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Research Article

Development of Standard Operating Procedures (SOPs), Standardization, TLC and HPTLC Fingerprinting of a Polyherbal Unani Formulation

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ABSTRACT

Background: Unani System of Medicine (USM) has its origin to Greece. To ensure and develop the quality, authenticity of Unani drugs, standardization on modern analytical parameter is essential requirement for drugs. **Objectives:** The aimed of the present study was to develop a standard profile of *"Qurṣ-e-Mafasil*" by systematic study through authenticated ingredients, pharmacognostic identification followed by physicochemical, TLC, HPTLC fingerprinting analysis as per standard protocol. **Material and Methods:** In this study three batches of *"Qurṣ-e-Mafasil*" QM were prepared by standard method as per UPI had been followed by organoleptic properties of formulation such as appearance, color, odor, taste. Powder Microscopy and physicochemical studies were carried out such as Uniformity of weight, Friability, Disintegration time, hardness, LOD, ash vales and extractive values in like aqueous, alcohol & hexane. Further qualitative tests such as Thin-Layer Chromatography (TLC), and High-Performance Thin Layer Chromatography (HPTLC) studies were also carried out to develop fingerprint pattern of the alcoholic solvent extract of QM. Phytochemical screening was carried out in different solvent extracts such as alcoholic, aqueous and chloroform extracts to detect the presence phytoconstituents in the formulation QM. Heavy metals, Microbial Load Contamination and pesticidal residues were also determined. **Results:** *Qurṣ-e-Mafasil* showed tablet-like appearance, light brown colour, mild pungent odour and acrid taste. Uniformity of weight (mg), friability (rpm), and hardness (kg/cm) and disintegration time was ranged between (500 to 503), (0.0340 to 0.038), (8.40 to 8.67) and (4-5 minutes) respectively for the three batches. Loss in weight on drying at 105°C was ranged between (8.3425 to 8.7346). Extracted values were calculated in distilled water ranged between (30.9091 to 31.4358), hexane (1.1419 to 1.4281), and alcohol (3.3352 to 3.3962). The ash values recorded were ranged between (3.7336 to 3.8378), and acid insoluble ash (0.5859 to 0.6112).

Keywords Standardization, *Qurṣ-e-Mafasil* (QM), Standard Operating Procedures (SOPs), TLC, HPTLC.

INTRODUCTION

Unani System of Medicine the name itself signifies its origin to Greece (Yunan), and as the time passes it went to several transformations. After Hippocrates, Galen (129-200 AD) the Roman scholar is one of the most illustrious scholars made valuable addition to medicine by conducting experiments and contributed a lot to the Unani Medicine (Anonymous, 2016

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and Anonymous, 2012) (Anonymous. UNANI SYSTEM OF MEDICINE (The Science of Health and Healing); (New Delhi: Ministry of AYUSH, Govt. of India), 2016, pp. 1-14. Anonymous. Standard Unani Medical Terminology. Introduction (xv), Central Council for Research in Unani Medicine, (New Delhi: Ministry of AYUSH, Govt. of India), 2012, pp. 123, 290). It is also observed that about 80% of the world population is using medicinal plants primarily in the developing countries for treating different diseases, due to their safety, efficacy, cultural acceptability and lesser side effect. It is important for herbal formulations to get the quality assurance by the conventional system of medicine, so that they can be justified, accepted and must be beneficial for the ailing masses of the mankind (Madhav NVS *et al.,* 2011). According to the WHO, the quantity, quality, safety and efficacy data on traditional medicine (TM) are not sufficient to meet the criteria needed, so some of the major policy challenges include safety, efficacy, quality, and enlightened the perception for the use of TM. Various policy measures have been applied for a clear-eyed view of the use of TM, in order to increase its safety, efficacy and acceptability (G. Bodeker and G. Burford, 2007). As there is increase demand of herbs and herbal products especially Unani medicinal products, run across many problems like non-availability of good quality of raw materials, proper methodology for standardization. In consequence to ensure and develop the quality, authenticity of Unani formulations, the standardization of single as well as compound drugs on modern analytical parameter is basic requirement for drugs. Before studying pharmacological activity of any drug physico-chemical characteristics is necessary for its authenticity (Alam A *et al.,* 2019).

Qurṣ is a solid, flat and circular medicinal preparation of varying size and weight for oral administration. Its possible English equivalent term is tablet (Anonymous, 2012; Ali HSS, 2010) *Qurs* is the type of tablet which is made flattened by compression, instead of round (Hakeem Zillur Rahman, June 2002). *Qurṣ-e-Mafasil* (QM) is a kit-Medicine developed by the Central Council for Research in Unani Medicine New Delhi and used in General out Patient Department (GOPD) of its peripheral unit across India. Although it is found very effective in treatment of various joint pain conditions successfully. Hence, the Unani poly herbal formulation, *Qurṣ-e-Mafasil* is identified and chosen for standardization as well as its Standard Operating Procedures (SOPs) for manufacturing the quality drug (Anonymous, YNM, Unani Kit Medicine).

