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PHYSICOCHEMICAL AND PHYTOCHEMICAL INVESTIGATIONS OF A POLY HERBAL UNANI FORMULATION: *QURSE MAFASIL*

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ABSTRACT

'*Qurse mafasil*' is a Unani poly herbal formulation mentioned in *Qarabadeen-e-Majeedi* (which is now a part of National Formulary of Unani Medicine, India), commonly prescribed for rheumatic conditions. This formulation contains three ingredients *Curcuma longa* Linn., *Colchium luteum* Baker. and *Acacia arabica* Willd. These herbals have anti-inflammatory, analgesic, anti oxidant, etc. properties. In present context, the adulteration has become a major problem due to unavailability of standards relating to genuineness of herbal drugs. The WHO has emphasized the need to certify the quality of herbal products by adopting various standard parameters. In this study efforts have been made to analyze the physicochemical and phytochemical parameters such as extractive values, ash values, loss on drying, pH, swelling index, fluorescence analysis, powdered drug reaction with different reagents, disintegration test, uniformity of mass/ weight variation and estimation of total phenolics and flavonoids content were carried out in *Qurse mafasil* tablet. HPTLC fingerprint profiles of different extracts of the drug also developed. This would serve as a standard reference for identification, authentication and for further studies in this Unani formulation.

INTRODUCTION

In the present era, resurgence in the use of herbal products, a booming market for natural products and a rapidly growing consumer acceptance of complementary medicine, it is imperative to urgently evolve sensitive modern standards for the quality, safety and efficacy of traditional medicines[1]. Standardization is an essential measurement for ensuring the quality control of the drugs. In synthetic drug formulations, chemical and instrumental analyses are routinely used to confirm its authenticity. In contrast, especially for poly-herbal formulations, there is no chemical or analytical method available. However, the herbal formulations in general can be standardized schematically as to formulate the preparation with better clinical efficacy by adopting pharmacognostical, physicochemical, phytochemical analysis for routine checkup[2].

Today quality assurance is thrust area for the evaluation of traditional used medicinal plants and herbal formulation. Traditional medical practitioners prepared the medicines by themselves according to the need of their patients on individual basis. In order to maintain the quality standards of the herbals and its formulations, they have adopted various organoleptic methods. However, in present context, there is a need to change in this way of approach, because large number of herbal drugs is manufactured and available in the market. In this regard, availability of high-quality & standard drugs became the major problem. Hence, the World Health Organization (WHO) has emphasized the necessity to make sure the quality of medicinal plant products by adopting standard scientific techniques. By that way, concrete methods of quality standards can be developed for the herbal formulation.

'*Qurse mafasil*' (QM) is a Unani herbal formulation mentioned in *Qarabadeen-e-Majeedi*, (which is now a part of National Formulary of Unani Medicine, recognized by Indian government, published by Department of AYUSH, Ministry of Health & Family Welfare) prescribed for arthritis (*waja-ul- mafasil*)[3]. This formulation contains three ingredients i.e. *Chob Zard*- Turmeric (*Curcuma longa Linn.), Surinjan Talkh*- Golden collyrium (*Colchium luteum* Baker.) and *Samaghe Arabi*- gum Arabic (*Acacia arabica* Willd.). *Colchium luteum* (Surinjan) is the drug, which is highly recommended for rheumatism by almost all the Unani physicians[4-5] and it is given in conditions like arthritis, gout, backache, sciatica, etc.[6-11] *Curcuma longa* Linn. (Chob zard/ Haldi) is having anti-inflammatory, analgesic and antioxidant[6-13] properties which are responsible for its continuous use in this formulation. *Acacia arabica* (*Samaghe arabi*) is used as base (binding property); in addition to that it has antioxidant properties, hence it can be used in inflammatory conditions [8, 10-11, 14]. Therefore, it is clear that the combination of these three drugs will effectively alleviate the disease process.

Since, adulteration and substitution have become a major problem due to the absence of standards; the purpose of standardization of medicinal plant products is obviously to ensure the therapeutic efficacy. Therefore, efforts have been made to obtain and maintain the high quality of this formulation. Hence, following protocol has been prepared for quality standardization of herbal formulation QM.

