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Pharmacognostical and physico-chemical studies of Unan formulation Zuroor-E-Qula

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Abstract

Unani system of medicine originated in ancient Greece, with the teachings of and was introduced in India by the Mughal emperors. Oral health is given special emphasis in Unani medicine due to the understanding that diseases of the mouth affect eating and nutrition which may affect all systems of the body. Many drugs described in Unani literature have been used since centuries as folk and traditional medicine in various oral health problems. Oro-dental diseases are described extensively in Unani literature in a systematic manner and many drugs such as *Aqar Qarha* and *Kath Safaid* etc are mention for their treatment. Zuroor-e-qula, a powdered poly herbal Unani formulation, known to possess sialorrhic, antimicrobial and anti-inflammatory properties is recommended in cases of stomatitis and gastric ulceration.

Keywords: USM, buqrat, oral health, unani formulation and Zuroor-e-qula

1. Introduction

Plants are used for treating various diseases through ancient time in most part of the world especially in developing countries and dependence on traditional medicine for various diseases [1]. Unani system of medicine dealing with traditional system of medicine, which has its own theory of health and disease [2]. Unani drugs have been used in both single as well as compound formulations to manage numerous health problems. Various ailments of oral cavity can be treated by Unani medicines like toothache, gingivitis, stomatitis, bleeding gums, plaque, tooth decay and dental caries etc. Numerous single and compound drugs have been mentioned in Unani medicine which in a variety of oro-dental disorders [3-4]. In Al-Qanoon, the chapter on oral diseases is divided into three sections, viz (i) diseases of tongue, (ii) diseases of teeth and (iii) diseases of gums and lips. In the section of teeth disorders, Ibn Sina first describes the measures to prevent teeth disorders. Then, there is a description of the general line of treatment of teeth disorders. He states that teeth have a *Barid Mizaj* (cold temperament). Therefore, the medicines used should be such that they restore the original temperament. In addition, most of the drugs prescribed in teeth disorders have a *mujaffif* (desiccant) action. Drugs are prescribed for both oral and local use [5]. Zuroor-e-Qula, a powdered polyherbal Unani formulation containing ingredients such as Gul-e-Surkh (*Rosa damascena* Mill), Gulnar Farsi (*Punica granatum* L.), Kath Safaid (*Acacia leucophloea* Willd.), Dana Heel Khurd (*Elettaria cardamomum* Maton.), Kabab-e-Khandan (*Zanthoxylum alatum* Roxb.), Tabasheer (*Bambusa bambos* Druce.), Kafoor (*Cinnamomum camphora* Nees & Eberm.) The formulation is known to possess sialorrhic, anti-microbial and anti-inflammatory properties and is used in cases of stomatitis and gastric ailments [6]. The present research work aims to develop quality parameters and evaluate the data to lay down the pharmacopoeial standards of 'Zuroor-e-Qula'. The conventional parameters such as organoleptic parameters, microscopy, macroscopy and physico-chemical evaluations (water & alcohol soluble extractives, total ash, acid insoluble ash, moisture content, pH values). The quality control parameters such as heavy metals estimation, pesticide estimation, microbial load and aflatoxins were also analyzed in order to assess the quality of the formulation.

2. Materials and Methods

2.1 Preparation of Formulation

All the ingredients were procured from local raw drug dealer and were identified botanically by using pharmacognostical methods [7-8]. The ingredients were further validated by comparing with the monographs available in different volumes of UPI [9-10].

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All the ingredients of pharmacopoeial quality were taken. The ingredients were cleaned and dried under shade to remove the moisture if any. The ingredient no. 1-7 (Table-1), were grind and sieved through mesh no. 100 to obtain fine powder. The

coarse powders were further ground in a grinder to get their fine forms. Mixed the powders of ingredient no.1-7 thoroughly and prepared a homogenous form of drug. Stored the drug in a glass/ plastic container free from moisture.