Qurṣ-e-Mafasil is composed of *Zanjabeel* (*Zingiber officinale* Rosc.), *Suranjaan* (*Colchicum luteum* Baker.*), Filfil Siyah* (*Piper nigrum* Linn.) and *Asgand* (*Withania somnifera* Dunal.), and is prepared by mixing all the ingredients with *samagh e arabi* (*acacia arabica*) as binding agent. It is useful in *Waja'ul-Mafasil* (Musculoskeletal disorders, joint pains) as mentioned in Unani Kit Medicine developed by CCRUM (Anonymous. Unani Medicine Kit, YNM). These ingredients have their own therapeutic importance for the effect of almost all type of joint pains and musculoskeletal disorders and are mentioned in various authentic Unani literature books (Kabeerudddin HM, 2006; Khan MA, 2006; Tabri ASR, jun-2010; Anonymous, 2001). The formulation had not been standardized so far on organoleptic, microscopic, physicochemical parameters, phytochemical screening, TLC, HPTLC profile, aflatoxin, microbial load and heavy metal analysis.

METHODOLOGY

Collection of material: Plant ingredients of the formulation of *"Qurṣ-e-Mafasil"* QM were procured from the GMP certified pharmacy, identified and authenticated by the botanist at National research Institute of Unani Medicine for Skin Disorders, Hyderabad, India.

Preparation of the Formulation: The study formulation of QM was prepared according to its composition mentioned in Unani Medicine Kit developed by CCRUM, department of AYUSH, ministry of Health & Family welfare New Delhi and prepared as per the standard procedure mentioned in the UPI and NFUM and other reference books and the following SOPs developed in accordance with the preparation of formulation.

Ingredient	Botanical name	Part used	Quantity
Zanjabeel	*Zingiber officinale* Rosc.	Dried rhizome	One part
Suranjaan	*Colchicum luteum* Baker.	Dried corms	Two parts
Filfil Siyah	*Piper nigrum* Linn.	Dried fruit	One part
Asgand	*Withania somnifera* Dunal.	Dried fruit	One part

According to pharmaceutical procedure and standardization, the conventional and modern analytical techniques were used to standardize *"Qurṣ-e-Mafasil"* (QM). In this study three batches of QM were prepared by standard method as per UPI (Anonymous, 2010) had been followed by organoleptic properties of formulation such as appearance, color, odor, taste. Powder Microscopy and physicochemical studies were carried out such as Uniformity of weight, Friability, Disintegration time, hardness, LOD, pH of 1% and 5% aqueous solution, ash vales and extractive values in different solvents like aqueous, alcohol & hexane. Further qualitative tests such as Thin-Layer Chromatography (TLC), and High-Performance Thin Layer Chromatography (HPTLC) studies were also carried out to develop fingerprint pattern of the alcoholic solvent extract of QM. Phytochemical screening was carried out in different solvent extracts such as alcoholic, aqueous and chloroform extracts to detect the presence phytoconstituents in the formulation QM. Heavy metals, Microbial Load Contamination, Aflatoxins and pesticidal residues were also determined. QM was analyzed for heavy metal at DSRI, Ghaziabad. QM was analyzed for the identification of heavy metals on an Atomic Absorption Spectrophotometer. Flame atomization has been applied for detection of Lead (Pb) & Cadmium (Cd) and Hydride generator was used for the detection of the elements Arsenic (As) & Mercury (Hg) (WHO, 2011). The microbial load analysis was carried out in three different samples of QS. The media used are Soyabean Casein Digest Agar Media, Sabouraud Dextrose Agar with Chloramphenicol Media, HiCrome TM E. Coli Agar Media, HiCrome Raj Hans Medium, modified (Salmonella Agar, Modified). Aflatoxins can be harmful to health even though they are consumed in minute quantities in herbal medicines. This research was performed to identify the possible presence of B1, B2, G1 and G2 aflatoxins in QM (WHO, 2011). QM was analyzed for pesticidal residue at Bureau Veritas Testing laboratory, Hyderabad. The procedure and methods for analysis were followed on the protocol of WHO issued guidelines (WHO, 2011).

