

# Physico- phytochemical Standardization of a Unani Herbo-Mineral Drug, 'Sunnon- e-Zard': A remedy for Odontalgia and Gingivitis

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## Abstract

Sunnon-e-zard, a herbo-mineral drug, valued in the Unani System of Medicine for its anti-inflammatory actions and therefore, utilized widely to relieve odontalgia (Waj-ul-Asnan) and gingivitis (waram-e-Lissa). It contains six ingredients, out of which only one is of mineral origin. Present communication deals with the physico-phytochemical and thin layer chromatographic evaluations of this multi-action drug with an aim to bring out its pharmacopoeial standards and also to develop its standard operating procedure.

**Keywords:** Physico-phytochemical standardization, SOP, Odontalgia, Gingivitis

## Introduction

Since last few decades the Traditional Systems of Medicine (TSM) has attained much recognition globally and often proved tremendously effective to relieve a variety of common human ailments. It is because of our vast and deep knowledge embodied in the classical literature of these systems and age-old practices, there lies a potential source of a new brand of herbal drugs which could provide safe and effective alternatives and adjuncts to the available armamentarium of herbal drugs. The herbal drugs have already gained universal acceptance but there is still need for developing the methods to ascertain their standards to provide quality products with standardized dosage forms and proof of their safety and efficacy (Premilla, 1989; Akerele 1984 and; Hsu 1958).

Dental complaints are common both among rural and urban population. Some of the problems commonly recorded by the dentists are, toothache, pyorrhoea, tooth cavity, loose teeth, due to weakness of gums and also bleeding gums etc. The validity of numerous drugs are assured in such complaints, free from adverse drug reaction and are available at an affordable cost and can be promoted for the management of oral and dental problems. Razi (1977) and Joshi and Halde (1984) have recommended specific tooth sticks and suggested various measurements for the prevention of dental and oral complaints (Girach et al., 2011; and Punjabi, 1988).

Sunnon-e-zard is a poly herbo-mineral unani drug used to relieve a variety of dental complaints like Waj-ul-Asnan (Odontalgia) and waram-e-Lissa (Gingivitis), due to its two chief actions i.e. Mohallil-e-warm (Anti-inflammatory) and Musakkin-e-Alam (Analgesic). It contains five drugs of herbal origin and

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a mineral ingredient (Plate-1). The present communication thus highlights detailed physico-phytochemical assessment and thin layer chromatographic validations of the laboratory sample prepared as per NFUM-I (Anonymous, 2006), to lay down the quality standards for the identification and authentication of the drug. So that it could provide maximum therapeutic potential and efficacy. The standards of few other unani formulations viz. Sunoon-e-Tambaku and Darhald have already been reported by Zuberi and Tajuddin (2008) and Siddiqui et al. (2009).

### Methodology

All the ingredients used in the prepared of laboratory samples of the drug were of standard pharmacopoeial quality and identified botanically by the experts. Post-e-Anar was collected from the local juice vendors and fresh gulnar farsi was procured from our medicinal plants nursery, dried in shade and kept in



