

Development of Standard Operating Procedures and Quality Control Standards of Roughan-a-Amla and Roughan-e-Surkh : Unani Oil Preparations

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Abstract

The analytical standards of two highly medicinal and frequently used oil preparations viz. Roughan-e-Amla and Roughan-e-Surkh have been established in order to maintain their quality, safety and efficacy while taken for a commercial preparation. The standardization is based on the pharmacopoeial parameters such as organoleptic features, physico-chemical validation, thin layer chromatography, estimation of microbial load, pesticide residue, aflatoxins and heavy metals of both these compound drugs. Accordingly, their standard operating procedure (SOP) are also developed.

Key words: SOP, Physico-chemical validation, Thin layer chromatography

Introduction

Both Roughan-e-Amla and Roughan-e-Surkh are important polyherbal Unani formulations classified under the category of "Roughaniyat" in NFUM-I (Anonymous, 2006). Therapeutically both these oil preparations are used externally to relieve a variety of human ailments. As Roughan-e-Amla consisting of four herbal ingredients, is used frequently as a hair tonic (Muqawwi-e-Shar), for blackening of hair (Musawwid-e-Shar) and more often to control the falling of hair (Intesar-e-Shar) while Roughan-e-surkh being highly antiinflammatory (Mohalil-e-Waram) is prescribed generally to relieve Rheumatism (Waj-ul-mafasil), sciatica (Irqun-nisa) and Gout (Niqras) according to Arzani (1880), Kabiruddin (1967), Sina (1994), Anonymous (2006) and many others.

A review of literature revealed that a large number of unani formulations have already been standardized and their SOPs also developed accordingly. Some of such studies on Unani formulations such as sufoof, Majoon, Habbs and Sunoon are reported by various workers viz., Aminuddin and Siddiqui (2007), Bagul *et al.* (2006), Goel *et al.* (2007), Hashmi and Zuberi (2010), Khan *et al.* (2010), Negi *et al.* (2009, 2010), Ramaswamy *et al.* (2009), Sajwan *et al.* (2010), Siddiqui *et al.* (1991), Zuberi and Tajuddin (2008) and many others. However, no such work appears to be taken up on any of these two oil preparations thus far. It was therefore considered essential to take up the investigation of two oil preparations in order to develop certain standards for the commercial producers.

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Materials and Methods

In order to develop SOP and the pharmacopoeial standard, sufficient quantity of all the raw ingredients of Roughan-e-Amla and Roughan-e-surkh were procured from three different sources i.e. one from Dawakhana Tibbiya College, AMU Aligarh and the other two from authorized local druggists. The botanical identity of all these raw ingredients were established with the help of parameters as described in API, UPI and IP (1966). Foreign matter if any in the raw ingredients was removed and dried further. Three batches of both these formulations were prepared in pharmacognosy lab of RRIUM, Aligarh, following the ingredient ratio and methodology given in NFUM-I (2006) as described below in Table 1 and 2 respectively.

Table 1: Formulation Composition of Roughan-e-Amla

S.No.	Unani Name	Botanical Name	Part used	Quantity
1.	Aab-e-Amla Taza	<i>Emblica officinalis</i> Gaertn	Amla water	1 lit
2.	Berg-e-Moorad	<i>Myrtus communis</i> L.	Leaf	125 g
3.	Berg-e-Hina	<i>Lawsonia inermis</i> L.	Leaf	125 g
4.	Roughan-e-Kunjad	<i>Sesamum indicum</i> L.	Seed oil	3.75 lit.

Table 2: Formulation Composition of Roughan-e-surkh

S.No.	Unani Name	Botanical Name	Part used	Quantity
1.	Majeeth	<i>Rubia cordifolia</i> L.	Stem	200 g
2.	Saleekha	<i>Cinammomum cassia</i> L.	Bark	80 g
3.	Kaiphah	<i>Myrica nagi</i> Buch	Stem bark	80 g
4.	Charela	<i>Parmelia perlata</i> Ach	Lichen	80 g
5.	Saad kufi	<i>Cyperus rotundus</i> L.	Rhizome	80 g
6.	Waj-e-Turki	<i>Acorus calomus</i> L.	Rhizome	80 g
7.	Qaranful	<i>Syzygium aromaticum</i> L.	Flowerbud	80 g
8.	Narakachoor	<i>Zingiber zerumbet</i> (L.) Sm	Rhizome	80 g
9.	Roughan-e-Sarshaf	<i>Brassica campestris</i> L.	Mustard oil	150 g
10.	Roughan-e-kunjad	<i>Sesamum indicum</i> L.	Sesame oil	150 g
11.	Aab-e-Aahak	Water	–	QS

The physico-chemical estimations which include parameters such as Acid, Iodine, Peroxide and the saponification value and also the thin layer chromatographic analysis of the two oil preparations have been carried out

as per WHO guidelines (Anonymous, 1998). Determination of microbial load, pesticide residue, Aflatoxins and the heavy metals on the other hand done following Anonymous (2000).