High performance thin layer chromatography (HPTLC) analysis

Alcoholic (ethanol) extract of the sample was applied on the TLC plate and developed under various detection system viz., UV 254nm, UV 366nm and detection after derivatizing with vanillin sulfuric acid at λ 580 nm. For the procedure to be done. Five grams of powdered sample is taken and reflux with 200 ml of ethanol using Soxhlet apparatus on a water bath for 30 minutes. Filter the extract and concentrate to Five ml then the sample obtained is used for thin layer chromatography. Thin layer chromatography was carried out on pre-coated Silica gel aluminum plates. The sample alcoholic extract was applied on the TLC plate and developed with the selected mobile phase. The R_f values for the spots obtained in the TLC plate were calculated.

HPTLC Instrument and Method conditions:

Made/ Make of Instrument	Desaga Sarstedt Gruppe (Germany),
Development Chamber	20 X 10 cm,
Twin-trough chamber Stationary phase	Pre coated silica gel 60 F_{254} Aluminium plates ((Merck, KgaA Germany)
Plate thickness	0.2 mm
Plate size	100 x 100 mm
Distance from starting	20 mm
Distance from bottom	10 mm
Volume applied	5 µl
Band length	10 mm
Distance between tracks	20 mm
Development distance	70 mm
Solvent used	HPLC grade
Software	Proquant 1.6 version
Mobile phase solvent system	*toluene: ethyl acetate (8:2, v/v)*
Detection system	UV at λ 366 nm, λ 254 nm and after derivatized with vanillin sulfuric acid reagent and scanned at λ 580 nm.

RESULT and DISCUSSION

In this research work, standardization of QM was carried out in terms of its physicochemical, phytochemical and safety profile. The physicochemical study of *Qurṣ-e-Mafasil* (QM) includes the parameters such as organoleptic properties of formulation such as appearance, color, odor, taste and physicochemical studies were carried out such as Uniformity of weight, Friability, Disintegration time, hardness, LOD, pH of 1% and 5% aqueous suspension, ash vales and extractive values in different solvents like aqueous, alcohol & hexane. Further tests such as Thin-Layer Chromatography (TLC), and High-Performance Thin Layer Chromatography (HPTLC) studies were also carried out to develop fingerprint pattern of the alcoholic solvent extract of QM. Phytochemical screening was carried out in different solvent extracts such as alcoholic, aqueous and chloroform extracts to detect the presence of phytoconstituents in the formulation QM. Heavy metals, Microbial Load Contamination and pesticidal residues were also determined.

Quality assurance is an important part of all systems of medicine to establish the standard quality pharmaceutical drugs. Thus, there is high-priority requirement for the evaluation of parameters that can be adopted by the pharmaceutical industries for quality assessment of traditional preparations. Powder microscopy was done for the detection of microscopic structures present in that specific drug. The efficacy of a drug mainly depends upon its physical and chemical properties, so need of physicochemical characters is necessary for authenticity of the drug. Physicochemical study is also important, because it helps in characterization of constituents or groups of constituents that frequently lead to establish the structure activity relationship as well as mechanism of drug action. The phytochemical constituents present in the drug may vary, not only from plant to plant but also among the different sample of same species, depending upon various atmospheric conditions, storage, and drying conditions. If there is any deviation in terms of quality and quantity, that may alter the efficacy of the drug. In spite of quality assurance, adulteration is another factor that may contribute to variability.

Organoleptic properties: *Qurṣ-e-Mafasil* shows light brown color mainly due to presence of *Zanjabeel, Suranjan talq*. Shape of the tablet was obtained slightly biconvex. Odor was mild pungent due to presence of pungent & aromatic essential oils of Fil fil Siyah and Zanjbeel. It is an essential parameter for fast identification and consumer acceptance. Poor organoleptic properties not only lack aesthetic appeal and non-uniformity of content.

Powder microscopy: The following salient features of raw drugs were observed. Epidermis in surface view filled with dark brown pigment, fragments of cork cells, oleoresin cells; spiral vessels. Abundant simple, compound, spherical or oval shaped starch grains, tracheid, prismatic crystals of calcium oxalate, crossing the thin wall xylem fibers, cortical parenchymatous cells were also observed (Anonymous, 2016; Iyengar MA, 1980; Evans WC, 1983).

Uniformity of weight: The mean value of randomly selected 20 tablets was found to be in the range between 500 ± 8.69 to 503 ± 7.55 mg. The deviation from the average weight of each tablet was found within percentage limit of 5%.

Friability Test: The mean percentage of friability was found to be in the range between 0.0340 ± 0.001 to 0.038 ± 0.001 among the three different batches. Friability of tablets is done to check the tablet's strength. Improper handling, careless coating and packaging will tend to break down the tablet into powder, chip and fragment will lead to lack of consumer acceptance (Anonymous, 2018). It can also affect the uniformity of tablet and weight variation. Conventional compressed tablets that lose 0.5 to 1% of their weight are generally acceptable.