Post-e-Anar



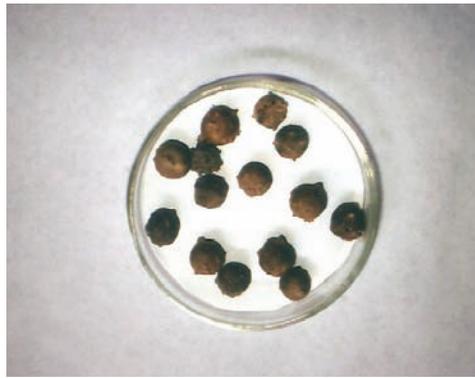
Gulnar



Sumaq



Zard chob

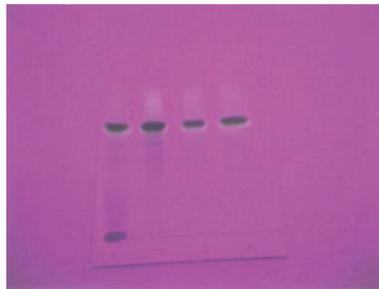


Mazu

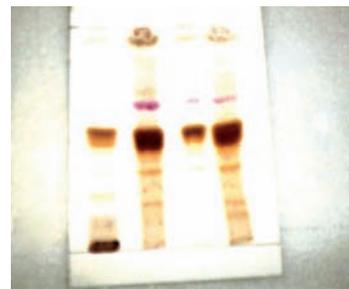


Shubb-e-Yamani

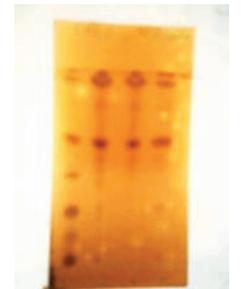
Plate 1. Ingredients of Sunoon-e-zard



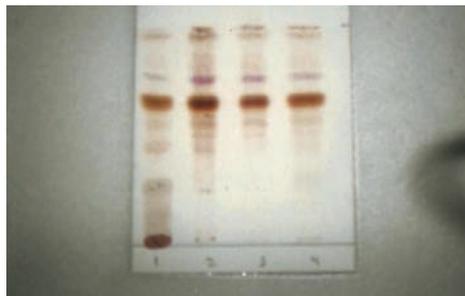
Under UV (365 nm)



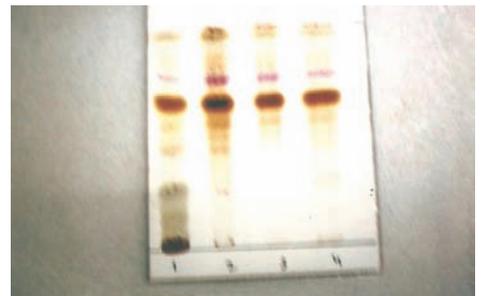
Under UV (365 nm) after derivatization with 10% Eth.H2SO4



On exposure to Iodine vapour



In visible light



Under UV (365 nm) after spraying with 10% Eth. H2SO4 and heating at 110°C

Plate-2. TLC profile of Sunoon-e-zard

containers. Rest of the ingredients were collected from Dawakhana Tibbiya College AMU, Aligarh. The raw drugs were separately powdered using an electric grinder and finally passed through 60 mesh size sieve. The powders of five ingredients thus obtained were mixed as per formulation composition shown in Table-1 and then subjected to physico-chemical, phytochemical estimations, thin layer chromatography, microscopic investigation and behavior of powder drug with different chemical reagents and their observations in day light and UV (365 nm) have been recorded as already reported earlier in similar studies (Hashmi and Zuberi, 2010; Zuberi and Hashmi, 2010, 2011, 2012). Crude protein estimation (Lowry et al., 1951), the crude fibre, total nitrogen and tannins were also estimated accordingly (Anonymous, 2009).

#### Description

Sunoon-e-zard is a powdered drug in appearance, reddish brown in colour having an aromatic odour and a light saltish taste tending astringent.

#### Observations

Microscopy : The powdered drug showed epidermal cells in surface view with anomocytic stomata, druses of calcium oxalate crystals; epidermal cells in surface view with occasional anomocytic stomata; parenchymatous zones of thin walled cells, containing angular fragments of tannins, abundant starch grains with crystals of calcium oxalate; characteristic horn shaped multicellular trichomes, small and large palisade cells from testa, fragments of fruit walls with cicatries; testa of the seeds; embryo and oil globules, brick shaped cork cells showing striations in surface view, starch grains in form of big rounded pasty masses, oleoresin cells with brownish content and the xylem vessels having reticulate and spiral thickenings.

The observations related to physico-chemical and phytochemical estimations, thin layer chromatography of different extracts and the behavior of powdered drug are thus recorded in Tables 2-9.

#### Discussion and Results

Quality is important in the preparation of every product and its vitality depends upon the genuinity and quality of the raw materials used in the preparation of the polyherbal drugs, so the physico-chemical studies and other investigative parameters are important tools in highlighting for a drug to be genuine, potent and hence efficacious.

Quality control is a concept which strives to prevent and eliminate errors at various stages of production along with standard operation procedures. With the growth of herbal pharmaceutical industries and research organizations involved in the physicochemical, phytochemical validations has been in rapid progress in the field of standardization and physic-botanical analyses, involving complex instrumentations alongwith simple procedures and guide lines for formulations, is a matter of foremost importance. The tests of quality control and quality assurance are primarily designed to evaluate the aspects of reliability, reproducibility and accuracy of results depending upon the environmental, infrastructural, technical and also the instrumentational facilities.