MATERIAL AND METHODS

Source of the drug

The herbal formulation, *Qurs-e-mafasil* is a product of Hamdard (*Wakf*) Lab., Delhi (GMP certified) and this is dispensed free of cost by Unani pharmacy, Majeedia Hospital, Jamia Hamdard. Batch number: M 029.

Extractive values

The drug was extracted with different solvents to get the correct and dependable values. Petroleum ether, chloroform, methanol and water extractive values are taken into consideration for fixing the standards of this drug. Extractive values such as cold extraction, hot extraction & successive extraction were determined by using the method described by Indian pharmacopeia[15].

Ash value

Different types of ash figures such as total ash, acid insoluble ash and water-soluble ash were calculated as described by Indian Pharmacopeia[15].

Effect of different chemical reagent on drug powder

The powdered drug was treated with different reagents and acids like sodium hydroxide, picric acid, hydrochloric acid, ferric chloride, nitric acid etc. and the color shown by that treatment was noted as described by Arya, et al.,[16].

Fluorescence analysis

Many herbs fluorescence when cut surface or powder is exposed to UV light and this help in their identification. The fluorescence character of the powders (40 mesh) was studied both in daylight and UV light after treatment with different reagents[17].

Preliminary phytochemical screening

The powdered drug was subjected to preliminary phytochemical investigation for detection of alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids, proteins and amino acids. Qualitative test of alkaloids were done by taking the powdered drug in 5 ml of 1.5% hydrochloric acid and filtered. The filtrate was then tested with reagents Dragendorff's reagent, Hager's reagent, Wagner's reagent, Mayer's reagent[18]. Phytochemical screening of carbohydrates was done by using Molisch test, Fehling's reagent test[18]. Glycosides was screened by using the

Borntrager's test[18]. Tests for phenolic compounds was carried out by using the methods described by Ali,[19]. Tests for flavonoids was performed by using Ammonia test[20]. Proteins and amino-acids were tested by Millon's test, Biuret test, Xanthoprotein test & Ninhydrin test[21].

Loss on drying

This parameter is used to determine the amount of moisture present in the drug. The powdered drug 1g was placed on a tarred evaporating dish. This was dried at 105 ^{o}C for 6 h and weighed. It was continued until two successive reading matched each other or the difference between two successive weighing was not more than 0.25% of the constant weight.

Determination of pH

pH 1% and 10 % solution of drug was prepared in distilled water and filtered; pH of filtrate was checked with standard glass electrode[17].

Determination of swelling index

The swelling index is the volume in ml taken up by the swelling of 1 g of herbal material under specified conditions. Swelling index was determined by using the method described by WHO guidelines[17]. Three readings were taken and mean value obtained.

Disintegration test

This test determines whether tablets disintegrate within the prescribed time when placed in an immersion fluid under prescribed experimental conditions. Unless otherwise specified in the individual monograph, water is used as the immersion fluid at a temperature of 37 ± 2 °C. One tablet is placed in each of the six tubes. The apparatus is operated for the specified period of time, withdraw the assembly, and examined the state of the tablets[22].

Uniformity of mass/ Weight variation

20 tablets were weighed and calculated the average mass. When weighed singly, the deviation of individual masses from the average mass should not exceed the limits as given in table 1. If the tablets meet the requirement of this test, they can be considered acceptable[22].

Table 1: Uniformity of mass test[22]

Average mass of tablet	Deviation %	Number of tablets
Less than 80 mg	±10.0	minimum 18
	±20.0	maximum 2
80 mg to 250 mg	±7.5	minimum 18
	±15.0	maximum 2
More than 250 mg	±5.0	minimum 18
	±10.0	maximum 2

Quantitative chemical evaluation

Phytoconstituents in herbal medicine are important to manage pathological conditions of diseases. Hence, quantitative estimation of phenolic & flvonoids content were determined.