Disintegration test: The mean value of disintegration time in aqueous medium was found to be in the range between of 4 - 5 minutes. Disintegration test is used to determine whether tablet disintegrate within the prescribed time when placed in a liquid medium at the experimental conditions. Uncoated USP tablets have disintegration time minimum 5 min. and maximum 30 min (Kokate CK, *et al.,* 2012).

Hardness test: The mean value of three batches were found to be in range between of 8.40 ± 0.53 to 8.67 ± 0.42. Hardness test is done to check the resistance of a solid dosage form for mechanical deforming. To hold up mechanical distress during manufacturing, packaging, storage and transportation hardness test is an essential. It is an important quality control check in tablet manufacturing (Lachman L, *et al., 1987*).

Loss of weight on drying at 105 ^{0}C: The mean percentage of loss in weight on drying at 105^{0}C is ranged between 8.3425 ± 0.16 to 8.7346 ± 0.05.

This parameter helps to indicate the amount of water and volatile substances present in that particular drug. After drying at 105^0 C if the drug shows more loss of weight which indicated that particular drug is more prone to infection. The moisture content varies from drug to drug, but most of the times vegetative drugs are hygroscopic and excessive moisture content becomes an ideal medium for the growth of bacteria and fungi. They subsequently spoil the purity of drug (Anonymous, 2010).

pH value: pH was determined in 1% and 5% suspension and the values were found to be in the range of 5.8 to 5.9 respectively. In turn, these chemical species often affect the stability, therapeutic activity (through drug absorption) (Anonymous, 2010).

Extractive value: The mean percentage values of Alcohol, Water and Hexane were found to be ranged between 3.3352 ± 0.093 to 3.3962 ± 0.053, 30.9091 ± 0.13 to 31.4358 ± 0.16 and 1.1419 ± 0.08 to 1.4281 ± 0.04 respectively. The amount of the extract in a particular drug yield in a solvent is an approximate measure of the amount of a certain constituents present in that drug. Extractive value of a drug in a definite solvent indicates its purity and plays a major role to determine adulteration. Hence extractive values play a vital role for the establishment of standard of that particular drug (Anonymous, 2010).

Ash value: The mean percentage values of total ash and acid insoluble ash were found in the range between 3.7336 ± 0.010 to 3.8378 ± 0.027 % and 0.5859 ± 0.085 to 0.6112 ± 0.047 % respectively.

Estimation of ash values is a dominant parameter for the detection of impurities and adulteration. This establishes the quality and purity of the drug. The ash value indicates the residue remaining after incineration. It usually determines the inorganic substances present in the drug. It can also detect the nature of the material, added in that particular drug for the purpose of adulteration. Hence, determination of ash value provides criteria for judging the identity and purity of the drug (Anonymous, 2010). The mean percentage values of total ash and acid insoluble ash were found in the range between 3.7336 ± 0.010 to 3.8378 ± 0.027 % and 0.5859 ± 0.085 to 0.6112 ± 0.047 % respectively.

Thin layer chromatography: TLC studies of Alcoholic extract of *Qurṣ-e-Mafasil* was performed and R_f values of various spots appeared in *toluene: ethyl acetate (8:2, v/v)* solvent system was noted. Alcoholic (ethanol) extract of the sample was applied on the TLC plate and developed with solvent system *toluene: ethyl acetate (8:2, v/v)* as the mobile phase. The TLC plate show eight major spots under UV 366 nm at R_f values 0.14 (light blue), 0.23 (light blue), 0.30 (light blue), 0.40 (pale yellow), 0.51 (blue), 0.63 (light blue), 0.66 (pale yellow), 0.83 (light blue); and show nine major spots under UV 254 nm at R_f values 0.14, 0.31, 0.36, 0.39, 0.47, 0.53, 0.63, 0.69, 0.76 (all black); and detection after derivatizing with vanillin sulfuric acid reagent and heating at 110^{0}C show five major spots at R_f values 0.31 (brown), 0.41 (purple), 0.54 (purple), 0.67 (purple), 0.76 (purple).

For the detection of adulterant and determination of quality of drug TLC is an important technique. If the drug is adulterated there might be appearance of other compound present in adulterant that may increase the number of spots on TLC plate. On the other way deteriorated of exhausted drugs may lose the component and number of spots might appeared less.

The qualitative test: Phytochemical screening revealed the presence of Carbohydrates, Phenols, Proteins, Glycosides, Tannins, Phytosterols / Terpenes, Steroids, Saponin and Resins in the sample. The phytochemical screening is required for the detection of chemical constituent of drugs, which shows the presence of metabolites such as alkaloids, glycosides, flavonoids, saponins, tannins, starch, resins, proteins and polyphenolic compounds etc. In the present study phytochemical screening of alcoholic, aqueous and chloroform extract of *Qurṣ-e-Mafasil* was done for qualitative determination of different chemical constituents present in the sample. Different chemical tests were done to detect the presence of these compounds (Anonymous, 2007).

