2ml of acetate anhydride were added to 0.5 g ethanol extract of each sample with 2ml of sulphuric acid	No change in colour	-

Key : + positive, - Negative

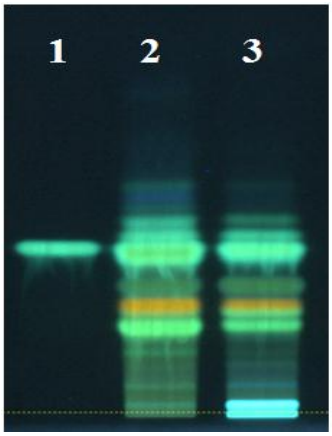


Fig 1: HPTLC fingerprint.

1 – Standard Thymol
2 – Methanolic extract of Trachyspermum ammi
3- Arq Zeera extract for thymol

CONCLUSION

Quality control parameters are of key importance if traditional medicines are to be given credibility as modern medicine has.In order to have consisitancy and uniformity in the production of these medicines on large scale,there is a need to set a standard protocol for preparation and for assesment of quality,efficacy. Unani formulation Arq Zeera has been standardized by intervention of modern scientific quality control measures in the traditional preparation described in classical texts. Pharmacognostic characters established for the formulation could be employed as Q.C. standards for evaluating its identity and can be used for routine analysis. It can be concluded that it is possible to survive with traditional medicinal systems if we establish proven efficacy of these medicines on scientific lines.

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