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Research Article

QUALITY STANDARDS OF *SAFOOF-E-MUHAZZIL*, AN ANTI-OBESITY UNANI FORMULATION

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ABSTRACT

Objective: The objective of this study is to establish the quality standards of *Safoof-e-Muhazzil* (*SEMZ*), a polyherbal Unani formulation for the treatment of overweight and obesity. Methods: The quality standards of *SEMZ* were established as per WHO guidelines including heavy metal residues, pesticides residue, aflatoxins, microbial load, GC-MS analysis and HPTLC fingerprinting analysis. Results: The results of standardization parameters showed total ash (10.73±1.23 %), acid-insoluble ash (1.47±0.21%), water-soluble ash (2.91±0.11%), alcohol-soluble extractive (24.95±1.47%), water-soluble extractive (37.46±2.14%), loss on drying (10.03±0.42 %), pH-value for 1% solution (6.01) and 10 % solution (5.3) at 25±2 ºC, fat content (14.13±0.10 %), resin content (9.475±1.2 %) and heavy metals residue, pesticides residue, aflatoxins, microbial load complies with official limits. Conclusion: Data's evolved in this investigation may be used in laying down Pharmacopoeial standards for the formulation studied, as standardization of herbal medicines as well as polyherbal formulations are absolutely essential and is the need of the hour.

Keywords: *Safoof-e-Muhazzil*, Standardization, Unani System of Medicine, WHO guidelines.

INTRODUCTION

The World Health Organization (WHO) has estimated that about 80 % of World's population relies on traditional medicines for primary health care[1]. India is the richest source of medicinal and aromatic plants. Herbal drugs are gaining popularity again day by day in the World since last decades because of its efficacy and lower toxicity as compared to allopathic drugs. *Safoof-e-Muhazzil or Sufoof-e-Mohazzil* (*SEMZ*) is a polyherbal formulation used in the Unani System of Medicine for treatment of obesity since ages. The formulation given in National Formulary of Unani Medicine[2] contains ajwain (Nankhwah-*Trachyspermum ammi* Linn., seeds), ajmoth (Tukhm-e-Karafs-*Apium graveolens* Linn., seeds), jatamansi (Sumbul-ut-Teeb-*Nardostachys jatamansi* DC, rhizomes), red rose (Gul-e-Surkh-*Rosa damascena* Mill., petals), oregano (Marzanjosh- *Origanum vulgare* Linn., whole plant) and lakh maghsool (Natural resin-animal origin *Laccifer lacca* Kerr.)[2,3]. *Trachyspermum ammi* syn. *Carum copticum* (Apiaceae) commonly known as ajwain, is a glabrous annual plant. Its fruits are aromatic, stems hollow, striated, much branched[4]. Thymol, the major phenolic compound present in ajwain, has been reported to be a germicide, antispasmodic and antifungal agent[5]. *Apium graveolens* L. (Apiaceae), known as celery, is a common European plant that is now grown, consumed and used as a popular vegetable and spice all over the world. Due to its pleasant odor and nutritional, pharmacological and medicinal values[6,7], this plant product is deemed to be suitable for humans, pets and the environment[8].

Nardostachys jatamansi DC. is a small, perennial, dwarf, hairy, rhizomatous, herbaceous, endangered and most primitive species within family Valerianaceae, distributed in the Himalayas from Pakistan, India (Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Sikkim) to Nepal, Tibet and China between 3300 to 5000 m asl[9]. *Rosa damascena* Mill belongs to the family Rosaceae, many species and variety of which are cultivated throughout the world as an ornamental plant[10,11]. *Origanum vulgare* L. belonging to mint family Lamiaceae, is an aromatic plant with a wide distribution throughout the Mediterranean area and Asia[12]. Major components are carvacrol and thymol that constitute about 78 to 82% of the total oil[13]. Lac (Lakh Maghsool) is the only natural resin of animal origin, produced by a red coloured tiny Lac insect, *Laccifer lacca* (Kerr) found throughout India. Fourteen species of insect *Laccifer* are reported in India on a number of plants both wild and cultivated in various regions[14]. The present study deals with the evaluation of quality parameters of *SEMZ*.

MATERIAL AND METHODS

Plant Material and Authentication

All the drugs were purchased from Samsi Dawakhana, Ballimaran, Delhi and authenticated by Dr. H.B. Singh, Scientist F and Head, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. Voucher specimens of drugs were deposited in the Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, with reference number Ref. NISCAIR/RHMD/consult/-2010-11/1705/05.