Test for microbial load contamination and specific pathogen: Total bacterial count was found 5x 102 /g, 10x 102 /g and 15x 102 /g in sample 1, 2 and sample 3 respectively and total fungal count were found nil in sample 1, 2 and sample 3. Specific pathogen like E. coli, Salmonella spp. were found absent (Anonymous, 2010).

Heavy metals contamination: In the formulation, heavy metals (lead, mercury, cadmium, arsenic) are found absent and complies with the permissible limit prescribed by WHO guidelines, indicating that the formulation is free from any unwanted contaminations and safe for consumption. Quantitative determination of heavy metals in herbal drugs are very important in the present situation as high quantity of these can lead to a number of health hazards. These heavy metals are usually accumulated in the plant through soil, contaminated water or air pollution. Consumption of such contaminated plant products may lead to various consequences in human's physiological system like renal damage, high blood pressure, change in heart rhythm or paralysis and possibly death. Hence, it was recommended by WHO that every herbal product or mineral based drugs should be examined tor the heavy toxic metals. (Anonymous, 2009).

Pesticidal Residue: Any substance or mixture of substances considered for preventing, destroying or controlling any pest, unwanted species of plants or animals causing harm during the production, processing, storage, transport and marketing of plant origin drugs is called a pesticide. They include growth-regulators, defoliants or desiccants and any substances applied to crops either before or after the harvest to protect from deterioration during transport and storage (Anonymous, 2009).

Table 1. Physicochemical parameters of the compound formulation QM

Parameters	Batch 1 (Mean ± SD)	Batch 2 (Mean ± SD)	Batch 3 (Mean ± SD)
Uniformity weight of Tablet (mg)	502 ± 8.77	500 ± 8.69	503 ± 7.55
Friability Test (%)	0.0340 ± 0.001	0.0382 ± 0.001	0.0348 ± 0.001
Disintegration Time in aq. medium (min)	5 ± 1	5 ± 1	5 ± 1
Hardness test (kg/cm)	8.67 ± 0.42	8.67 ± 0.31	8.40 ± 0.53
Loss in weight on drying at 105˚C (% w/w)	8.7346 ± 0.05	8.4576 ± 0.26	8.3425 ± 0.16
pH of 1% (aqueous suspension)	5.9167 ± 0.045	5.8567 ± 0.049	5.8533 ± 0.078
pH of 5% (aqueous suspension)	5.8467 ± 0.006	5.8300 ± 0.010	5.8333 ± 0.006
Alcohol soluble matter (%w/w)	3.5551 ± 0.015	3.3352 ± 0.093	3.3962 ± 0.053
Water soluble matter (%w/w)	31.4358 ± 0.16	30.9091 ± 0.13	30.9239 ± 0.34
Hexane soluble matter (%w/w)	1.1419 ± 0.08	1.4281 ± 0.04	1.3339 ± 0.17
Total Ash values (% w/w)	3.7336 ± 0.010	3.8378 ± 0.027	3.8209 ± 0.016
Acid Insoluble Ash (% w/w)	0.6091 ± 0.047	0.6112 ± 0.047	0.5859 ± 0.085

Table 2. Analysis of phytochemical constituents of QM

Phytochemical constituents	Observation		
	Alcoholic extract	Aqueous extract	Chloroform extract
Alkaloid	+	+	-
Carbohydrate	+	+	+
Phenols	+	++	-
Proteins	+	-	-
Flavonoids	-	-	-
Tannin	+	-	-
Saponin	-	-	+
Steroids	+	-	+
Starch	-	-	-
Fixed	-	-	-

Table 3. Microbial Load Contamination of QM

Sl. No.	Parameter Analyzed	Results			Permissible Limits as per WHO
		Sample – 1	Sample – 2	Sample – 3	
1.	Total Bacteria Load	05 X 10^2	10 X 10^2	15 X 10^2	Not more than 10^5/g
2.	Salmonella Spp.	Nil	Nil	Nil	Nil
3.	Escherichia Coli	Nil	Nil	Nil	Nil
4.	Total Fungal Count	Nil	Nil	Nil	Not more than 10^3/g

Table 4. Aflatoxin Contamination of QM

Sl. No.	Parameter Analyzed	Results			Permissible Limits as per WHO
		Sample – 1	Sample – 2	Sample – 3	
1.	B1	Nil	Nil	Nil	Not more than 0.50 ppm
2.	B2	Nil	Nil	Nil	Not more than 0.10 ppm
3.	G1	Nil	Nil	Nil	Not more than 0.50 ppm
4.	G2	Nil	Nil	Nil	Not more than 0.10 ppm