The primary phytochemical screening of the extract of the test drug revealed the presence of alkaloid only with dragendorff's reagent, but a very weak positivity of the test was observed, while the test with mayer's reagent was negative. The tannins were screened with ferric chloride reagent. Steroids did not respond to salkowski's test. The behavior of the powdered drug with different chemical reagents are noteworthy due to the sharp fluorescent golden yellow colour on treating the drug with picric acid solution. The test of aluminium for shibb-e-yamani was found quite compatible (Anonymous 1970, Anonymous 1987). The physic-chemical data pertaining to the quality assessment was recorded as mean values of triplicate readings for alcoholic soluble matter (31.39%), water soluble matter (54.33%), total ash (6.84%), water soluble ash (2.37%), pH of 1% aq. solution (2.87) and 10% aq. solution (2.32). Moisture contents as taken by the loss on drying at 105°C was found not more than 9.80 as a mean value.

## **Conclusion**

On the basis of wide therapeutic importance and actions of the ingredients, the test drug "sunoon-e-zard" has become a vital unani remedy for dental complaints. Its systematic standardization has been therefore carried out to lay down the quality standards of the laboratory sample and a concept to provide adequate informations to the consumers about the credibility and safety of the herbal drugs.

**Table 1:** Formulation composition of Sunoon-e-Zard

Name of Unani Drug	Botanical name/ mineral composition	Family	Part used	Quantity
Post-e-Anar	Punica granatum Linn.	Punicaceae	Fruit rind	100 g
Gulnar farsi (abortive flowers)	Punica granatum Linn. (male variety)	Punicaceae	Flower	100 g
Haldi (zard chob)	Curcuma longa Linn.	Zingerberaceae	Rhizome	100 g
Sumaq	Rhus coriaria Linn.	Anacardiaceae	Seed	100 g
Mazu Sabz	Quercus infectoria Oliv.	Fagaceae	Gall	100 g
Shib-e-yamani	Potassium Aluminium Sulphate KAl(SO <sub>4</sub> ) <sub>2</sub> .12H <sub>2</sub> O	-	-	100 g

**Table 2:** Physico-chemical quality standards of Sunoon-e-zard

Quality standards/ parameters	Values	Mean	Range
Alcohol soluble matter (95%) %, w/w)	41.34, 31.45, 31.38	31.39	31.34 – 31.45
Water soluble matter (% w/w)	54.28, 54.34, 54.38	54.33	54.28 – 54.38
Successive Extractive (% w/v)			
(i) Peter Ehter (60-80o)	1.96, 2.04, 1.94	1.97	1.94-2.04
(ii) Benzene	0.44, 0.48, 0.52	0.48	0.44-0.52
(iii) Ethyl acetate	2.38, 2.44, 2.35	2.39	2.35-2.44
(iv) Chloroform	0.82, 0.78, 0.80	0.80	0.78 – 0.80
(v) Ethanol	31.91, 32.02, 31.97	31.96	31.91 – 32.02
(vi) Water	44.88, 44.92, 44.83	44.87	44.83 – 44.92
pH (1% aq. Solution)	2.87, 2.88, 2.87	2.87	2.87 – 2.88
pH (10% aq. Solution)	2.31, 2.33, 2.32	2.32	2.31 – 2.33
Moisture contents (loss on drying at 105oC) %, w/w	9.78, 9.73, 9.89	9.80	9.73 – 9.89
Total ash (% w/w)	6.84, 6.82, 6.88	6.84	6.82 – 6.88
Water soluble Ash (% w/w)	2.34, 2.37, 2.40	2.37	2.34 – 2.40
Tannins	1.242, 1.248, 1.244	1.240	1.242-1.248
Resin	4.92, 4.84, 4.88	4.88	4.84 – 4.92
Crude fibre	3.24, 3.27, 3.21	3.24	3.21 – 3.27
Total nitrogen	0.044, 0.046, 0.041	0.043	0.041 – 0.046
Crude protein	0.275, 0.2875, 0.256	0.272	0.256 – 0.2875

**Table 3:** Preliminary phytochemical screening of sunoon-e-zard

S. No.	Organic phytochemicals	Test reagents	Inferences
1.	Alkaloids	Mayer's reagent Dragendorff's reagent	-ve
2.	Glycosides	Sodium hydroxide test	-ve
3.	Resin	Acetic anhydride	+ve
4.	Tannins/Phenols	Ferric chloride test	+ve
5.	Flavonoids	Shinoda test	-ve
6.	Steroids	Salkowski's test	-ve
7.	Protein	Xanthoproteic test	+ve
8.	Carbohydrates	Anthrone test	-ve