Observations and Result

Based on the preparation of three batches of each, Roughan-e-Amla and Roughan-e-Surkh their standard operating procedures (SOP) have been developed as described below -

SOP of Roughan-e-Amla

In order to get required quantity of Aab-e-Amla taza sufficient green, fresh, healthy Amla fruits were taken and the pulp separated from the seed. It was grinded and then squeezed through a muslin cloth to get the Aab-e-Amla taza. The other two ingredients already being soaked overnight in water was boiled for half an hour. After cooling it was smashed again and filtered through a muslin cloth. The decoction thus obtained was mixed with Aab-e-Amla taza, Roughan-e-kunjad in the same proportion was then added to the above decoction and boiled continuously for several hours till all water evaporates from the preparation. After slight cooling, the viscous portion was decanted in another container and filtered finally before packing.

SOP of Roughan-e-Surkh

The required quantity of all the eight herbal ingredients were first of all grinded coarsely (Neem kafta) one by one in the ratio as per NFUM-I and then soaked together overnight in Aab-e-Amla, already prepared earlier in sufficient quantity. Next day, the soaked drugs were boiled till only half of the water left in it. After slight cooling, it was smashed slowly and then filtered through a double fold of Muslin cloth. The extract thus obtained was heated again until reduced to half. A mixture of Roughan-e-sarshaf and Roughan-e-kunjad in 1:1 ratio was then added to the condensed extract and boiled again for several hours till water evaporates completely and finally the viscous portion decanted carefully and filtered finally to ensure its' transparency.

After the preparation of three batches, the organoleptic examination of both the drugs was conducted and the features recorded below in Table 3.

Table 3

Organoleptic feature	Name of drug	
	Roughan-e-Amla	Roughan-e-Surkh
Appearance	Viscous liquid	Viscous liquid
Colour	Light brown	Bright red
Taste	Not specific	Not specific
Odour	Agreeable	Agreeable

Physico-chemical Study

The physico-chemical estimation of two oil preparations which includes the parameters such as determination of acid value, iodine value, saponification value, ester value, refractive index and weight per ml have been recorded in Tables 4 & 5 and the test for presence of various adulterant oils in Table 6 accordingly.

Thin Layer Chromatography (TLC)

TLC of the petroleum ether (60-80°) extract of both Roughan-e-Amla and Roughan-e-Surkh was developed on precoated Aluminium plates (silica gel 60 F₂₅₄) using Toluene-Ethyl acetate-Formic acid (9:1:1) as mobile phase, and the chromatogram were viewed under visible light as well as under the UV (365 nm).

Further on spraying the plate with vanilline-H₂SO₄ reagent and heating at 110°C till optimum spot development. The number of spots, the R_f values and the colour zones observed for the two oil preparations are recorded in Table 7 & 8 and their chromatograms presented in Plate-3.

The observations about the determination of microbial load and pesticide residue are shown in Table 9 and the estimation of Aflatoxins and heavy metals in Table 10.

Conclusion

Based on the present study of two highly medicinal oil preparations of the unani system of medicine, a number of physico-chemical standards of both Roughan-e-Amla and Roughan-e-Surkh have been established besides their standard operating procedures (SOPs).



