

Standardisation of *Sunoon-e-Mukhrij-e-Ratubat* (SMR) - A Herbo-Mineral Unani Remedy for Periodontitis

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DOI: <https://doi.org/10.52403/ijrr.20260327>

ABSTRACT

Now a days, the use of herbal pharmaceuticals has become a global trend among people returning to natural therapies. According to WHO guidelines, every herbal product should be standardized to ensure its quality, efficacy and safety. In view of this present study was done to standardize Sunoon-e-Mukhrij-e-Ratubat; (SMR) a herbo-mineral formulation prescribed by the Unani physicians to cure periodontitis which is commonly known as dental pyorrhea; a severe dental infection characterized by the inflammation and bleeding of the gums. The main aspect of the study was subjected to various parameters like taxonomic identification of the ingredients, organoleptic studies and identification of the ingredients in the formulation, physicochemical studies such as loss in weight, total ash, acid insoluble ash, pH of 5 % solution, water and alcohol soluble matter and TLC studies. Besides this heavy metal studies, pesticidal residue and microbial contamination studies were also performed so as to set the quality standards of the formulation.

Key Words: Standardization; Sunoon-e-Mukhrij-e- Ratubat; SMR; Herbo - mineral unani formulation

INTRODUCTION

Periodontitis which is also known as dental pyorrhea is a common and serious gum disease that affects millions of people around the world. It is a form of periodontal disease that primarily affects the tissues surrounding and supporting the teeth, including the gums, ligaments, and bone. If left untreated, it can lead to tooth loss and other severe health complications. [1-3]

The use of herbal medicines has increased remarkably in line with the global trend of people returning to natural therapies.[4] Herbal formulations have reached widespread acceptability as therapeutic agents for diabetics, arthritis, liver diseases, cough remedies, memory enhancers and adoptogens. [5] Now a days the people became more conscious about the side-effects of fluoride and totally chemical based toothpastes and hence there is a demand for a safe and effective herbal product. Due to its excellent therapeutic effect Sunoon-e-

Mukhrij-e- Ratubat (SMR) serves as an ideal choice among the physicians in the treatment of Periodontitis. This herbo-mineral formulation is categorized under Sunoon in the National Formulary of Unani Medicine; Part I. The main ingredients of this formulation are aaqarqarha, filfil siyah and shibb-e-yamani. [6]

Aaqarqarha (*Anacyclus pyrethrum* DC) [7-9] is a highly valued herb with a wide range of uses. Its roots are reported as anti-inflammatory and sialagogue in ancient Unani literature and very effective in treating neurological and oral health issues. [10-13]. Filfil siyah (*Piper nigrum* L) is popularly known as 'The King of Spices' throughout the world.[14] Its fruits find application in treating toothache in the Unani System of Medicine. [11, 12, 15, 16] Shinn-e-Yamani (Alum) commonly known as phitkari in Unani medicine is a mineral origin drug used for its astringent, antiseptic and haemostatic properties. It is therapeutically used in the treatment of conditions like inflammation and bleeding of the gums and

teeth and as a mouth freshener. [17- 21] Sunoon-e-Mukhrij-e- Ratubat (SMR) is the natural finished powdered product consisting of all these ingredients in equal quantity and shows excellent therapeutic effect in the management of oro-dental disorders. According to WHO guidelines, a herbal product needs to be standardized to ensure its quality, efficacy and safety. [22] In view of this present study was done on Sunoon-e-Mukhrij-e- Ratubat so as to set its quality standards.

METHODOLOGY

RAW MATERIALS

All the ingredients i.e. aqarqarha (root), filfil siyah (fruit) and shibb-e-yamani (alum) were procured from the authentic local raw drug dealers and were identified botanically using pharmacognostical methods. [23, 24] Sunoon-e-Mukhrij-e- Ratubat (SMR) was prepared at DSRU; New Delhi as per the formulation composition (Table - I) given in National Formulary of Unani Medicine, Part I [6]

TABLE - I: FORMULATION COMPOSITION

S. No	Ingredient	Botanical Name	Part used	Qty.
	Aaqarqarha	<i>Anacyclus pyrethrum</i> DC	Root	50g
	Filfil siyah	<i>Piper nigrum</i> L.	Fruit	50g
	Shibb-e-Yamani	Alum (White)	Mineral	50g



AQARQARHA



FILFIL SIYAH



SHIBB-E-YAMANI

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 the 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 # 100 to get a fine powder. Then all the powdered ingredients were mixed to get SMR; a herbo-

mineral formulation. Further it was preserved in a tightly closed container free from moisture and kept in a cool, dry place.