**Table 4:** Behaviour of powdered drug with different chemical reagents.

S. No.	Chemical Reagents	Observations	
		Day Light	UV (366 nm)
1.	Conc. Sulphuric acid	Dark brown	Dark violet
2.	Conc. Hydrochloric acid	Reddish brown	Dark brown
3.	Conc. Nitric acid	Creamish yellow	Violet
4.	Iodine solution (5% aq. Solution)	Bluish black	Dark brown
5.	Acetic acid (glacial)	Pale red	Fluorescent golden yellow
6.	Formic acid	Reddish pale yellow	Bright golden brown
7.	Vanilline-H <sub>2</sub> SO <sub>4</sub> reagent	Pale brown	Golden yellow
8.	Ferric chloride solution (5% aqueous)	Violet value	Dark bluish black
9.	Picric acid solution (commercial)	Reddish yellow	Violet green
10.	Orthophosphoric acid (commercial)	Cherry red	Dark reddish brown
11.	Ethanol-H <sub>2</sub> SO <sub>4</sub> reagent (10%)	Reddish	Fluorescent golden yellow
12.	Drug as such	Reddish yellow	Bright golden yellow

**Table 5:** TLC profile of Different Extracts of Sunoon-e-Zard in visible light

S. No.	Extracts	Solvent system	Detection/ Spray treatment	No. of spots	Rf values
1,	Methanol	Toluene-Ethyl acetate-Methanol-Formica cid (6:3:0.5:0.5)	Visible light	6	0.18, 0.31 (dull gray), 0.43 (faint yellow), 0.50 (brownish yellow), 0.56 (light yellow), 0.68 (brownish yellow)
2.	Chloroform	Toluene-Ethyl acetate-Methanol-Formica cid (6:3:0.5:0.5)	Visible light	5	0.43, 0.56 (light yellow), 0.68 (golden yellow), 0.71 (light yellow), 0.81 (yellow)
3.	Benzene	Toluene-Ethyl acetate-Methanol-Formica cid (6:3:0.5:0.5)	Visible light	3	0.56 (yellow), 0.68 (dark brownish yellow), 0.75 (light yellow)
4.	Ethyl acetate	Toluene-Ethyl acetate-Methanol-Formica cid (6:3:0.5:0.5)	Visible light	4	0.47 (light yellow), 0.58 (yellow), 0.67 (golden yellow),

**Table 6:** TLC Evaluations of Different Extracts of Sunoon-e-Zard under UV (365 nm) radiation

S. No.	Extracts	Solvent system	Detection/ Spray treatment	No. of spots	Rf values
1.	Methanol	T o l u e n e - E t h y l acetate- Methanol-Formica cid (6:3:0.5:0.5)	UV (365 nm)	7	0.21 (dark brown), 0.25 (light red), 0.32 (dark brown), 0.42 (light yellow), 0.50 (light pinkish yellow), 0.54 (fluorescent pale yellow), 0.61 (golden yellow)
2.	Chloroform	T o l u e n e - E t h y l acetate- Methanol-Formica cid (6:3:0.5:0.5)	UV (365 nm)	11	0.18 (light brown), 0.21 (light golden yellow), 0.25 (golden yellow), 0.28 (red), 0.34 (light golden yellow), 0.42 (pale yellow), 0.47 (reddish yellow), 0.57 (dark golden brown), 0.71 (light yellow), 0.75, 0.82 (fade yellow)

3.	Benzene	Toluene-Ethyl acetate-Methanol-Formica cid (6:3:0.5:0.5)	UV (365 nm)	4	0.40 (yellow), 0.47 (light yellow), 0.54 (pale yellow), 0.61 (golden yellow)
4.	Ethyl acetate	Toluene-Ethyl acetate-Methanol-Formica cid (6:3:0.5:0.5)	UV (365 nm)	7	0.17 (fade brown), 0.22 (golden yellow), 0.25 (light golden yellow), 0.36 (golden yellow), 0.48 (pale red), 0.57 (golden brown), 0.77 (light yellow)