Phenolic content

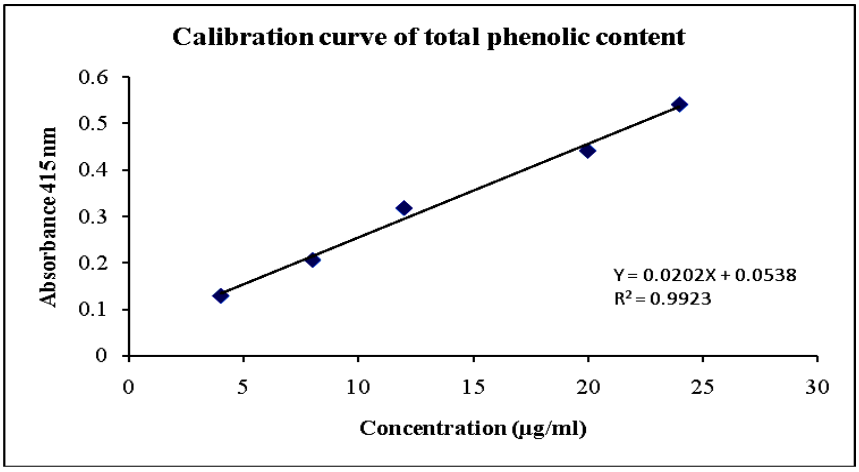
The phenolic content was determined in powdered drug by Folin Ciocalteu (FC) method[23]. This test is based on the oxidation of phenolic groups with phosphomolyblic and phosphotungstic acids. After oxidation a green-blue complex is formed which is measured at 765nm in UV.

Standard stock solution was prepared by dissolving 25 mg of catechin standard in 100 ml distilled water. Different concentrations of the standard solutions were prepared for standard calibration curve starting from 4 to 24 µg/ml in water. The commercial FC reagent was diluted (1: 10) with distilled water on the day of use. 1M sodium acetate was prepared by dissolving 82 g of sodium acetate in 1000 ml distilled water

Sample preparation - 500 mg of the samples were taken in 50 ml volumetric flasks and added around 25 ml of distilled water and sonicated for 10 minutes then made up the volume with water.

Method - Take 3ml of each standard and sample solution in a 10 ml test tube and to this add 3 ml of FC reagent and 3 ml of sodium carbonate solution. A blank solution was prepared by adding 3 ml each of distilled water, sodium carbonate solution, and FC reagent in test tube. Keep the solution in dark for 30 minutes for color development. Absorbance was taken at 765nm against blank solution. After taking the absorbance of standard dilutions, calibration curve was plotted (Fig. 1). Phenolic contents in drug were calculated by using standard calibration curve[23].

Figure 1: Calibration curve of standard catechin for total phenolic contents

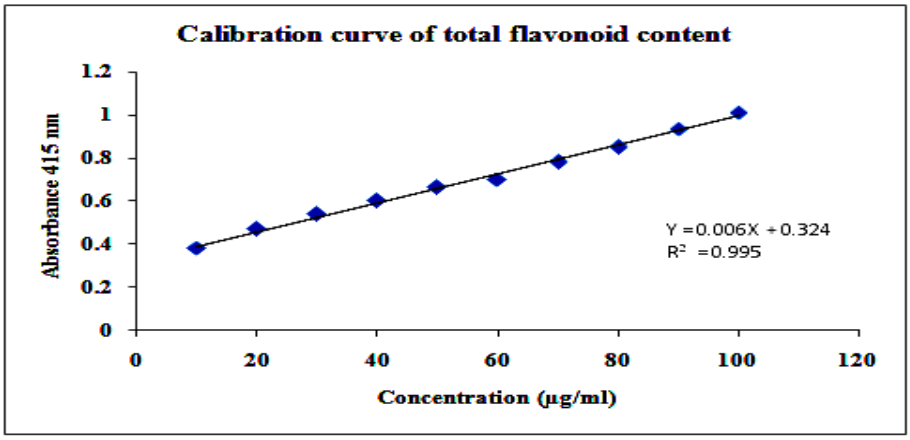


Flavonoids

The flavonoids content of the powdered drug were determined by using following method. $AlCl_3$ (0.1 g/ml), CH_3COONa (1M), diluted Rutin solution (100 µg/ml) were prepared. 500 mg of the samples were taken in 50 ml volumetric flask and added around 25 ml of methanol and sonicated for 30 minutes then made the volume with methanol.