Table 1: Formulation composition

| S. No. | Unani Name | Botanical/English Name | Part used | References | Qty. |
|--------|-----------------|--|-------------------|-----------------------------|------|
| 1. | Gul-e-Surkh | <i>Rosa damascena</i> Mill. | Flower | UPI, Part I, Vol. III, p.31 | 100g |
| 2. | Gulnar Farsi | <i>Punica granatum</i> L. | Flower | UPI, Part II, Vol. I, p.94 | 100g |
| 3. | Kath Safaid | <i>Acacia leucophloea</i> Willd. | Resin | UPI, Part I, Vol. VI, p.40 | 100g |
| 4. | Dana Heel Khurd | <i>Elettaria cardamomum</i> Maton. | Seed | UPI, Part I, Vol. I, p.34 | 100g |
| 5. | Kabab-e-Khandan | <i>Zanthoxylum alatum</i> Roxb. | Fruit | API, Part I, Vol.IV,p.130 | 100g |
| 6. | Tabasheer | <i>Bambusa bambos</i> Druce. | Silica concretion | UPI, Part II, Vol. I, p.256 | 100g |
| 7. | Kafoor | <i>Cinnamomum camphora</i> Nees & Eberm. | Natural Camphor | API, Part I, Vol. VI, p.210 | 25g |

2.2 Microscopy

About 5 g powder was taken and stirred thoroughly in 50% acidic acid for some time to remove all the salts. The supernatant was discarded and residue washed with slightly hot distilled water. A little residue was stained with safranin, fast green, iodine solution and then mounted in 50% glycerin. Some of the residue was heated in chloral hydrate solution and mounted in 50% glycerin. A little residue was boiled in 2% potassium hydroxide solution, washed with distilled water and mounted in 50% glycerin [11].

2.3 Physico-chemical analysis

The physico-chemical parameters of Zuroor-e-Qula' viz. moisture content, extractive values (solubility in water and ethanol), Ash values (total ash and acid insoluble ash), pH values (1% & 10% aqueous solution), bulk density and sugar content (reducing & nonreducing) were analyzed as per standard methods.

2.4 Quality control parameters

The herbal medicines specially Unani medicines are being used by millions of people as effective remedies assuming that they have lesser side effects or no side effects at all. People's faith in Unani medicines leads to concern over their

quality assurance. Regardless of the form of Unani preparation, their quality check is of greatest importance. So, the different quality control parameters like microbial loads, heavy metals, aflatoxins and pesticide residues were carried out to check the level of quality of Zuroor-e-Qula. Estimation of microbial load was conducted as per standard method [12]. Heavy metal analysis and aflatoxins were carried out by respective use of Atomic Absorption Spectrophotometer (LABINDIA) [13]. Pesticide residues were analyzed using GC-MS system (Agilent) equipped with mass selective detector as per standard methods [12, 14].

3. Results and Discussions

The microscopic features of studies of all single ingredient of Zuroor-e-Qula are mention in table-1 and macroscopic characters are in table-2 &3.

Table 2: Microscopic feature (Fig.1-4)

| Name of the Drug | Macroscopic feature |
|------------------|--|
| Zaroor-e-Qula | It is a light brown powder with pleasant smell and having slightly pungent taste |

Table 3: Microscopic feature (Fig.1-4)

| Name of the Drug | Microscopic feature |
|------------------|---|
| Gul-e-Surkh | It showed various microscopic characters such as vessel cell, parenchyma cells, stomata, cortical parenchyma cells, thick walled parenchyma cells, trichomes, fibre cells with narrow lumen and parenchymatous cells (Fig. 1 A-H). |
| Gulnar Farsi | This flower showed various microscopic characters in Zuroor-e-Qula such as cortex cells with of calcium oxalate crystals, pith cells, sclereid cells, vessel cells and parenchyma cells filled with yellowish content. All these characters are depicted in Fig. 2 A-F. |
| Dana Heel Khurd | This drug showed various microscopic characters such as sclerenchymatous fibers, perisperm cell containing rounded starch grains, parenchyma cells filled with aleurone grains, endosperm cells, palisade cells and group of rectangular cells with volatile oils (Fig. 3 A-F). |
| Kabab-e-Khandan | This drug showed various microscopic characters such as fragments of endocarp cells with calcium oxalate crystals, vessel cells, fibre cells, scleried cell, tangentially elongated cell filled with reddish brown content and parenchymatous cells containing oil globules (Fig. 4 A-F). |