**Table 7:** TLC profile of various Extracts of Sunoon-e-Zard on Exposure to Iodine vapours

S. No.	Extracts	Solvent system	Detection/ Spray treatment	No. of spots	Rf values
1.	Methanol	Toluene-Ethyl acetate-Methanol-Formica cid (6:3:0.5:0.5)	I2 vapours	5	0.11 (light brown), 0.17, 0.24 (light yellowish brown), 0.50 (light brown), 0.57 (brown)
2.	Chloroform	Toluene-Ethyl acetate-Methanol-Formica cid (6:3:0.5:0.5)	I2 vapours	6	0.18 (light brown), 0.38 (dull brown), 0.50 (light brown), 0.54 (dark brown), 0.82, 0.94 (light brown)
3.	Benzene	Toluene-Ethyl acetate-Methanol-Formica cid (6:3:0.5:0.5)	I2 vapours	4	0.37 (fade brown), 0.52 (dark brown), 0.81 (light brown), 0.94 (light brown)
4.	Ethyl acetate	Toluene-Ethyl acetate-Methanol-Formica cid (6:3:0.5:0.5)	I2 vapours	7	0.20, 0.25, 0.38 (light brown), 0.48, 0.51 (dark brown), 0.80, 0.94 (brown)

**Table 8:** TLC profile of Different Extracts of Sunoon-e-Zard after spraying with 10% Ethanolic H<sub>2</sub>SO<sub>4</sub> reagent and viewed under UV (365 nm)

S. No.	Extracts	Solvent system	Detection/ Spray treatment	No. of spots	Rf values
1.	Methanol	Toluene-Ethyl acetate- Methanol-Formica cid (6:3:0.5:0.5)	10% Ethanolic-H <sub>2</sub> SO <sub>4</sub> reagent & heating at 110oC	6	0.22, 0.31 (gray), 0.53 (yellow), 0.56 (brown), 0.68 (light pink), 0.95 (dull gray)
2.	Chloroform	Toluene-Ethyl acetate- Methanol-Formica cid (6:3:0.5:0.5)	10% Ethanolic-H <sub>2</sub> SO <sub>4</sub> reagent & heating at 110oC	6	0.28, 0.41 (light golden brown), 0.56 (dark brown), 0.68 (pink), 0.72 (light gray), 0.92 (dark gray)
3.	Benzene	Toluene-Ethyl acetate- Methanol-Formica cid (6:3:0.5:0.5)	10% Ethanolic-H <sub>2</sub> SO <sub>4</sub> reagent & heating at 110oC	4	0.41 (light brownish yellow), 0.56 (dark yellowish brown), 0.68 (light pink), 0.97 (light gray)
4.	Ethyl acetate	Toluene-Ethyl acetate- Methanol-Formica cid (6:3:0.5:0.5)	10% Ethanolic-H <sub>2</sub> SO <sub>4</sub> reagent & heating at 110oC	7	0.22 (light gray), 0.28 (light brown), 0.41 (yellowish brown), 0.60, 0.62 (golden brown), 0.68 (light pink), 0.97 (gray)

**Table 9:** TLC profile of Different Extracts of Sunoon-e-Zard under uv (365 nm) radiation after heating the chromatogram at 110°C

S. No.	Extracts	Solvent system	Detection/ Spray treatment	No. of spots	Rf values
1.	Methanol	Toluene-Ethyl acetate- Methanol-Formica cid (6:3:0.5:0.5)	Under UV (365 nm) after heating at 110oC	10	0.22 (dark brown), 0.25 (light red), 0.27 (dark brown), 0.33 (fade yellow), 0.46 (pale yellow), 0.52 (golden yellow), 0.65 (pinkish red), 0.68 (light grayish red), 0.91 (light reddish yellow), 0.93 (light sky blue)

2.	Chloroform	Toluene-Ethyl acetate- Methanol-Formica cid (6:3:0.5:0.5)	Under UV (365 nm) after heating at 110oC	9	0.18 (light golden brown), 0.22 (sharp golden brown), 0.31, 0.40 (pale yellow), 0.47 (dark brown), 0.62 (pinkish brown), 0.69 (grayish yellow), 0.81 (light pinkish red), 0.87 (brownish yellow)
3.	Benzene	Toluene-Ethyl acetate- Methanol-Formica cid (6:3:0.5:0.5)	Under UV (365 nm) after heating at 110oC	8	0.29 (yellow), 0.35, 0.40 (light yellow), 0.37 (brownish pale yellow), 0.65 (light pink), 0.68 (light grayish yellow), 0.78 (pinkish red), 0.87 (grayish yellow)
4.	E t h y l acetate	Toluene-Ethyl acetate- Methanol-Formica cid (6:3:0.5:0.5)	Under UV (365 nm) after heating at 110oC	9	0.17 (light grayish brown), 0.21 (brownish gray), 0.33, 0.42 (pale yellow), 0.46 (dark brown), 0.60 (pinkish brown), 0.71 (grayish yellow), 0.82 (pinkish red), 0.88 (light yellowish brown)

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