Table 5. Heavy Metals

S. No.	Parameters analyzed	Results	WHO Permissible Limits
1	Lead-(Pb)	ND	10 ppm
2	Cadmium- (Cd)	ND	0.3 ppm
3	Arsenic- (As)	ND	3.0 ppm
4	Mercury- (Hg)	ND	1.0 ppm

TLC and HPTLC analysis of an alcoholic (ethanol) extract of study formulation QM

Thin layer Chromatography of QM was carried out in alcoholic (ethanol) extract of the sample was applied on the TLC plate in three different batches and develop with solvent system *toluene: ethyl acetate (8:2, v/v)* as the mobile phase. QM was detected under following system as follows; Under UV 366nm - The Chromatogram profile showed eight major spots at R_f values 0.14 (light blue), 0.23 (light blue), 0.30 (light blue), 0.40 (pale yellow), 0.51 (blue), 0.63 (light blue), 0.66 (pale yellow), 0.83 (light blue); Under UV 254 nm - The chromatogram profile showed nine major spots at R_f values 0.14, 0.31, 0.36, 0.39, 0.47, 0.53, 0.63, 0.69, 0.76 (all black); and under the visible region detection after derivatizing with vanillin sulphuric acid reagent and heating at 110 ^{0}C show five major spots at R_f values 0.31 (brown), 0.41 (purple), 0.54 (purple), 0.67 (purple), 0.76 (purple). The TLC plate gets scan under the densitometer to obtain the corresponding HPTLC densitograms having peak areas for the spots. The corresponding data were presented in table 6-8 and corresponding densitograms obtained were shown in Fig 1- 4.

Table 6. Peak list of alcoholic extract of *Qurṣ-e-Mafasil* (QM) at UV λ 366 nm

Peak no	Y-Pos	Area	Area %	Height	R_f value
1	10.7	1744.01	15.74	595.21	0.02
2	19.8	840.50	7.59	290.76	0.15
3	23.4	215.52	1.95	106.07	0.20
4	26.1	173.33	1.56	71.01	0.24
5	32.8	5591.07	50.46	1202.98	0.33
6	38.7	2331.55	21.04	864.83	0.41
7	53.1	115.20	1.04	38.27	0.61
8	56.6	69.64	0.63	29.11	0.66

Table 7. Peak list of alcoholic extract of *Qurṣ-e-Mafasil* (QM) at UV λ 254 nm

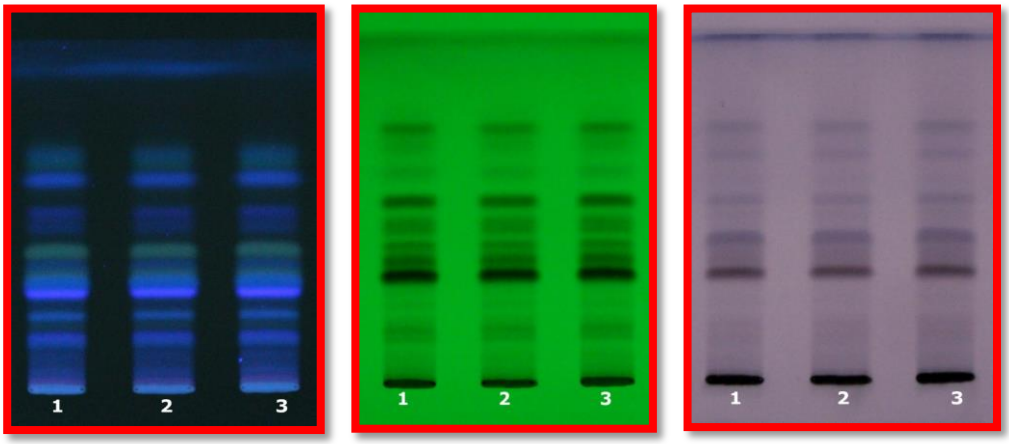
Peak no	Y-Pos	Area	Area %	Height	R_f value
1	10.7	2365.72	17.87	903.96	0.02
2	21.3	1080.69	8.16	233.95	0.17
3	26.4	220.56	1.67	104.16	0.24
4	32.6	3366.12	25.42	1079.15	0.33
5	35.3	1147.24	8.66	539.35	0.37
6	38.4	817.77	6.18	377.60	0.41
7	42.4	1170.71	8.84	299.46	0.46
8	47.5	1429.85	10.80	518.49	0.53
9	53.2	177.04	1.34	66.33	0.61
10	62.3	989.89	7.48	248.84	0.74
11	80.1	476.36	3.60	160.28	0.99

Table 8. Peak list of alcoholic extract of *Qurṣ-e-Mafasil* (QM) upon derivatized with vanillin sulfuric acid reagent at λ 580 nm.

