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Quality standards & safety profile of saffof-e-ood– A multiple herb mixture used in Unani system of medicine

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Abstract
In Unani System of Medicine, Safoof-e-Ood is a multiple herb mixture in fine powdered form frequently used by the unani physicians to cure the patients suffering from Zof-e-Meda (weakness of the stomach) and Ishal (diarrhoea). Present study aimed to develop quality standards of Saffof-e-Ood using various botanical; phyto-chemical and physio-chemical parameters such as organoleptic and microscopical studies, ash values, extractive values, pH and TLC profile etc. Besides these heavy metals, aflotoxins, pesticidal residue and microbial load studies have also been reported so as to develop a safe and efficacious drug for the global market.

Keywords: safoof-e-ood, multiple herb mixture, quality standards, safety profile

Introduction
The use of traditional medicine has been very popular in most of the countries of Asia, Africa, Latin America and some other parts of the world from the time immemorial [1]. But for the last few years the developed nations too have been tremendously growing interest in the use of traditional medicines. In India, traditional system of medicine especially Unani System of Medicine has been practiced to great advantage in the treatment of various diseases for centuries. Today everyone wants a safe and effective treatment for himself. These expectations of the people lead to concern over the quality of these medicines. Thus the quality standardization of the traditional medicine is evidently essential in the present scenario [2-4].
The standardization of different herbal and polyherbal unani formulations have been reported by various researchers from time to time [5-16]. But still there are many formulations which require standardization. Literature survey reports that Safoof-e-Ood is a multiple herb mixture in fine powdered form that has been used by the unani physicians to cure the patients suffering from Zof-e-Meda (weakness of the stomach) and Ishal (diarrhoea). As no standardization work on this drug has been reported uptill now; present study aimed to develop quality standards in Saffof-e-Ood using various botanical; phyto-chemical & physio-chemical parameters such as organoleptic and microscopical studies, ash values, extractive values, pH and TLC profile etc. Besides these heavy metals, aflotoxins, pesticidal residue and microbial load studies have also been reported so as to develop a safe and efficacious drug for the global market.

Methodology
Raw Materials
All the ingredients i.e. Sumbul-ut-Teeb (rhizome), Mastagi (resin), Qaranful (flower buds), Kababa (fruit), Ood (wood) and Shakar Tabarzad (purified sugar) were procured from the local raw drug dealers and were identified botanically using pharmacognostical methods [17, 18]. Safoof-e-Ood was prepared at DSRU; New Delhi as per the formulation composition given in National Formulary of Unani Medicine, Part IV [19].

Ingredients

Table 1

S.No	Ingredients	Botanical Name	Part used	Quantity
1.	Sumul-ut-teeb	*Nardostachys jatamansi* Dc.	Rhizome	10 g
2.	Mastagi	*Pistacia lentiscus* Linn.	Resin	10 g
3.	Qaranful	*Syzygium aromaticum* Merr.& L.M.Perry	Floral bud	15 g
4.	Kababa	*Zanthoxylum alatum* Roxb	Fruit	15 g
5.	Ood	*Aquilaria agallocha* Roxb	Wood	30 g
6.	Shakar Tabarzad	Purified Sugar	Crystals	Equal wt. of the drug

Method of preparation
All the ingredients of pharmacopoeial quality were taken and made free from all physical impurities and dried under the shade to remove moisture, if any.
All the ingredients were crushed separately in an iron mortar to obtain coarse powder. The coarse powder was processed further in a grinder and sieve through mesh # 80 to get a fine powder.
Then all the powdered ingredients were mixed to get Safoof-e-Ood; a multiple herbal mixture. Further it was preserved in a tightly closed plastic container free from moisture and kept in a cool, dry place.

Microscopic studies

5 g of powder was taken and stirred thoroughly in ethanol for some time to remove mastagi and sugar. The supernatant was discarded and the residue was washed with distilled water. A little residue was stained with iodine solution and mounted in 50% glycerin. Some of the residue was cleared by heating in chloral hydrate solution which was washed with distilled water, stained with safranine and then mounted in 50% glycerin. A little residue was stained with ferric chloride solution and then mounted in 50% glycerin [17, 20]. The representative photographs were taken from the computer with microscopic attachment.

Phyto-chemical studies

Preliminary phyto-chemical screening tests were carried out by using standard methods [21]. The obtained data shown in Table I.