MICROSCOPIC ANALYSIS

Weigh 5 g of the sample and mix it with 50 ml. of water in a beaker with gentle warming, till the sample gets completely dispersed in water. Centrifuge the mixture and decant the supernatant. Wash the sediment several times with distilled water, centrifuge again and decant the supernatant. Take a few mg of the sediment in a watch glass adds a few drops of phloroglucinol and concentrated hydrochloric acid, mount in glycerine to locate lignified cells. [23, 25] The representative photographs were taken from the computer with microscopic attachment.

PHYSIO – CHEMICAL ANALYSIS

The physio-chemical parameters of SMR were analyzed by standard methods as per the WHO guidelines [26] 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 [27]. The obtained data are shown in Table II.

THIN LAYER CHROMATOGRAPHY AND HPTLC ANALYSIS [28-34]

2g. drug sample was extracted in 25 ml. of ethanol under reflux of water bath for 30 minutes and filtered. Further the filtrate was concentrated upto 5ml. and used for thin layer chromatography [28,29]

SAFETY PROFILE ANALYSIS

The safety profile parameters like microbial load and aflotoxins, heavy metals and pesticide residues were carried out using standard methods of WHO and AOAC guidelines. [35]. The results are shown in Table IV, V, VI, VII respectively.

RESULTS AND DISCUSSION

ORGANOLEPTIC PROPERTIES

Appearance	:	Fine Powder
Colour	:	Light brown
Odour	:	Characteristic aromatic
Taste	:	Astringent

MICROSCOPIC OBSERVATIONS (Fig. 1 – 6)

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

Fragment of cork cells in surface and sectional view, pieces of fibers and vessels with reticulate and scalariform thickenings (Aaqarqarha); beaker shaped stone cells of the endocarp and group of parenchyma cells filled with compact mass of round- oval starch grains measuring 1μ - 5μ in diameter and oil globules (Filfil Siyah) and certain diagnostic features common to ingredients are a group of parenchyma cells and pieces of vessels of various shape and size.