Preparation of Formulation

All the drugs were dried in shade and powdered and passed through sieve no. 100. The formulation was prepared as the method described in National Formulary of Unani Medicines[2]:

1. Ajwain (*Tachyspermum ammi* L.)Seeds10 g.
2. Ajmoth (*Apium graveolens*) Seeds 10 g.
3. Jatamansi (*Nardostachys jatamansi*) Roots10 g.
4. Luk Maghsool (Purified *Laccifer lacca*)Resin10 g.
5. Gul-e-Surkh (*Rosa damascena*) Petals 25 g
6. Marzanjosh (*Origanum vulgare*) Whole plant 25 g

Morphological studies

Proper examination of the each ingredients of the *SEMZ* was carried out under diffused sunlight and artificial source similar to day light (Fig 1)[15].

Powder microscopy

The microscopic examination of powdered *SEMZ* was performed. Slides of powdered *SEMZ* were prepared and stained with chloral hydrated, phloroglucinol and HCl. Microphotography on different magnifications was carried out with Motic microscopic unit (Hong Kong). Polarized light was used for the study of crystals, starch granules and lignified cell[15].

Physico-chemical standardization

The various physico-chemical values of *SEMZ* such as ash values (Table 1), extractive values[16] (Table 2), loss on drying were determined according to the Pharmacopoeial method[17].

Powdered drug reaction with different reagents

The powdered *SEMZ* was treated separately with different reagents and acids like, picric acid, hydrochloric acid, nitric acid, iodine, ferric chloride, and sodium hydroxide the colour shown by that treatment is noted as such and under the microscope[18] (Table 3).

Fluorescence Analysis

Many herbs fluorescence when cut surface or powder is exposed to UV light and this can help in their identification method. The fluorescence character of the *SEMZ* powder (40 mesh) was studied both in daylight and UV light (254 and 366 nm) and after treatment with different reagents like sodium hydroxide, picric acid, acetic acid, hydrochloric acid, nitric acid, iodine, ferric chloride etc (Table 4)[19,20].

Phytochemical screening

The phytochemical evaluation of *SEMZ* was carried out as per the method described[21]. Powdered *SEMZ* (5 g) were extracted in a Soxhlet apparatus with petroleum ether, chloroform, acetone, alcohol and water. The extracts were evaporated to dryness under vacuum. These extract were used for the analysis of different phyto-constituents *viz*. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, and resins etc (Table 5)[16].

Loss on drying

The powdered *SEMZ* (10 gm) without preliminary drying was placed on a tarred evaporating dish and dried at 105 ºC for 6 hours and weighed. The drying was continued until two successive reading matches each other or constant weight was reached when two consecutive weighing after drying for 30 minutes in a desiccator, showed not more than 0.01 gm difference (Table 1)[22].

Determination of pH

The pH of 1 % and 10 % aqueous solution of *SEMZ* were checked (Table 1) by standardized glass electrode (Hanna pH meter, USA)[23].

Determination of fat content

A weighed quantity of *SEMZ* sample (3 g) is extracted with anhydrous ether in a continuous extraction apparatus for six hours the extract is filtered into a clean dry weighed flask. The extraction flask is rinsed with small quantity of ether, filtered and added to the weighed flask. The solvent is evaporated and dried to constant weight at 105 ºC (Table 1)[24].

Determination of resin content

The accurately weighed *SEMZ* sample (5 g) was rapidly refluxed with acetone (3 X 200 ml) for 6 h to exhaust the drug for the resin content. The excess solvent was removed by distillation on a water bath. The residue so obtained was suspended in water and transferred to a separating funnel, repeatedly extracted the suspension with solvent ether (2 X 200 ml) to extract all the resin contents. The ether extracts were cooled out dried over anhydrous sodium sulphate and excess ether removed over a water bath. It was transferred to a weighed beaker and the final weight is noted (Table 1)[24].

Determination of microbial load (Table 6, fig 3), heavy metal residues (Table 7) and pesticides residue contents (Table 8) and aflatoxins (Table 9) were also done as per WHO guidelines[15].

GC-MS analysis of *SEMZ*

The analysis of the volatile constituents were run on a Shimadzu QP-2010 GC-MS system equipped with AB-Innowax 7031428 WCOT column (60 m x 0.25 mm x 0.25 µm) directly coupled to the MS. The carrier gas was helium with a flow rate of 1.21 ml/min oven temperature was programmed as 50 ºC for 1 min and subsequently held isothermal for 2 min injector port: 250 ºC, detector: 280 ºC, split ratio 1: 50, volume injected: 1 µl of the oil. The recording was performed at 70 eV, scan time 1.5 s; mass range 40-750 amu. Software adopted to handle mass spectra and chromatograph was a Chem station. Further identification was made by comparison of fragmentation pattern of mass spectra obtained by GC-MS analysis with those stored in the spectrometer database of NBS 54 K.L, WILEY 8 libraries and published literature[25-30].

