Safety evaluation of a polyherbal formulation, Zuroor-e-Qula

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Abstract
Zuroor-e-Qula, a powdered polyherbal Unani formulation, known to possess anti-microbial and anti-inflammatory properties is recommended in cases of stomatitis and gastric ulceration. The freshly prepared formulation was evaluated for its mineral contents, microbial count, aflatoxins and pesticide residues. The results revealed that this formulation is free from such contaminants and its use is safe.

Keywords: Zuroor-e-Qula, Polyherbal formulation, Unani medicine, Mineral content, Microbial count, Aflatoxins, Pesticide residues.

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Introduction
Herbal drugs have found wide spread use in many countries not only because they are easily available and are cheaper but an important reason has been the notion that they are safer than synthetic drugs which may not always be true. According to WHO guidelines, an herbal product needs to be standardized with respect to safety before releasing it into the market.

Zuroor-e-Qula, a powdered polyherbal Unani formulation containing ingredients such as Kathsafoad [extract of Acacia leucophloea Willd. (Mimosaceae) 4 parts], Tabasheer [siliceous deposits from the culms of Bambusa arundinacea (Rezt.) Roxb. syn. B. bambos Druce (Poaceae) 4 parts], Kafoor [Cinnamomum camphora (Linn.) Nees & Ebrem. (Lauraceae) 1 part], Danaheelkhurd [seeds of Elettaria cardamomum Maton var. major (Zingiberaceae) 4 parts], Gulnafarsi [flowers of Punica granatum Linn. (Punicaceae) 4 parts], Gule-surkh [flowers of Rosa × damascena Mill. (Rosaceae) 4 parts], Kababe kandan [seeds of Zanthoxylum armatum DC. (Rutaceae) 4 parts] was evaluated for its mineral content (calcium, magnesium, aluminium, arsenic, cadmium, zinc, lead, chromium and mercury), microbial count (Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, total viable aerobic bacteria, yeasts and mould), aflatoxins (B1, B2, G1, G2) and pesticide residues (Aldrin, Benzene hexachloride, DDT, Dieldrien and Malathion). The mineral elements were determined by using atomic absorption spectrophotometer, Shimadzu, AA-640-13. In microbial count the colonies obtained on specified media, were subjected to suitable biochemical test along with the pure strain to know the presence or absence of microbes. Test for aflatoxins and pesticide residues were done by qualitative thin layer co-chromatography with reference samples. The formulation is known to possess anti-microbial and anti-inflammatory properties and is used in cases of stomatitis and gastric ailments. Fresh formulation prepared in the laboratory was tested for the presence of above-mentioned elements, microbes, aflatoxins and pesticide residues.

Materials and Methods

Determination of minerals
Clean dried ingredients were used for preparation of powdered formulation and hence were devoid of any foreign materials. It was heated at 150°C to a constant weight. About 5g of the powder was then incinerated in a muffle furnace at 400°C and the ash obtained was moistened with 0.5ml of sulphuric acid and re-heated on a heating mental till it gets free from fumes of sulphuric acid. The sulphated ash obtained was then incinerated for 2 to 3 hours in a muffle furnace at 600°C till its weight remained constant and then dissolved in 100ml of 5% hydrochloric acid4. This acid solution
of ash was then subjected to determination of various mineral elements by using atomic absorption spectrophotometer, model, Shimadzu, AA-640-13 (Ref 5, 6).

Standard solutions of all the mineral elements were prepared, the instrument was calibrated and the concentration of minerals in ppm was measured directly in the test solution.

**Microbial count**

Determination of microbial count was done at Analytical Testing Division, Arbopharmaceuticals Ltd, Industrial Area, New Delhi as per the WHO guidelines7, 8.

**Qualitative detection of Aflatoxins**

**Sample preparation**

About 50g of the powdered drug was weighed into a mixing jar; 25ml of saturated sodium chloride solution and 250ml of methylene chloride were added in the jar and blended for 3 minutes at high speed. It was then filtered through high porosity folded paper into 50ml-graduated cylinder and 50ml filtrate...
is transferred to 250ml glass stoppered Erlenmeyer flask. The extract was evaporated to near dryness on steam bath and methanol: 5% sodium chloride: hexane (50:50:50) were added to it; shaken for 10 minutes on wrist action shaker and transferred to 250ml separating funnel. It was allowed to stand for 5-10 minutes and lower aqueous layer is drained into another 250ml separating funnel. To the aqueous layer, added 50ml of carbon tetrachloride and shaken vigorously for one minute and then allowed to separate the layer. Methylene chloride (50ml) was added to retain aqueous layer and shaken for one minute then methylene chloride layer is drained into 250ml Erlenmeyer flask and extracted aqueous layer with additional 25ml methylene chloride. Combined the methylene chloride extracts and evaporated to near dryness on steam bath.