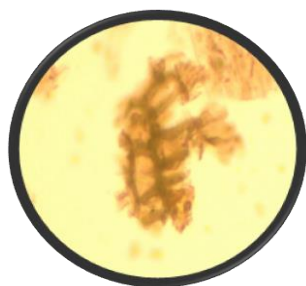


Fig.1

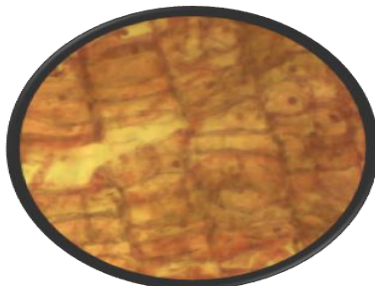


Fig. 2

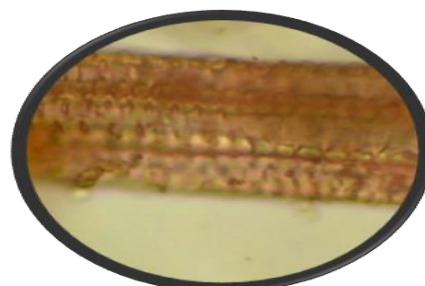


Fig. 3

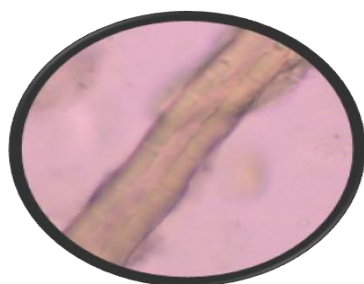


Fig. 4

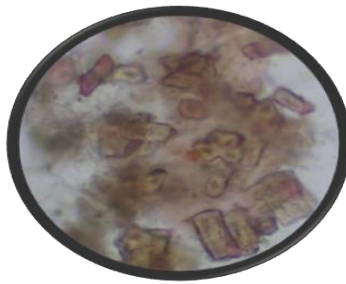


Fig. 5

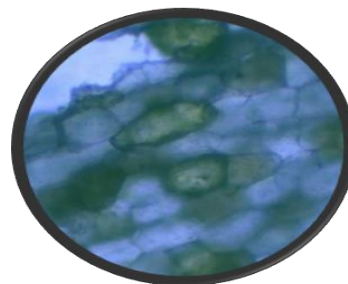


Fig. 6

Microscopic Analysis of SMR: Fig. 1: x10 Cork cells of Aaqarqarha (surface view); Fig. 2: x10 Cork cells of Aaqarqarha (sectional view); Fig. 3: x40 Vascular elements of Aaqarqarha; Fig. 4: x40 Piece of fibre of Aaqarqarha; Fig. 5: x10 Group of sclereids of Filfil Siyah; Fig. 6: x10 Parenchyma cells of Filfil Siyah filled with starch grains & oil globules

PHYSICO CHEMICAL ANALYSIS

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

chemical parameters such as alcohol and water-soluble extractives; total ash, acid insoluble ash, loss in wt. on drying at 105°C of the formulation SMR were carried out and the results are tabulated in TABLE II.

TABLE - II: PHYSICO CHEMICAL ANALYSIS

Parameters	Values	Mean	Range
Alcohol Soluble extractive (%)	5.45; 5.38; 5.31	5.38	5.31 – 5.45
Water Soluble extractive (%)	36.43; 36.50; 36.80	36.61	36.43 – 36.80
Total ash (%)	8.06; 8.00; 7.90	7.98	7.90 – 8.06
Acid Insoluble ash (%)	2.51; 2.50; 2.52	2.51	2.50 – 2.52
Loss in wt on drying at 105°C (%)	17.60; 17.50; 18.05	17.77	17.50 – 18.05
pH of 5% aqueous solution	3.97; 3.99; 4.00	3.98	3.97 – 4.00

T.L.C. ANALYSIS

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 SMR in methanolic extract shows 3 spots with Rf values 0.21 (blue), 0.73 (grey), 0.94 (brown) as tabulated in Table – III.

TABLE - III: T.L.C. ANALYSIS

Extract	Solvent system	Detection/Spray treatment	Number of Spots	Rf values
Methanol	Chloroform: Methanol (1: 1)	1% Ethanolic vanillin sulphuric acid	03	0.21 (Blue) 0.73 (Grey) 0.94 (Brown)

MICROBIAL LOAD AND AFLOTOXIN LEVEL ANALYSIS

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 SMR reveals that it is safe for

therapeutic use as it is free from any microbial contamination.

Aflotoxins are the highly toxic metabolites derived from the polyketides produced by fungal species such as *Aspergillus flavus* and *A. parasiticus*. These aflatoxins cause serious threat to human health through various complications such as hepatotoxicity, teratogenicity and immunotoxicity. Aflatoxins studies were conducted for SMR and their absence

ensures that this herbal formulation is safe for therapeutic purpose. The results of the study are tabulated in Table – IV.

TABLE - IV: MICROBIAL LOAD AND AFLOTOXIN LEVEL ANALYSIS

S. No	Parameters	Results	Reference strain (standard)
1	Total aerobic bacterial Count (TABC)	3.8×10 ³ CFU/gm	Not applicable
2	Total yeast and molds count (TYMC)	2.2×10 ² CFU/gm	Not applicable
	Enterobacteriaceae members		
3	<i>Escherichia coli</i>	ND	<i>E. Coli</i>
4	<i>Salmonella sp.</i>	ND	<i>Salmonella enterica</i>
5	<i>Shigella spp.</i>	ND	<i>Shigellasonnei</i>
6	<i>Klebsiella sp.</i>	ND	<i>Klebsiella sp.</i>
	Specific objectionable pathogens		
7	<i>Pseudomonas aeruginosa</i>	ND	<i>Pseudomonas aeruginosa</i>
8	<i>Staphylococcus aureus</i>	ND	<i>Staphylococcus aureus</i>
9	<i>Candida albicans</i>	ND	<i>Candida albicans</i>
	Aflatoxin producing fungi		
10	<i>Aspergillus flavus</i>	ND	<i>Aspergillusflavus</i>
11	<i>Aspergillus parasiticus</i>	ND	<i>Aspergillusparasiticus</i>