Method - 0.5ml of each standard and sample solution was taken in a 10 ml test tube and added 1.5 ml methanol, then 0.1ml of $AlCl_3$ and 0.1 ml of CH_3COONa reagents, 2.8 ml Distilled water were added and kept for 30 minutes. A blank solution was prepared by adding 2 ml of methanol, 0.1ml of $AlCl_3$, 0.1ml of CH_3COONa reagents and 2.8 ml Distilled water. Absorbance was taken at 415 nm against blank solution. After taking the absorbance of standard dilutions, calibration curve was plotted (Fig 2). Flavonoid contents in drug were calculated by using standard calibration curve.

Figure 2: Calibration curve of standard rutin for total flavonoid content



HPTLC analysis

Precoated plates and auto sampler were used for precision and to achieve significant separation. UV, Visible and fluorescent scanner were used for qualitative estimation. Extract were obtained by using different solvent (petroleum ether, chloroform, and methanol), then extracts were remained after recovering the solvents. Extracts were stored in deep freezer at -20C^0 until further application. For TLC and HPTLC samples were prepared by dissolving each extract in their respective solvent to get the concentration of 40 mg/ml.Solutions were further pass through syringe filter to remove any impurities and applied on TLC plate for finger printing analysis.

The extract was applied on TLC aluminum sheets silica gel 60 F 254 (Merck) 10 µL each with band length 6 mm using Linomat 5 (CAMAG, Switzerland) sample applicator set at a speed of 100 nl/sec. Toluene, Ethylacetate, Formic acid (TEF) solvent systems were used in different

ratio for separation of constituents of different extracts. The chromatograms were developed in twin trough chamber for 20 minutes up to the distance of 80 mm. Scanning was performed by CAMAG HPTLC Densitometer (Scanner 3) in absorbance mode at both 254 and 366 nm using deuterium and tungsten lamp with slit dimension 6.0 x 0.45 macro[24].

RESULTS AND DISCUSSION

World Health Organization supports, advocates and promotes alternative systems of medicines (Traditional herbal remedies) in primary health care systems, because these are used since the time of immemorial period and people have faith in them. In addition, herbal medicines are comparatively safe, effective and easily available. Standardization provides a more consistent, potent and effective product by chemical analysis to authenticate the quality and quantity of plant metabolites or pharmacologically active components in the herbs. Hence, need of hour to utilize modern techniques in standardization of herbal drugs. Keeping in view importance of quality control,drug QM was standardized according to WHO guidelines for herbal drugs.

In present investigation various standardization parameters such as organoleptic evaluation, physicochemical analysis, phytochemical screening, phenolic & flavonoid contents and HPTLC analysis were carried out to develop the standard of QM.

Qurse mafasil is a compressed, uncoated, circular tablet, yellow in color, astringent taste and the weight of a tablet is 500 mg.

The extractive value is used to determine the amount of active constituents. The petroleum ether extract contains fixed oil, resins and volatile substances, but when the extract is heated at 105^{o}C until constant weight, the volatile substances are volatized leaving only resin, coloring matter and fixed oil. Alcohol can dissolve almost all the substances, but is generally used for determining the extractive index for those drugs, which contain glycosides, resins, alkaloids etc. Water is used for the drugs containing water-soluble substances as chief constituents[15].

Ash values are used to determine the extraneous matters like sand and soil. This parameter was used for the determination of inorganic materials such as carbonates, silicates, oxalates and phosphates; with the help of this parameter we could detect the extent of adulteration, exhausted drugs and excess of sandy or earthy material.

Many herbal materials are of specific therapeutic or pharmaceutical utility because of their swelling properties, especially gums and those containing an appreciable amount of mucilage, pectin or hemicelluloses[17].