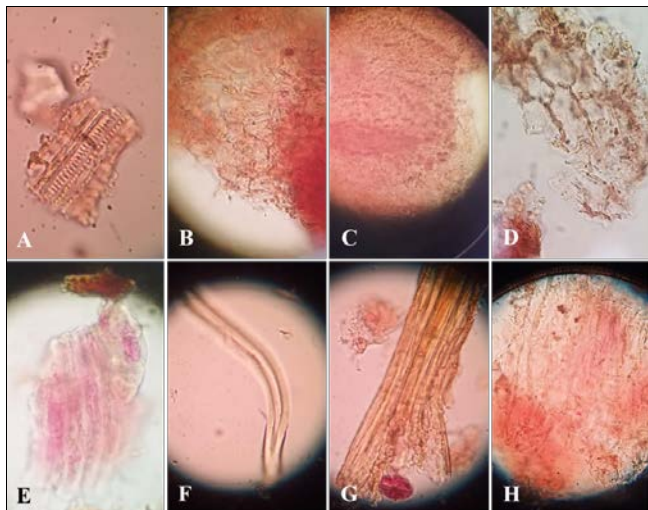


Fig 1: (A-H): Identification of microscopic characters of Gul-e-Surkh (Flower of *Rosa damascena*) in Zuroor-e-Qula drug formulation.

- a) Vessel cell with scalariform thickening along with xylem parenchyma cells (40x)
- b) Fragment of floral epidermis layer with stomata (40x)
- c) Fragment of thick wall petal cells (20x)
- d) Cortical parenchyma cells (40x)
- e) Thick walled parenchyma cells (40x)
- f) Trichome (40x)
- g) Group of fibres with narrow lumen (40x)
- h) Parenchymatous cells (40x)

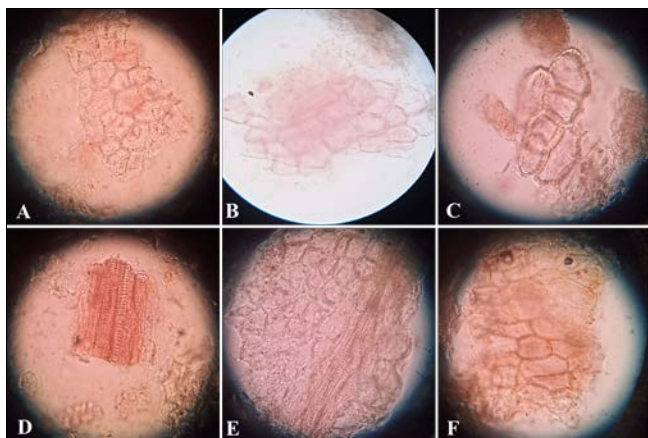


Fig 2: (A-F): Identification of microscopic characters of Gulnar Farsi (Flower of *Punica granatum*) in Zuroor-e-Qula drug formulation.

- a) Cortex cells containing druses of calcium oxalate and prismatic crystals (40x)
- b) Pith parenchyma cells (40x)
- c) Group of sclereid cells with pitted walls (40x)
- d) Group of xylem vessels (40x)
- e) Fragment of cortex cells with xylem vessel (40x)
- f) Thick walled parenchyma cells filled with yellowish content (40x)

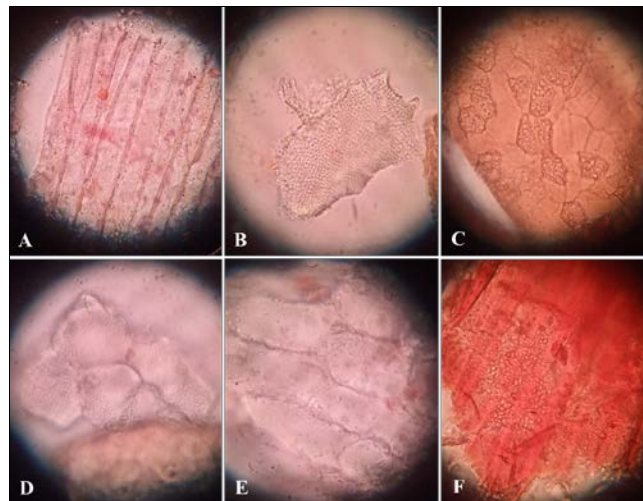


Fig 3: (A-F): Identification of microscopic characters of Dana Heel Khurd (Seeds of *Elettaria cardamomum*) in Zuroor-e-Qula drug formulation.