HPTLC fingerprinting analysis of *SEMZ*

The weighed quantity (20 g) of *SEMZ* was extracted in a Soxhlet apparatus for 6 h using twice the amount of solvent (hexane) at a controlled temperature. The dried extract was dissolved in the respective solvent (2 mg/ml). HPTLC was performed on 5 cm × 10 cm aluminum backed plates coated with silica gel $60F_{254}$ (Merck, Mumbai, India). Standard solution of thymol and sample solution were applied to the plates as bands 5.0 mm wide of the same chromatographic plate by use of a Camag (Muttenz, Switzerland) Linomat V sample applicator equipped with a 100 µl Hamilton (USA) syringe. Ascending development to a distance of 80 mm was performed at room temperature (28 ± 2°C), with toluene: ethyl acetate, 9 : 1 (*v/v*), as mobile phase, in a Camag glass twin-trough chamber previously saturated with mobile phase vapour for 20 min. After development, the plates were dried and sprayed with anasaldehyde-sulphuric acid reagent and then scanned at 513 nm with a Camag TLC Scanner with WINCAT software, using the deuterium lamp (Fig.3) [31].

RESULTS AND DISCUSSION

Morphological characters

Proper examination of the untreated samples of ingredients of *SEMZ* was carried out under diffused sunlight and artificial source similar to day light (Fig. 1).