The results of extractive values, ash values, moisture content (loss on drying), pH value, swelling index of *Qurse mafasil* were summarized in table 2. In each case, three readings were

taken which was converted in to percentage, then the mean value was calculated and recorded with standard error of mean (*SEM*). Utilizing these standards the formulation can be differentiated from adulterants.

Table 2: Summary of physico-chemical and phytochemical results of *Qurse mafasil*

B.	**Individual extractive values cold extract**	**Extractive value***
1.	Petroleum ether extract	1.6 ± 0.15 % *w/w*
2.	Chloroform cold extract	3.8 ± 0.25 % *w/w*
3.	Methanol cold extract	9.5 ± 0.47 % *w/w*
4.	Aqueous extract	2.93 ± 0.24 % *w/w*
C.	**Individual extractive values hot extract**	**Extractive value**
1.	Petroleum ether extract	4.1 ± 0.17 % *w/w*
2.	Chloroform extract	4.17 ± 0.07 % *w/w*
3.	Methanolic extract	19.29 ± 0.94 % *w/w*
4.	Aqueous extract	17.16 ± 0.68 % *w/w*
D.	**Successive extraction**	**Extractive value**
1.	Petroleum ether extract	4.09 ± 0.2 % *w/w*
2.	Chloroform extract	1.49 ± 0.06 % *w/w*
3.	Methanol extract	13.75 ± 0.79 % *w/w*
4.	Aqueous extract	14.12 ± 0.81 % *w/w*
E.	**Ash value**	**value**
1.	Total ash	8.07 ± 0.29 % *w/w*
2.	Acid insoluble ash	4.87 ± 0.41 % *w/w*
3.	Water soluble ash	2.0 ± 0.12% *w/w*
F.	**Loss on drying (%)**	8.87 ± 0.18 % *w/w*
G.	**pH of the drug 1%**	6.78 ± 0.009
H.	**pH of the drug 10%**	6.65 ± 0.003
I.	**Swelling index**	3.57 ± 0.044 ml

*Values are given in mean with *SEM*

Disintegration is defined as the state in which no residue of the tablet, except fragments of undissolved coating or capsule shell, remains on the screen of the test apparatus or, if any other residue remains, it consists of a soft mass having no palpably firm, unmoistened core[22]. All six tablets of QM have passed disintegrated test.

To test the uniformity of mass of the tablets 20 tablets were weighed. The mean weight of the QM tablet was 506.95 ± 3.097 (95 % Confident Interval, 500.47 to 513.43). Acceptable deviation of single tablet was 506.95 ± 5% (upper limit is 532.3 mg and lower limit is 481.6 mg). When weighed singly, all 20 tablets were not exceed the limits. Hence, *QM* tablets were uniformed in weight, and the weight variants were not significant.

The preliminary phytochemical screening was carried out using the powder to determine the presence of different types of chemical constituents, which are responsible for various therapeutic effects. Therefore, the powder of QM was analyzed for detection of alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids, proteins and amino acids. It was found that these chemical constituents were present in the formulation.

QM was treated with different chemical reagents, it showed dark brown, red, orange, brown, black, dark brown, brownish yellow and yellow with 10% Aq. NaOH, con. HNO_3, con. H_2SO_4, con. HCl, Iodine, glacial acetic acid and picric acid (saturated) respectively.

Fluorescence is an important parameter of pharmacognostical evaluation. Various chemical constituents present in plant material exhibit different fluorescence as such or when react with various chemicals. Fluorescence analysis of powdered drug with distilled water, dil. HNO_3, dil. H_2SO_4, dil. HCl, ethyl acetate, 5% ferric chloride, ammonia, methanol, chloroform, petroleum ether, 10% aq. NaOH and glacial acetic acid gave different characteristic color under ultraviolet (254 and 366 nm) and under normal ordinary light. These are summarized in table 3.