- a) Group of sclerenchymatous fibers (40x)
- b) Perisperm cell packed with minute rounded starch grains (40x)
- c) Thin wall parenchyma cells and some of them containing aleurone grains (40x)
- d) Endosperm cells of parenchyma containing protein as granular hyaline mass in each cell (40x)
- e) Palisade sclerenchyma cells (40x)
- f) Rectangular parenchymatous cells containing volatile oils (40x)

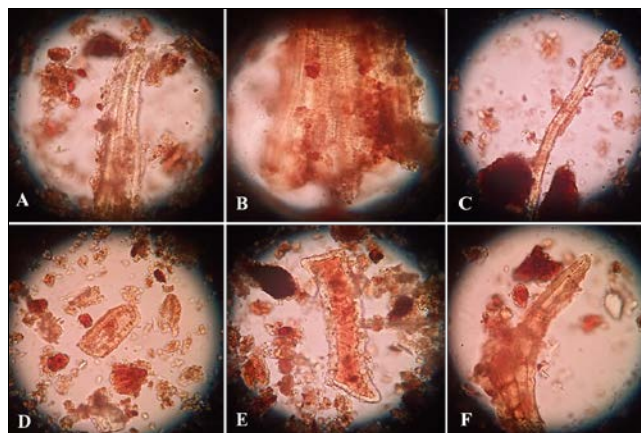


Fig 4: (A-F): Identification of microscopic characters of Kabab-e-Khandan (fruits of *Zanthoxylum alatum*) in Zuroor-e-Qula drug formulation.

- a) Endocarp cells with calcium oxalate crystals (40x)
- b) Vessel cells with spiral thickening (40x)
- c) Fibre cell with narrow lumen (40x)
- d) Scleried cell from epicarp (40x)
- e) Tangentially elongated tubular cell filled with reddish brown content (40x)
- f) Parenchymatous cells filled with oil globules (40x)

3.1 Physico-Chemical analysis

The physico-chemical data of the drug Zuroor-e-qula are shown in Table-4. The quantitatively evaluated data revealed that the moisture content of the drug ranged between 11.69-11.92 %, which is appropriate in case of Zuroor (tooth powder). The total ash content is high and range between 31.33% and acid insoluble ash is 29.39% which mark the amount of siliceous matter in the drug sample. The water extractive values turned out to be on lower side, ranged between 17.78% which marks the presence of inorganic constituents in the drug sample and the ethanol soluble extractive values were low and ranged between 9.03 -9.31% which indicates the extraction of polar constituents. The aqueous extract of the drug was acidic in nature as pH value falls in the range of 5.92-5.96 (1%) and 5.99-6.01(10%).

3.2 Quality control parameters

3.2.1 Heavy metal analysis

The results of heavy metal estimation are given in Table-5. Heavy metals are toxic or poisonous to human health even at low concentrations and may cause many deadly diseases. The heavy metal content in Zuroor-e-Qula was found to be below detection limit which indicated that the drug was free from heavy metal contamination.

3.2.2 Microbial load

Traditional medicines are largely spoiled due to microbial growth. Prevention of microbes can extend the shelf life of the drug. The estimation of microbial load indicates whether the drug contains disease causing and spoilage micro-organisms in permissible limits. The assessment is done for evaluating the total bacterial count, total fungal count, count of bacteria belonging to the Enterobacteriaceae family, count

of pathogens like *E. coli*, *Staphylococcus aureus*, *Salmonella* spp. and *Pseudomonas aeruginosa*. The results of microbial load are shown in Table-6 which indicates that the drug is safe for human consumption.

3.2.3 Aflatoxins

The results of aflatoxins analysis of the drug are given in Table-7. Aflatoxins are toxic metabolites produced by a variety of molds such as *Aspergillus flavus*, *A. parasiticus* and *A. nomius*. The results do not show the presence of any of the aflatoxin contents (B1, B2, G1, and G2) in Zuroor-e-Qula.

3.2.4 Pesticide residues

The results of pesticide residues are given in Table-8. Excessive use of pesticides to protect the crops from insects or diseases has created hazardous conditions for human food chain. They have to be checked not only in food items but also in traditional medicines. In order to estimate the pesticide residues, the drug was analyzed on GC-MS. The results indicated that the drug is free from pesticide residues and safe for use.