Column chromatography
A bed of glass wool was placed in the bottom of chromatographic column and one cm high anhydrous sodium sulphate is added to give base for silicone gel. Methylene chloride (up to 3cm high) was added to retain silicone and slowly added 2cm bed of anhydrous sodium sulphate to it.

The extract was dissolved in 5ml methylene chloride and charged the extract solution to the column and sequentially eluted at maximum flow rate with 40ml methylene chloride, 40ml benzene-acetic acid (9:1), 40ml hexane and 40ml anhydrous ether and discarded the elutes. Chloroform-acetone-isopropanol (85:10:5) was used as an eluting system for aflatoxins. The elute was collected and evaporated to dryness on steam bath under nitrogen and reserved for thin layer chromatography.

Aflatoxin reference samples
Different aflatoxin reference samples were prepared in benzene acetonitrile (98:2).

Particulars of TLC
Thin layer plates: Precoated silica gel G F₂₅₄ plates (10 × 20cm) of uniform thickness (0.2mm).
Chromatographic chamber: Glass tank with a lid.
Solvent system: Chloroform-acetone-isopropanol (85:10:05).
Detection: Under UV chamber fitted with 15-watt long wave ultraviolet lamp.

Qualitative detection of pesticide residues
Test sample
Acetonitrile-water mixture (650:350) was added to 20-50g of the drug powder and blended for 5 minutes at high speed and filtered. The filtrate was transferred into one litre separating funnel and 100ml of light petroleum ether was added to it. The contents were shaken for one to two minutes and added 10ml of sodium chloride (400g/l) and 600ml of distilled water. Separating funnel was shaken vigorously for 30-45 seconds and allowed the solvent layer to get separated. Collected the petroleum ether layer, washed thrice with water, and then treated with anhydrous sodium sulphate. The extract was subjected to column chromatography. The column was packed with activated florosil and the column was eluted with petroleum ether. Collected three fractions of 200ml each.

The first elute contains chlorinated pesticides like Aldrin, Benzene hexachloride, DDT, etc. While second elute contains Dieldrien, and the third elute contains Malathion. The elutes were concentrated to 10ml and used for the thin layer chromatography.

Standard samples: All the reference samples were prepared in petroleum ether.
Adsorbent: Precoated silica gel G F₂₅₄ plate (10 × 20cm) of uniform thickness (0.2mm).
Solvent system: n-hexane: acetone (7:3).
Detection: Under iodine treatment and UV chamber.

Results and Discussion
The percentage concentration (ppm) of calcium, magnesium, aluminum and zinc, 0.65, 0.47, 0.071 and 0.06%, respectively were found in Zuroor-e-Qula. While the heavy metals like arsenic, cadmium, lead, chromium and mercury were absent.

In case of determination of microbial contamination, the colonies obtained on specific media were subjected to suitable microbial test along with pure strain to know the presence or absence of microbes. The qualitative detection of pesticide residues revealed that, the formulation does not undergone either accidental or intentional contamination by the pesticides. The studies for microbial contamination revealed that the pathogenic microbes like S. typhi, P. aeruginosa and S. aureus were absent. The total viable aerobic bacteria, yeasts and moulds were more than 1100/g of the sample and E. coli was less than 10.
but more than 1/g of the sample. Aflatoxins (B$_1$, B$_2$, G$_1$, G$_2$) and pesticide residues (Aldrin, BHC, DDT, Dieldrein, Malathion) were also absent. The values obtained may be attributed to the environmental conditions, storage conditions, harvesting, handling, production methods and the soil in which the plants grown.

**Conclusion**

According to WHO guidelines herbal product should be standardized with respect to safety before releasing it into the market. Hence, the freshly prepared formulation of *Zuroor-e-Qula* (a powdered polyherbal Unani formulation) was evaluated for its mineral contents, microbial counts, aflatoxins and pesticide residues and found to be free from such contaminants.

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**References**