Table 3: Effect of different chemical reagents with crude drug powder on the fluorescence behavior

S.No.	Treatment	Day light	UV light (254 nm)	UV light (366 nm)
1.	Powder + distilled water	Yellow	Violet	Light green
2.	Powder + Dil.HNO_3	Orange	Black	Light green
3.	Powder + Dil. H_2SO_4	Brown	Black	Black
4.	Powder + Dil. HCl	Maroon	Black	Black
5.	Powder + Ethyl acetate	Light green	Light green	Yellow
6.	Powder + 5% Ferric chloride	Brown	Black	Dark brown
7.	Powder + Methanol	Light green	Light yellow	Light green
8.	Powder + chloroform	Yellow	Light yellow	Light green
9.	Powder + petroleum either	Light green	Violet	Light green
10.	Powder + 10% Aq. NaOH	Red	Dark red	Maroon
11.	Powder + Glacial acetic acid	Brownish Yellow	Fluorescence yellow	Brown
12.	Powder + Acetone	Light green	Light yellow	Light green
13.	Powder + Acetic acid	Yellow	Whitish	Light green

Compounds like phenolic and flavonoid are important plant metabolites, which exhibits several important functions in the herb such as maintaining the health, protecting plant from various diseases. Currently, phenolic and flavanoids are the topics of interest due to its role against oxidative damage diseases i.e. antioxidant activities. Besides this, some other important roles of these compounds are anti-allergic, anti-inflammatory, antimicrobial, anticancer activities, etc.[25-27]. Quantification of these compounds is important in determination of quality & efficacy of the drug. Thus, quantitative analysis of phenolic and flavonoid content of QM were carried out by UV spectroscopic method. The amount of phenolic content of 10 mg/ml of powder of *QM* was calculated with the help of standard calibration curve (Fig. 1) and found to be 0.497 ± 0.001 % (*w/w*). The amount of flavonoid content of 10 mg/ml of powder of *QM* was calculated with the help of standard calibration curve (Fig. 2) and found to be 2.34 ± 0.14 % (*w/w*).

HPTLC method is used for separation of the components present in the mixture both qualitatively and quantitatively. It is usually quicker and gives better separation[25]. HPTLC fingerprint profile of various extracts are used as markers for quality evaluation and standardization of the drug. The chromatograms obtained after development in different solvent systems are scanned at 254 and 366 nm in absorbance mode to depict the presence of number of substances in the extracts.

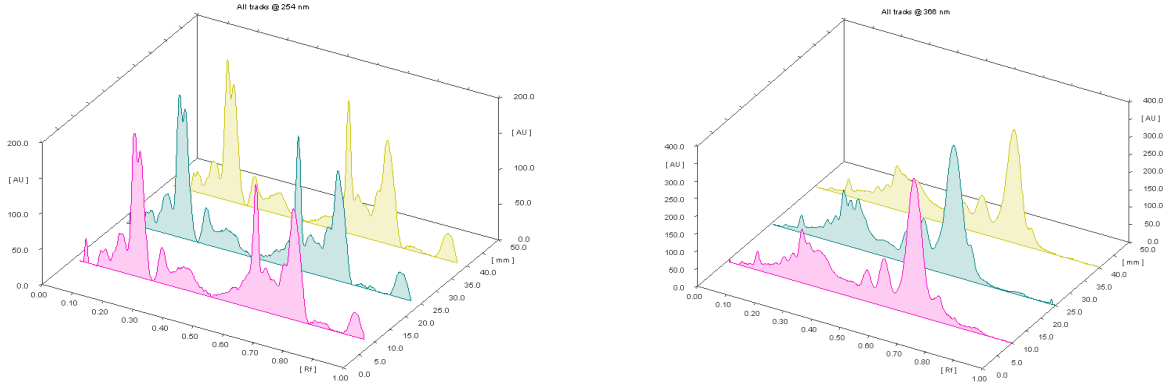
Results of HPTLC finger printing analysis of different extracts of QM such as methanolic extract, chloroform extract and petroleum ether extract carried out at 254 nm and 366 nm using different solvent systems are given in table 4.