Table 4: Physico-chemical analysis

| Parameters | Values |
|--|--------------|
| Ethanol soluble Content (%) | 9.03- 9.31 |
| Water soluble Content (%) | 17.64- 17.78 |
| Total Ash Value (%) | 31.16-31.33 |
| Acid insoluble Ash content (%) | 29.23- 29.39 |
| Loss of weight on drying at 105 °C (%) | 11.69-11.92 |
| pH of 1% aqueous solution | 5.92- 5.96 |
| pH of 10% aqueous solution | 5.99-6.01 |

Table 5: Heavy Metals Estimation

| S. No. | Element | Values | WHO Limits for internal use |
|--------|---------|--------|-----------------------------|
| 1. | Lead | ND | 10 ppm |
| 2. | Cadmium | ND | 0.3 ppm |
| 3. | Mercury | ND | 3.0 ppm |
| 4. | Arsenic | ND | 1.0 ppm |

Table 6: Microbial load

| S. No. | Microbial contaminant | Values | WHO Limits for internal use |
|--------|------------------------------|------------|-----------------------------|
| 1. | Total Bacterial load | < 10 cfu/g | 1x10 ⁵ cfu/g |
| 2. | Total Fungal count | < 10 cfu/g | 1x10 ³ cfu/g |
| 3. | Enterobacteriaceae | Absent | Nil |
| 4. | <i>Escherichia coli</i> | Absent | Nil |
| 5. | <i>Salmonella spp.</i> | Absent | Nil |
| 6. | <i>Staphylococcus aureus</i> | Absent | Nil |

Table 7: Aflatoxins

| S. No. | Aflatoxins | Results |
|--------|----------------|---------|
| 1. | B ₁ | ND |
| 2. | B ₂ | ND |
| 3. | G ₁ | ND |
| 4. | G ₂ | ND |

*ND – Not detected

Table 8: Pesticide Residue

| S. No. | Pesticide Residue | Result |
|--------|---------------------------|--------|
| 1. | Alachlor (mg/kg) | ND |
| 2. | Aldrin & Dieldrin (mg/kg) | |
| 3. | Azinophos-methyl (mg/kg) | |
| 4. | Bromopropylate(mg/kg) | |
| 5. | Chlordane (mg/kg) | |

| | |
|-----|---|
| 6. | Chlorfenvinphos(mg/kg) |
| 7. | Chlorpyrifos(mg/kg) |
| 8. | Chlorpyrifos-methyl(mg/kg) |
| 9. | Cypermethrin(mg/kg) |
| 10. | DDT (Sum of p,p,-DDT, p,p-DDE and p,p,-TDE (mg/kg) |
| 11. | Deltamethrin(mg/kg) |
| 12. | Diazinon (mg/kg) |
| 13. | Dichlorvos (mg/kg) |
| 14. | Dithiocarbamates(mg/kg) |
| 15. | Endosulfan (Sum of isomer and Endosulfan sulphate) (mg/kg) |
| 16. | Endrin(mg/kg) |
| 17. | Ethion(mg/kg) |
| 18. | Fenitrothion(mg/kg) |
| 19. | Fenvalerate(mg/kg) |
| 20. | Fonofos(mg/kg) |
| 21. | Heptachlor (Sum of Heptachlor & Heptachlor epoxide) (mg/kg) |
| 22. | Hexachlorobenzene(mg/kg) |
| 23. | Hexachlorocyclohexane isomer (other than γ) (mg/kg) |
| 24. | Lindane (γ -Hexachlorocyclohexane) (mg/kg) |
| 25. | Malathion(mg/kg) |
| 26. | Methidathion(mg/kg) |
| 27. | Parathion(mg/kg) |
| 28. | Parathion Methyl(mg/kg) |
| 29. | Permethrin(mg/kg) |
| 30. | Phosalone(mg/kg) |
| 31. | Piperonyl butoxide(mg/kg) |
| 32. | Primiphos Methyl(mg/kg) |
| 33. | Pyrethrins(mg/kg) |
| 34. | Quintozen (Sum of Quintozone, pentachloroaniline and methyl pentachlorophenyl sulphide) (mg/kg) |

4. Conclusions

Currently there is limited scientific evidence for the safety, efficacy and authenticity of traditional medicines which usually does not provide standards for assertive identification. Thus, standardization is vital for evaluation of the quality of traditional medicines. Zuroor-e-Qula was evaluated through pharmacopoeial parameters which certainly provide an assurance of quality of the drug. All the physico-chemical parameters, quality control parameters *viz.* heavy metals, microbial load, aflatoxins and pesticidal residue were found within permissible limits which conspicuously show that the drug Zuroor-e-Qula is free from harmful toxins and can be used safely. Hence, the present study contains high significance to ensure authenticity and quality of the drug.

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