Physio- chemical studies

The physio-chemical parameters of Safoof-e-Ood were analyzed by standard methods as per the WHO guidelines [22] like removal of foreign matters, water and alcohol solubilities, total ash, acid insoluble ash and water soluble ash, loss on drying at 105C, pH values for 1% and 10% solution [23]. The obtained data are shown in Table II.

Preparation of extract for TLC

2g. powder drug was extracted in 25 ml. of ethanol under reflux of water bath for 30 minutes and filtered. Further the filterate was concentrated upto 5ml.and used for thin layer chromatography [24, 25].

Thin Layer Chromatography

Thin layer chromatography was carried out on T.L.C. pre coated silica gel aluminium plate using Chloroform: Ethanol (7: 3) as mobile phase. On spraying with 5% ethanolic sulphuric acid followed by heating at 105 C for 10 minutes in an oven Rf of the spots were calculated by the following formula [26] and the data is shown in Table III

$$\text{Rf value} = \frac{\text{Distance travelled by the spot}}{\text{Distance travelled by the solvent}}$$

Safety Profile Studies

The safety profile parameters like microbial load, heavy metals, aflotoxin and pesticide residues were carried out using standard methods of WHO and AOAC guidelines [27]. The results are shown in Table IV, V, VI, VII respectively.

Results and Discussion

Organoleptic properties
Appearance: Powder
Colour: Greyish black
Odour: Aromatic
Taste: Slightly sweet

Microscopic Observations

On examination under the microscope, following cells/ tissues/ cell contents etc. were observed:-

- **Sumbul-ut-teeb:** Pieces of fibres either single or in groups, fragments of vessels with reticulate thickening (Fig. 1 & 2)
- **Qaranful:** Pollen grains triangularly lenticular approx. 15µ - 20 µ in diameter (Fig 3)
- **Kababa:** Parenchymatous cells filled with hesperidin and oil globules (Fig. 4)
- **Ood:** Pieces of aseptate fibres and pieces of simple pitted vessels with dark brown content (Fig. 5 & 6)

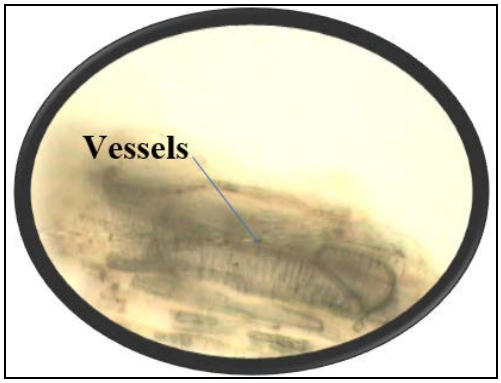

Fig 1: (x40)

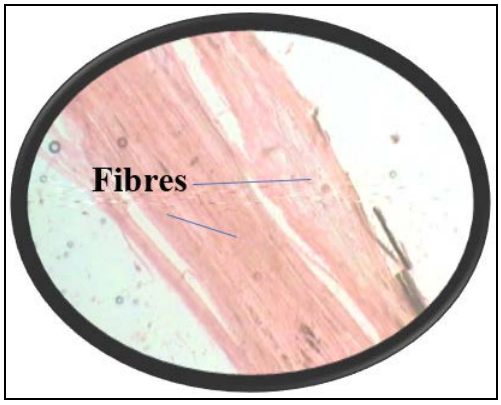

Fig 2: (x40)

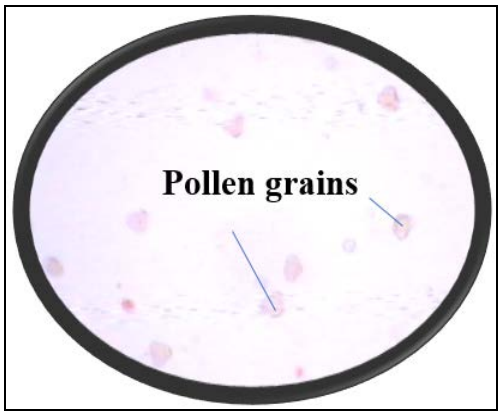

Fig 3: (x40)