Trachyspermum ammi

Apium graveolens

Nardostachys jatamansi

Rosa damascena *Origanum vulgare* *Lakh resin*

Fig. 1: Ingredients of *Safoof-e-Muhazzil*

Organoleptic characteristics of *SEMZ* powder

Colour: Dark brown

Odour: Characteristics, aromatic

Taste: Acrid

Powder microscopy

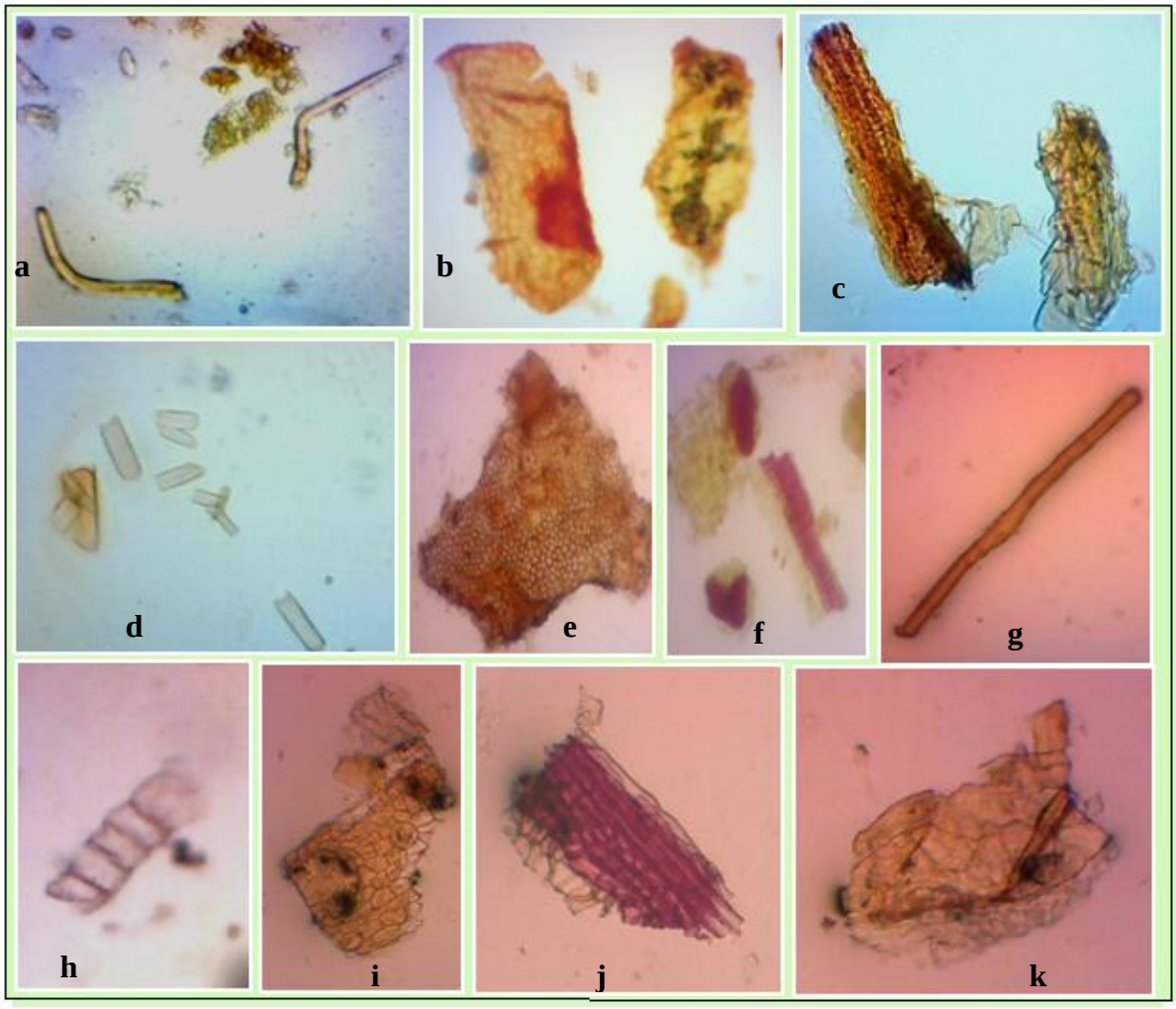

Fig. 2: Powder characteristics of *Safoof-e-Muhazzil*

a. Trichomes, parenchymatous cells, oil globules, calcium oxalate crystals; **b.** Parenchymatous cells of *R. damanscena* petals; **c.** Tangentially cut medullary rays of *N. jatamansi*; **d.** Calcium oxalate crystals; **e.** sclerenchymatous cells with oil glands; **f.** Endocarp of seeds; **g.** Fibre; **h.** Broken trichome; **i.** Cortex with vascular bundle; **j.** Xylem vessel; **k.** Stone cells.

Table 1: Physico-chemical parameters

Parameters	Values (% Mean ± SEM)
Total ash value	10.73 ± 1.23
Acid insoluble ash	1.47 ± 0.21
Water soluble ash	2.91 ± 0.11
Loss on drying	10.03 ± 0.42
Resin content	9.475 ± 1.2
Fat content	14.13 ± 0.10
pH values	6.1 (1 % solution)
	5.3 (10 % solution)

Extractive values

The extractive values of *SEMZ* were done as per procedure described above. All the procedure was repeated in triplicate (Table 1). The mean values ± SEM are given below:

Table 2: Extractive values of *SEMZ*

Extraction	Extractive value, Mean (%) ± SEM					
	Petroleum ether	Chloroform	Acetone	Alcoholic	Hydro alcoholic	Aqueous
Hot	8.83 ±0.72	4.86 ± 0.38	14.68±1.26	18.68± 2.73	21.36 ±1.37	23.18±1.52
Cold	7.07± 0.47	5.81 ± 0.18	7.81 ±0.38	17.10± 1.38	24.12±1.72	25.97± 1.12
Successive	5.67± 0.82	14.05± 0.94	16.92±0.75	24.95±1.47	44.65± 2.38	37.46±2.03

Table 3: Powdered drug reaction with different reagents

The powder of *SEMZ* was treated with different reagents which showed following colour:

Chemical treatment	Observation	Chemical treatment	Observation
Iodine	Dark brown	Sodium hydroxide (5%)	Light brown
50% HNO_3	Orange	Glacial acetic acid	Brown
1N H_2SO_4	Crimson red	Ferric chloride (5%)	Black
Lead acetate	Light green	1N HCL	Brown
Picric acid	Yellow	KOH (1%)	Orange

Table 4: Fluorescence analysis of *SEMZ*

The powder of *SEMZ* (mesh size 40) was examined under daylight and UV light. The observation was recorded as under:

Treatment with	Day light	UV light (254 nm)	UV (366 nm)
Powder as such	Light brown	Light brown	Dark brown
Distilled water	Light brown	Brown	Black
5% NaOH	Brownish red	Black	Black
H_2SO_4	Dark brown	Dark brown	Black
Conc. HCL	Dark brown	Black	Black
Acetone	Brown	Reddish brown	Dark brown
Chloroform	Greenish yellow	Dark brown	Black
Conc. HNO_3	Yellowish red	Dark brown	Black
Methanol	Yellowish green	Red	Light green
$FeCl_3$	Grey	Brown	Black