Sad-kufi



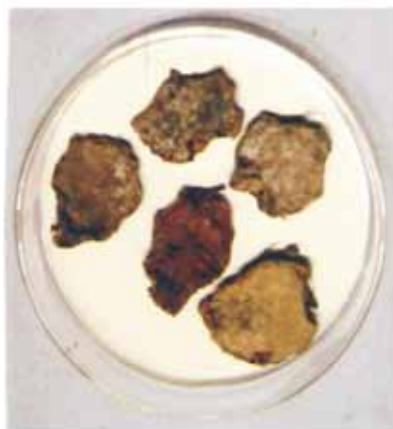
Majeeth



Charela



Saleekha



Narkchur



Wajturki

Plate 1. Ingredients of Roughan-e-Surkh and Roughan-e-Amla



Kaifal



Qaranful



Amla



Berg-e-Hina



Berg-e-Moorad

Plate 2. Ingredients of Roughan-e-Surkh and Roughan-e-Amla



Under UV 365 nm after spraying with VSA



Under UV 365 nm



Under UV 365 nm After spraying with VSA and heating at under 110°C



After spraying with VSA and heating at 110°C



After spraying with 10% Eth. H₂SO₄ reagent



Under iodine vapours



Under UV 365 nm and heating at 110°C



After spraying with VSA and heating with 110°C



After spraying with 10% Eth. H₂SO₄

Plate-3. TLC profile of Roughan-e-Surkh and Roughan-e-Amla

All the three batches of these two oil preparations when qualitatively analysed for the presence of any adulterant oil, it was found totally lacking. The petroleum ether extractive of Roughan-e-surkh was found to be 100%, while Roughan-e-Amla was found not less than 98.72%. The saponification value on the other hand ranging from 166.33 – 169.94 and 189.37 – 193.44 in Roughan-e-surkh and Roughan-e-Amla respectively is also an indication that all three samples of drugs under study are uniform.

Table 4: Physico-chemical Standards of Roughan-e-Amla

S.No.	Parameters	Sample I	Sample II	Sample III	Limit
1.	Acid value	2.47 2.42 2.51	2.82 2.77 2.88	2.37 2.32 2.35	Not more than 2.88
2.	Iodine value	112.28 112.31 112.23	116.23 116.42 116.29	114.45 114.41 114.33	Range 112.28 – 116.42
3.	Saponification value	190.88 190.82 190.78	193.38 193.44 193.33	189.44 189.46 189.37	Range 189.37 – 193.44
4.	Unsaponifiable matter (% w/v)	1.78 1.74 1.71	2.17 2.22 2.14	1.57 1.52 1.62	Not more than 2.22
5.	Ester value	188.41 188.40 188.27	190.56 190.68 190.45	187.07 187.14 187.02	Range 187.02 – 190.68
6.	Refractive index	1.4663 1.4661 1.4662	1.4732 1.4734 1.4731	1.4660 1.4662 1.4658	Range 1.4658 – 1.4734
7.	Weight per ml	0.9419 0.9421 0.9418	0.9632 0.9634 0.9633	0.9423 0.9424 0.9422	Range 0.9418 – 0.9634
8.	Petroleum Ether (60-80oC) extractive (% v/w)	98.47 98.39 98.32	98.66 98.72 98.69	97.84 97.88 97.81	Not less than 98.72

Table 5: Physico-chemical Standards of Roghan-e-Surkh

S.No.	Parameters	Sample I	Sample II	Sample III	Limit
1.	Petroleum ether (60-80o) extractive (%)	100.00	100.00	100.00	Not less than 100.00

S.No.	Parameters	Sample I	Sample II	Sample III	Limit
2.	Acid value	1.87 1.82 1.90	1.72 1.78 1.83	1.45 1.49 1.38	Not more than 1.90
3.	Iodine value	103.18 103.23 103.11	101.88 101.32 101.60	104.16 104.07 104.02	Range 101-32-104.16
4.	Peroxide value	8.51 8.45 8.58	8.49 8.60 8.52	7.24 7.32 7.38	Not more than 8.60
5.	Unsaponifiable matter (%)	4.12 4.19 4.24	4.22 4.28 4.13	4.08 4.13 4.21	Not more than 4.28
6.	Weight per ml (g)	0.882 0.884 0.884	0.872 0.873 0.872	0.867 0.866 0.867	0.866-0.884
7.	Saponification value	167.92 168.66 168.98	168.86 169.23 169.94	166.33 166.65 166.44	Range 166.33-169.94
8.	Ester value	166.84 166.05 167.08	167.14 167.45 168.11	164.88 165.16 165.06	Range 165.05-168.11
9.	Refractive index	1.4872 1.484 1.4869	1.4837 1.4835 1.4832	1.4773 1.4772 1.4775	

Table 6: Qualitative tests for presence of various oil

Name of Formulation	Qualitative Tests			
	Arachis oil	Cotton seed oil	Sesame oil	Mineral oil
Roughan-e-Amla				
Sample-I	-ive	-ive	+ive	-ive
Sample-II	-ive	-ive	-ive	-ive
Sample-III	-ive	-ive	+ive	-ive
Roughan-e-Surkh				
Sample-I	-ive	-ive	+ive	-ive
Sample-II	-ive	-ive	-ive	-ive
Sample-III	-ive	-ive	+ive	-ive