ND = Not Detected

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 SMR and the results were found within the permissible limit of WHO .as tabulated in Table – V.

TABLE - V: HEAVY METALS ANALYSIS

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

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 SMR was performed and no toxicity is reported in the formulation and the results are tabulated in Table – VI.

TABLE – VI: PESTICIDAL RESIDUE ANALYSIS

S. No.	Pesticide	Result (mg/Kg)	Permissible limit (mg/Kg)
1.	Alachlor	BLQ	0.02
2.	Aldrin (Aldrin and dieldrin combined expressed as dieldrin)	BLQ	0.05
3.	Azinophos-methyl	BLQ	1.0
4.	Bromopropylate	BLQ	3.0
5.	Chlordane (cis, trans and oxychlordane)	BLQ	0.05
6.	Chlorfenvinphos	BLQ	0.5
7.	Chlorpyrifos	BLQ	0.2
8.	Chlorpyrifos-methyl	BLQ	0.1

9.	Cypermethrin (and isomers)	BLQ	1.0
10.	DDT (all isomers, sum of p,p'-TDE (DDD) expressed as DDT)	BLQ	1.0
11.	Deltamethrin	BLQ	0.5
12.	Diazion	BLQ	0.5
13.	Dichlorvos	BLQ	1.0
14.	Dithiocarbamates (as CS ₂)	BLQ	2.0
15.	Endosulphan (sum of isomers & Endosulphansulphate)	BLQ	3.0
16.	Endrin	BLQ	0.05
17.	Ethion	BLQ	2.0
18.	Fenitrothion	BLQ	0.5
19.	Fenvalerate	BLQ	1.5
20.	Fonofos	BLQ	0.05
21.	Heptachlor (sum of Heptachlor & Heptachlor epoxide)	BLQ	0.05
22.	Hexachlorobenzene	BLQ	0.1
23.	Hexachlorocyclohexane isomer (other than γ)	BLQ	0.3
24.	Lindane (γ - Hexachlorocyclohexane)	BLQ	0.6
25.	Malathion	BLQ	1.0
26.	Methidathion	BLQ	0.2
27.	Parathion	BLQ	0.5
28.	Parathion methyl	BLQ	0.2
29.	Permethrin	BLQ	1.0
30.	Phosalone	BLQ	0.1
31.	Piperonylbutoxide	BLQ	3.0
32.	Pirimiphos methyl	BLQ	4.0
33.	Pyrethrins (sum of isomers)	BLQ	3.0
34.	Quintozen (sum of Quintozone, pentachloroaniline and methyl pentachlorophenylsulphide)	BLQ	1.0

BLQ – Below limit of quantification

CONCLUSION

In the present investigation, the microscopic studies establish the presence of each ingredient in the herbal formulation SMR. Various physico chemical parameters along with Rf values of the TLC profile are helpful in establishing the standards for SMR. 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.

Declaration by Authors

Ethical Approval: Approved

Acknowledgement: The authors are extremely thankful to the Director General, CCRUM, New Delhi, for his valuable guidance, encouragement and providing necessary research facilities.

Source of Funding: None

Conflict of Interest: No conflicts of interest declared.

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How to cite this article: Kiran Negi, Mustehasan, Anas Iqbal Alvi, R.P. Meena, Ghazala Javed. Standardisation of Sunoon-e-Mukhrij-e-Ratubat (SMR) - a herbo-mineral Unani remedy for periodontitis. *International Journal of Research and Review*. 2026; 13(3): 232-238. DOI: <https://doi.org/10.52403/ijrr.20260327>