Table 4: HPTLC fingerprint of various extracts of *Qurse mafasil*

Extracts	**Solvent system**	**No. of peak observed (Rf values) at 254 nm**	**No. of peak observed (Rf values) at 366**
Chloroform extract	Toluene : Ethyl acetate (8 : 2)	0.10, 0.14, 0.17, 0.20, 0.25, 0.28, 0.30, 0.36, 0.42, 0.46, 0.54, 0.64, 0.70, 0.86, 0.99	0.05, 0.11, 0.17, 0.20, 0.23, 0.26, 0.29, 0.32, 0.49, 0.55, 0.66, 0.74, 0.80, 0.87
Methanolic extract	Toluene : Ethyl acetate: Formic acid (6: 3: 1)	0.04, 0.13, 0.18, 0.22, 0.25, 0.29, 0.35, 0.41, 0.62, 0.72	0.04, 0.13, 0.18, 0.22, 0.25, 0.29, 0.35, 0.41, 0.62, 0.72
petroleum ether extract	Toluene : Ethyl acetate: Formic acid (8: 1.5: 0.5)	0.06, 0.12, 0.16, 0.18, 0.26, 0.39, 0.49, 0.59, 0.70, 0.81, 0.95	0.07, 0.16, 0.19, 0.27, 0.40, 0.49, 0.81

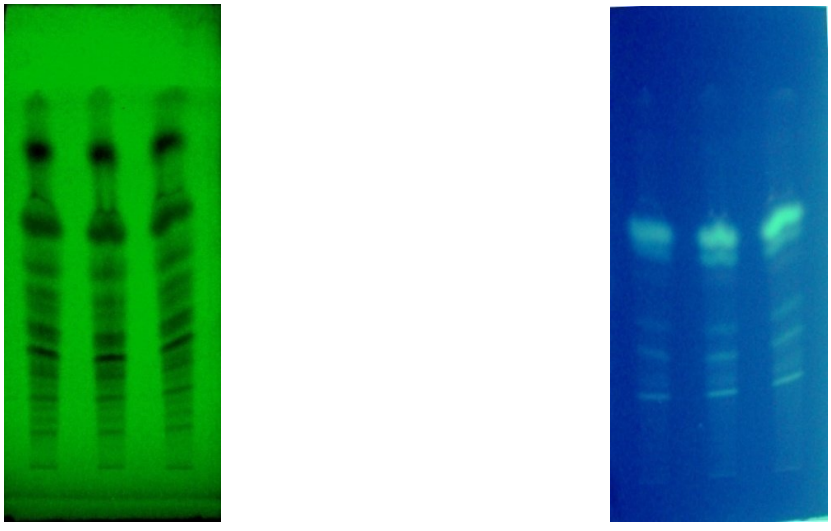
HPTLC fingerprint of methanolic extract of *QM* showed 10 spots at both 254 nm & 366 nm in Tolune: Ethyl acetate: Formic acid (6: 3: 1). HPTLC fingerprint of chloroform extract of *QM* showed 15 & 14 spots in Toluene: Ethyl acetate (8: 2) at 254 nm & 366 nm respectively. HPTLC fingerprint of petroleum ether of *QM* showed 11 & 7 spots in Tolune: Ethyl acetate: Formic acid (8: 1.5: 0.5) at 254 nm & 366 nm respectively. 3 D view of HPTLC fingerprinting analysis of different extracts of *QM* and chromatogram of different extracts of *QM* are shown in Fig. 3- 8.

Figure 3: 3D view of chloroform extract at 254 nm (A) and 366 nm (B).