Table 7: TLC Profile of Roughan-e-Amla and Roughan-e-Surkh with their Rf values

No.	Extracts	Solvent system	Detection	Rf values and their colour zones			
				No. of spots	Raughan-e-Surkh	No. of spots	Raughan-e-Amla
1.	Petroleum Ether (60-80oc)	Toluene-Ethylacetate-Formic Acid (9:1:1)	Visible light	4	0.06 (fade brown), 0.16 (light pinkish brown), 0.43 (light yellow), 0.56 (light pinkish yellow)	2	0.10 (grayish green), 0.17 (light brown)
2.	Petroleum Ether (60-80o)	Toluene-Ethyl acetate-formic acid (9:1:1)	UV (365 nm)	8	0.13 (pinkish brown), 0.15 (light blue), 0.35 (orange brown), 0.38 (light blue), 0.43 (light orange yellow), 0.58 (light yellowish green), 0.70 (light orange blue), 0.92 (orange brown)	3	0.07 (dark brown), 0.58 (fade orange red), 0.69 (light blue)
3.	Petroleum Ether (60-80o)	Toluene-Ethyl acetate-formic acid (9:1:1)	Vanilline-H2SO4 reagent	7	0.16 (reddish brown), 0.39 (orange yellow), 0.61 (light blue), 0.72 (fade brown), 0.81 (orange brown), 0.87 (light blue), 0.97 (orange yellow)	5	0.11 (brown), 0.27 (dark brown), 0.62 (dull red), 0.65 (light reddish brown), 0.70 (light blue)

Table 8: TLC Profile of Roughan-e-Amla and Roughan-e-Surkh with their Rf values

No.	Extracts	Solvent system	Detection	Rf values and their colour zones			
				No. of spots	Raughan-e-Surkh	No. of spots	Raughan-e-Amla
1.	Methanol	Petroleum Ether (60-80O)-Diethyl ether- Ethyl acetate-formic acid (4:4:1)	UV (365 m)	7	0.05, 0.19 (dull brown), 0.35 (fade gray), 0.39 (gray), 0.82 (light green), 0.85 (light brown), 0.98 (light orange brown)	3	0.07 (Light pink), 0.42 (light gray), 0.94 (dull pinkish brown)

No.	Extracts	Solvent system	Detection	Rf values and their colour zones			
				No. of spots	Raughan-e-Surkh	No. of spots	Raughan-e-Amla
2.	Methanol	Petroleum Ether (60-80O)-Diethyl ether- Ethyl acetate-formic acid (4:4:1)	Vanilline-H ₂ SO ₄ reagent	5	0.16 (light pink), 0.53 (pink), 0.67 (light violet brown), 0.77 (light orange red), 0.98 (violet pink)	2	0.10 (grayish green), 0.66 (pinkish brown)
3.	Methanol	Petroleum Ether (60-80O)-Diethyl ether- Ethyl acetate-formic acid (4:4:1)	I ₂ vapours	6	0.12 (light brown), 0.48 (fade brown), 0.62 (dark brown), 0.68 (light brown), 0.73, 0.81 (fade brown)	5	0.06 (brown), 0.44 (fade green), 0.51 (light brown), 0.88, 91 (fade brown)

Table 9: Determination of Microbial load and Pesticide residue of two drugs

Parameters studied	Roughan-e-Amla	Roughan-e-Surkh	WHO Limit
A. Microbial load			
Total Bacterial load	1x10 ²	16x10 ²	Not more than 10 ⁵ /g
Salmonella spp.	Nil	Nil	Absent
Escherichia coli	Nil	Nil	Absent
Total fungal count	1x10	1x10 ²	Not more than 10 ³ /g
B. Pesticide residue			
DDT	Nil	Nil	Nil
Endosulfan	Nil	Nil	Nil

Table 10: Determination of Aflatoxins and Heavy metals of two drugs

Parameters studied	Roughan-e-Amla	Roughan-e-Surkh	WHO Limit
A. Aflatoxins			
B1	Nil	0.0018	Not more than 0.50 ppm
B2	Nil	Nil	Not more than 0.10 ppm
G1	Nil	Nil	Not more than 0.50 ppm
G2	Nil	Nil	Not more than 0.10 ppm

Parameters studied	Roughan-e-Amla	Roughan-e-Surkh	WHO Limit
B. Heavy metals			
Arsenic	Nil	Nil	Not more than 3 ppm
Cadmium	Nil	Nil	Not more than 0.3 ppm
Lead	Nil	Nil	Not more than 10 ppm
Mercury	Nil	Nil	Not more than 1 ppm

Further, both the oil preparations are almost totally devoid of any microbial load, pesticide residue, Aflatoxin and heavy metals except found very much within the permissible limit in a few samples.