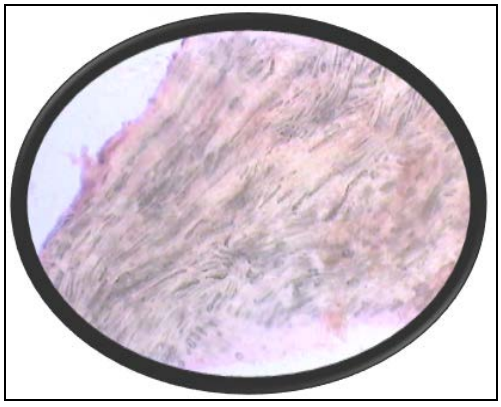

Fig 4: (x10)

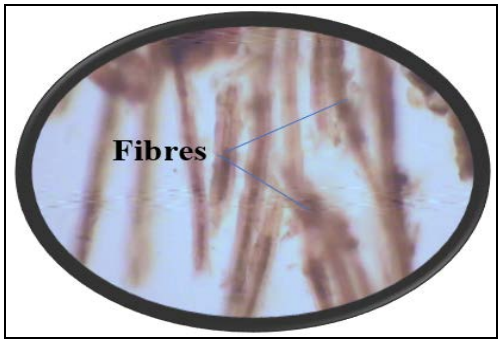

Fig 5: (x40)

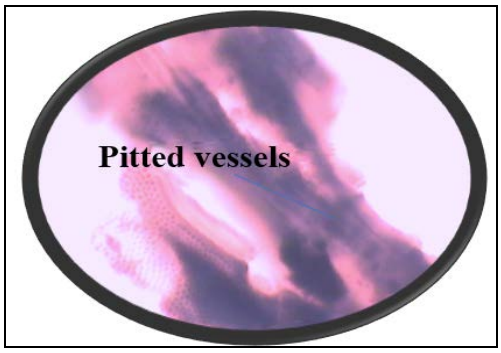

Fig 6: (x40)

Microscopical studies of Saffof-e-Ood: Fig. 1-6 pieces of reticulate vessels & group of fibres of Sumbul-ut-teeb in x40 (Fig. 1 & 2) Pollen grains of Qaranful in x40 (Fig. 3); Parenchyma cells filled with hesperidin &oil globules in Kababa in x10 (Fig. 4); Pieces of fibres & Pitted vessel of Ood in x40 (Fig. 5 & 6)

Phyto-chemical Parameters

Phyto-chemicals are the natural bioactive compounds present in the plants to provide protection against various diseases. The phyto-chemical analysis of Safoof-e-Ood reveals the presence of alkaloids, glycosides, tri terpenes, phenolics, flavonoids, tannin and resin in it hence confirming its therapeutic significance.

Table 2

S. No.	Phytoconstituents	Tests	Result
1.	Alkaloids	Dragandroff's Reagent	Present
2.	Glycosides	Libberman Test	Present
3.	Triterpenes	Selkawaski Test	Present
4.	Phenolic	Phenol	Present
5.	Flavonoids	Magnessium Test	Present
6.	Tannin	Ferric chloride Test	Present
7.	Resin	Acetic anhydride test	Present

Physico chemical Parameters

Physico- chemical screening is the preliminary and key aspect to establish a standard data for the quality control of any pharmaceutical formulation.

Water-soluble and alcohol soluble extractive values plays an important role in evaluation of finished herbal products. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage or formulating. The water soluble extractive value of Saffof-e-Ood was found to be 61.17% whereas alcohol soluble extractive value is 24.97%

The determination of ash is quite useful parameter in the detection of low-grade products, exhausted drugs and excess of sandy or earthy matter and is more applicable in powdered herbal formulations. High total ash value indicates the presence of inorganic constituents and very low value of acid insoluble ash denotes the presence of negligible amount of siliceous matter. The low value of total ash (1.67%) and acid insoluble ash (o.45%) indicates the presence of negligible amount of siliceous matter in Safof-e-Ood.

Moisture content should be minimum to prevent the degradation of any pharmaceutical product. Excess of water in a drug encourage microbial growth, presence of fungi or insects and deterioration following hydrolysis. As finished Safoof-e-Ood contains less amount of moisture, (7.50%) it can be expected that it will be stable/safe for a longer time.