Peak no	Y-Pos	Area	Area %	Height	R_f value
1	10.3	3116.07	40.80	1187.72	0.02
2	32.6	1684.90	22.06	496.71	0.32
3	39.6	1048.55	13.73	256.29	0.42
4	47.7	186.80	2.45	83.25	0.53
5	57.0	133.39	1.75	58.46	0.66
6	62.9	222.99	2.92	89.80	0.74
7	81.5	1245.54	16.31	443.29	0.99

Table 8. Peak list of alcoholic extract of *Qurṣ-e-Mafasil* (QM) upon derivatized with vanillin sulfuric acid reagent at λ 580 nm.

Peak no	Y-Pos	Area	Area %	Height	R_f value
1	10.3	3116.07	40.80	1187.72	0.02
2	32.6	1684.90	22.06	496.71	0.32
3	39.6	1048.55	13.73	256.29	0.42
4	47.7	186.80	2.45	83.25	0.53
5	57.0	133.39	1.75	58.46	0.66
6	62.9	222.99	2.92	89.80	0.74
7	81.5	1245.54	16.31	443.29	0.99



At UV 366nm At UV 254nm After derivatization

Fig 1. TLC plate of alcohol extract of *Qurs-e- Mafasil*

HPTLC analysis of alcoholic extract of *Qurṣ-e- Mafasil*

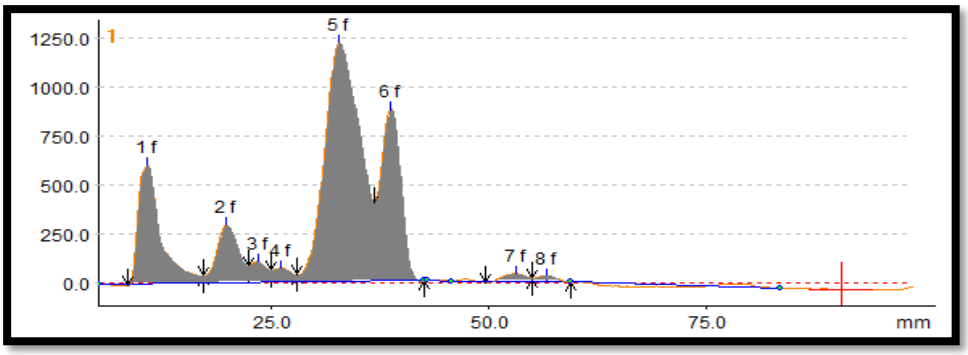


Fig.2 Densitogram of alcoholic extract of *Qurṣ-e-Mafasil* at UV λ 366 nm

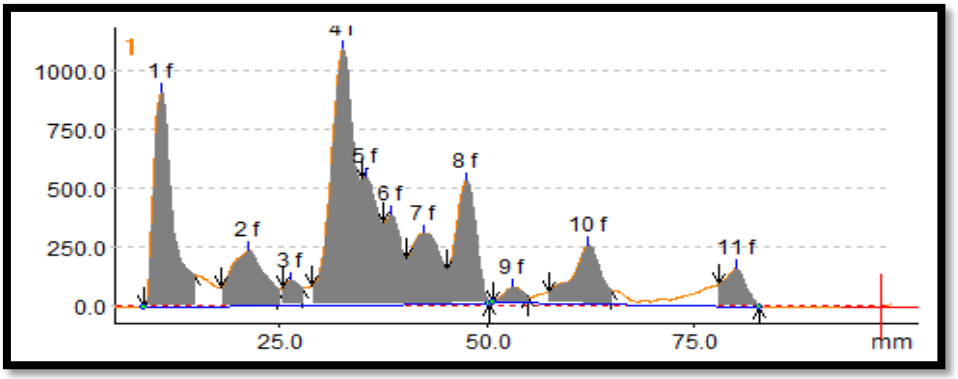


Fig.3 Densitogram of alcoholic extract of *Qurṣ-e-Mafasil* at UV λ 254 nm

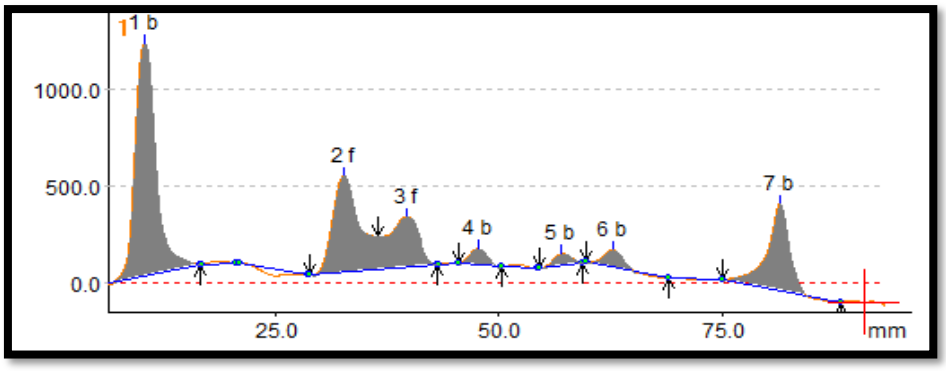


Fig.4 Densitogram of alcoholic extract of *Qurṣ-e-Mafasil* upon derivatized with vanillin sulfuric acid reagent at λ 580 nm.