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References

- Aminuddin and Siddiqui, M.K., 2007. Microscopic examination of Jawarish Podina – A polyherbal formulation in Unani system of medicine. *Hippocratic Journal of Unani Medicine* 2(2): 113-120.
- Anonymous, 1966. Indian Pharmacopoeia, Manager of Publications, Delhi, p. 293.
- Anonymous, 1989-2007. The Ayurvedic Pharmacopoeia of India, Part I, Vols. I-VI, Ministry of Health & Family Welfare, Government of India, New Delhi.
- Anonymous, 2007-2009. The Unani Pharmacopoeia of India, Part I, vols. I-VI, Ministry of Health & Family Welfare, Govt. of India, New Delhi.
- Anonymous, 1998. Quality control methods for medicinal plant materials, WHO, Geneva, p. 25-28.
- Anonymous, 2000. Official methods of analysis of the Association of official analytical chemists (AoAC), 17th Ed. Arlington USA, p. 38-60.
- Anonymous, 2006. National Formulary of Unani Medicine, part I, Ministry Health and Family Welfare, Govt. of India, New Delhi, p. 189, 200.
- Arzani, M.A., 1880. Qarabadeen Qadri, CCRUM, Repr. Ed. 2009, New Delhi, p. 501.

- Bagul, M.S., Pathak, S.B., Ravishankar, M.N. and Rajani, M., 2006. Phytochemical standardization of Sharbat-e-Aijaz, Proc. Of Natl. workshop on Institute-Industry interaction on research in Unani Medicine, Narasi Publication House, Delhi, p. 131-138.
- Goel, S., Ahmad, R. and Khan, M.S.Y., 2007. Microscopical examination of compound formulation – Majoon-e-Ispand Sokhtani. *Proc. Of Int. Conf. on Unani Drugs*, 8-11 Feb. 2005, CCRUM New Delhi, p. 817-819.
- Hashmi, S. and Zuberi, R.H., 2010. Botanical and physico-chemical standardization of sufoof-e-Bers – a polyherbal unani drug of repute. *Hippocratic Journal of Unani Medicine* 5(3): 131-139.
- Kabiruddin, 1967. Bayaz-e-Kabir, vol. II, Hikimat Book Depot, Hyderabad Repr. Ed. p. 85.
- Khan, N.A., Muzaffar, M., Qsim, J.A., Nasiruddin, M. and Haque, M.M., 2010. Physico-chemical and phytochemical studies on Majoon-e-Baladur. *Hippocratic Journal of Unani Medicine* 5(3): 15-19.
- Negi, K., Singh, V.K. and Siddiqui, M.K., 2009. Ingredients identity in Itrifal-e-Kishnizi – a polyherbal formulation of Unani System of medicine, *Hippocratic Journal of Unani Medicine* 4(1): 55-65.
- Negi, K., Sajwan, K. and Khan, M.S.Y., 2010. Standardization of Habb-e-Man-e-Hamad – A Unani contraceptive, *Hippocratic Journal of Unani Medicine* 5(3): 31-38.
- Ramaswamy, D., Meena, R.P., Khan, S.A., Arfeen, S., Mageshwari, S. and Sultana, G., 2009. Chemical standardization of Majoon-e-Rewnd chini – A Unani formulation, *Hippocratic Journal of Unani Medicine* 4(3): 59-67.
- Sajwan, S., Sajwan, K., Asim, S.M. and Agarwal, U.C., 2011. Standardization of Polyherbal unani formulation – Jawarish Kamooni. *Hippocratic Journal of Unani Medicine* 6(4): 1-9.
- Siddiqui, S.H., Zaidi, S.T.H., Khan, G. and Sharma, H.P., 1991. Standardization of Itrifal-e-Zamani and some of its constituents. *Ind. Jour. Unani Med.* 1: 37-42.
- Sina, B.A., 1994. Mujarribat-e-Bu Ali Sina, Almoor raj Tohfatul Aashqeen. Aijaz Publ. House, New Delhi, p. 200.
- Zuberi, R.H. and Tajuddin, 2008. Physico-chemical and phytochemical evaluation of Sunoon-e-Tambaku, *Hippocratic Journal of Unani Medicine* 3(4): 53-61.