Table 5: Results of phytochemical screening of *SEMZ*

Extract constituents	Petroleum ether	Chloroform	Acetone	Alcoholic	Hydro-alcoholic	Aqueous
Alkaloids	-	+	-	+	+	+
Glycosides	-	+	+	+	++	+
Tannin	+	++	++	++	+++	+++
Phenolics	+	++	+	+++	++	++
Flavonoids	-	+	++	+++	++	++
Proteins	-	-	-	+	+	+
Saponins	-	-	-	+	+	+
Resins	+	+	++	+	-	-
Sugars	+	+	+	++	++	+++
Lipids/fats	++	+	+	+	-	-

(+++) Strongly Positive, (+) Positive test, (-) Negative test

Table 6: Microbial load

Colony forming units on nutrient agar medium.

Test	Result
Total plate count cfu/ g	23181
Yeast/mould	886

Table 7: Heavy metal content of *SEMZ*

Heavy metal	Heavy metal in the formulation	Pharmacopoeial percentage limit of heavy metals in crude drugs
Lead	Not Detected	Not more than 10.0 ppm
Cadmium	Not Detected	Not more than 0.3 ppm
Mercury	Not Detected	Not more than 1.0 ppm
Arsenic	Not Detected	Not more than 10.0 ppm

Table 8: Pesticides residue of *SEMZ*

Pesticides	Test Method	Result	MDL
γ-BHC(Lindane)	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
δ-BHC	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
Heptachlor	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
Chlorpyrifos	AOAC 970.52/EPA 525.2	0.3 mg/kg	0.01 mg/kg
α-Chlordane	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
α-Endoulfan	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
β-Chlordance	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
Endrin	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
Total DDT	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
Aldrin	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
Methoxychlor	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg
Dieldrin	AOAC 970.52/EPA 525.2	Not Detected	0.01 mg/kg

Aflatoxins

Aflatoxin B1, Aflatoxin B2, Aflatoxin G1 and Aflatoxin G2 was found below the detection limit while minimum detection limit was 1.0 µg/kg.

GC-MS analysis

Fifty eight compounds representing 99.35 % of the oil were characterized. Consisting of thirteen monoterpenes (11.62 %), forty four sesquiterpenes (85.05 %), two diterpene alcohols (0.56 %), *trans*-phytol and 13(16),14-labdiene-8-ol, four aliphatic hydrocarbons *n*-eicos-15-enol, 9,12-octadecadienoic acid methyl ester, *n*-heptacosane and methyl-6-hydroxyoctadecanoate and two aromatic components 2-benzilidine and amyrolin. Eudes-4(14),11-diene (28.61 %), viridiflorol laurate (16.40 %), bisabolene (9.73 %), globulol (9.13 %), thymol (6.14 %), *t*-cadinol (4.15 %), *trans*-cadine-1,4-diene (2.08 %), 2E, 6E-farnesol (1.41), L-limonene (1.39 %), *δ*-cadinene (1.37 %) and *β*-gurjunene (1.28 %) were the predominant compounds[3].

HPTLC fingerprinting analysis

Table 9: TLC Details of Test Solution of *SEMZ* after derivatization with anasaldehyde-sulphuric acid reagent.

***SEMZ* oil**		***SEMZ* Hexane extract**	
R_f Value	**Colour of the band**	**R_f Value**	**Colour of the band**
0.10	Pink	0.06	Violet
0.19	Faint purple	0.13	Light pink
0.25	Faint purple	0.22	Light purple
0.27	Light blue	0.33	Light blue
0.29	Orange	0.40	Pink
0.35	violet	0.46	Light brown
0.40	Light brown	**0.58**	**Orange (Thymol)**
0.44	Light blue	0.65	Purple
0.58	**Orange (Thymol)**	0.75	Light brown
0.62	Pink		
0.69	Brown		
0.74	Light brown		

1 2 3 4

Fig. 3: TLC profile of test solution of *SEMZ* after derivatization with anasaldehyde-sulphuric acid reagent.

1: Thymol standard; 2-3: *SEMZ* oil; 4: Hexane extract.

CONCLUSION

The present study is related to pharmacognostical, physicochemical and preliminary phytochemical screening of *Safoof-e-Muhazzil*. Phytochemical study is also useful to isolate the pharmacologically active principles present in the drugs. This work may be used as standard monograph for identification and evaluation of the other such formulations[32,33].

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