The pH conventionally represents the acidity or alkalinity. Aquous extract with a pH range between 5-6 indicates that the formulation is acidic in nature

Table 3

Parameters	Values	Mean	Range
Alcohol Soluble matter (%)	25.20; 24.80; 24.90	24.97	24.80 – 25.20
Water Soluble matter (%)	61.21; 61.20; 61.10	61.17	61.10 – 61.21
Total ash (%)	2.00; 1.50; 1.50	1.67	1.50 – 2.00
Acid Insoluble ash (%)	0.50; 0.40; 0.45	0.45	0.40 – 0.50
Loss in wt on drying at $105^{O}C$ (%)	8.00; 7.00; 7.50	7.50	7.00 – 8.00
pH of 1% aqueous solution	6.02; 6.05; 6.04	6.04	6.02 – 6.05
pH of 10% aqueous solution	5.72; 5.60; 5.55	5.64	5.55 – 5.72

T.L.C. Results

TLC is used to monitor the progress of a reaction and identify the compound present in a given substance. It is used as an important and reliable tool to separate the identical compounds present in a herbal mixture.

TLC of Safoof-e-Ood in ethanolic extract shows 3 spots with Rf values 0.12 (black); 0.60 (violet) and 0.96 (violet).

Table 4

Extract	Solvent system	Detection/Spray treatment	Number of Spots	Rf values
Ethanol	Chloroform: Ethanol (7:3)	5% Ethanolic sulphuric acid	03	0.12 (Black) 0.61 (Violet) 0.96 (Violet)

Microbial Load

Presence of microbial contaminant such as bacteria and fungi alters the physiochemical characteristics and hence inactivates the therapeutic activity of any pharmaceutical product. Microbial load estimation provides an idea about the quality and safety assessment of the prepared formulation.

Microbial load investigation of the formulation Safoof-e-Ood reveals that the mixture is safe for therapeutic uptake as it is free from any microbial contamination.

Table 5

S.No.	Parameter Analysed	Result	Permissible Limits as per WHO
1.	Total Microbial load	Nil	Not more than 10^5/g
2.	*Salmonella spp*	Nil	Nil
3.	*Escherichia coli*	Nil	Nil
4.	Total *Fungal* count	Nil	Not more than 10^3/g

Heavy Metals

Unintentional or accidental contamination by heavy metals such as lead, cadmium, arsenic and mercury results an adverse effect to the human health even at very low concentration. As they are not easily mobilized by the body, they produce severe toxic effects by interfering the normal biochemical and metabolic processes. Estimation of the comprehensive risk of heavy metal contamination was conducted for Saffof-e-Ood and the results were found within the permissible limit of WHO and Pharmacopoeia Commission of Indian Medicine & Homeopathy (PCIM&H).

Table 6

S.No.	Parameter Analysed	Result	Permissible Limits as per WHO
1.	Lead	0.111 ppm	Not more than 10 ppm
2.	Cadmium	Nil	Not more than 3.0 ppm
3.	Arsenic	Nil	Not more than 3.0 ppm
4.	Mercury	Nil	Not more than 1.0 ppm

Aflotoxin Level

Aflotoxins are the highly toxic metabolites derived from the polyketides produced by fungal species such as *Aspergillus flavus*, *A. parasiticus* and *A nomius*. The major aflatoxins re B1, B2, G1 and G2 that posses serious threat to human health through various complications such as hepatotoxicity, teratogenicity and immunotoxicity. Aflatoxins studies were conducted for Saffof-e-Ood and their absence ensures this herbal mixture is safe for therapeutic intake.

Table 7

S.No.	Parameter Analysed	Result	Permissible Limits as per WHO
1.	B1	Nil	Not more than 0.50 ppm
2.	B2	Nil	Not more than 0.10 ppm
3.	G1	Nil	Not more than 0.50 ppm
4.	G2	Nil	Not more than 0.10 ppm

Pesticidal Residue

Pesticidal residue gets accumulated through various agricultural practices such as spraying, treatment of soils during cultivation and administering of fumigants during storage of herbal drugs. Hence it is important that the herbal products should be free from these chemicals. Pesticidal residue analysis of Safoof-e-Ood was performed and no toxicity is reported in the mixture.

Table 8

S.No.	Parameter Analyzed	Result	Permissible Limits as per WHO
1.	DDT	Nil	Not more than 1.00mg/kg
2.	Endosulfan	Nil	Not more than 3.00mg/kg

Conclusion

In the present investigation, the microscopic studies establishs the presence of each ingredient in the herbal mixture Safoof-e-Ood. Various phyto-chemical & physico chemical parameters along with Rf values of the TLC profile are helpful in establishing the standards for the herbal mixture. Besides microbial load contamination, heavy metal analysis, aflatoxins contamination, pesticide residue analysis was done and found absent justifying the quality of this efficacious drug for the global market.

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