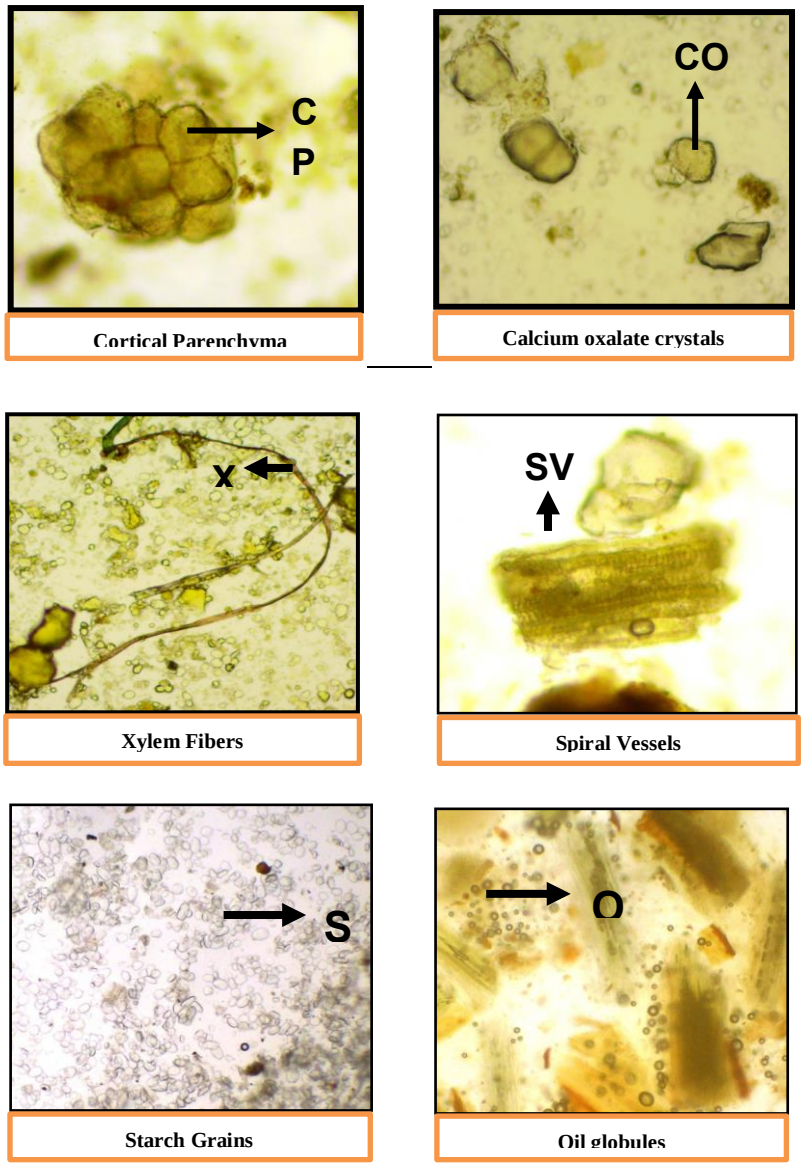


Fig.5 Powder microscopy

CONCLUSION

Qurṣ-e-Mafasil (QM) has been developed for Standard Operating Procedures and Standardized as there is no data is accessible regarding this. This formulation was Standardized for the first time to understand various standard parameters of Qurṣ-e- Mafasil. Powder microscopy has done, to check microscopic structures. The standard parameters applied for the standardization of *Qurṣ-e-Mafasil* includes organoleptic properties of formulation such as appearance, color, odor, taste and physicochemical studies were carried out such as Uniformity of weight, Friability, Disintegration time, hardness, LOD, pH of 1% and 5% aqueous solution, ash vales and extractive values in different solvents like aqueous, alcohol & hexane. Further TLC, HPTLC analysis that proves its identity and purity. Standardization of QM presents distinctive evidence - based research study. The formulation QM was standardized for the first time gives rise to various standard parameters. The results obtained may provide as a reference standard and could be beneficial for consideration of efficacious Unani formulation for future research endeavors.

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CONFLICT OF INTEREST-

The authors declare that there is no conflict of interest.

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