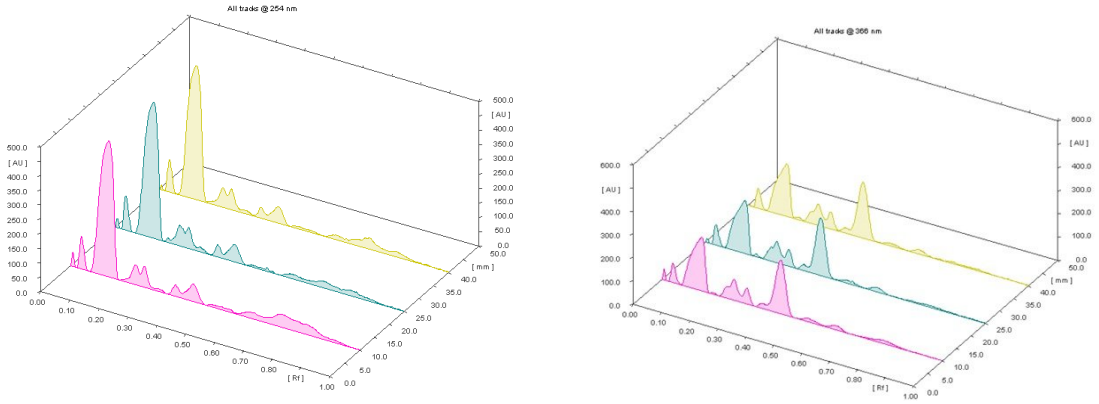
(A) **(B)**

Figure 4: Chromatogram of chloroform extract at 254 nm (A) and 366 nm (B)



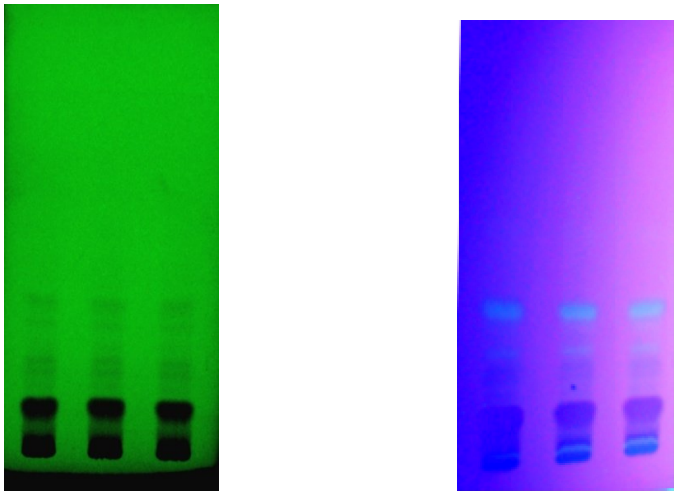
(A) **(B)**

Figure 5: 3D view of methanolic extract at 254 nm (A) and 366 nm (B)



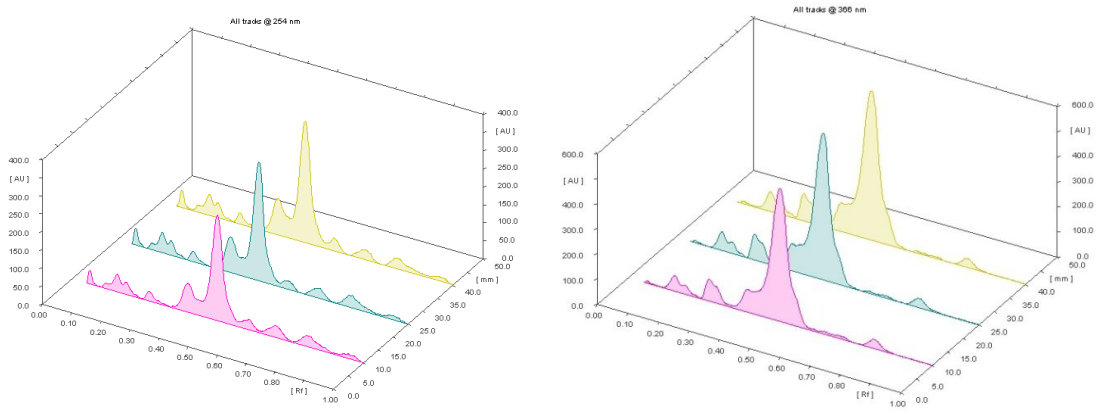
(A) **(B)**

Figure 6: Chromatogram of methanolic extract at 254 nm (A) and 366 nm (B)



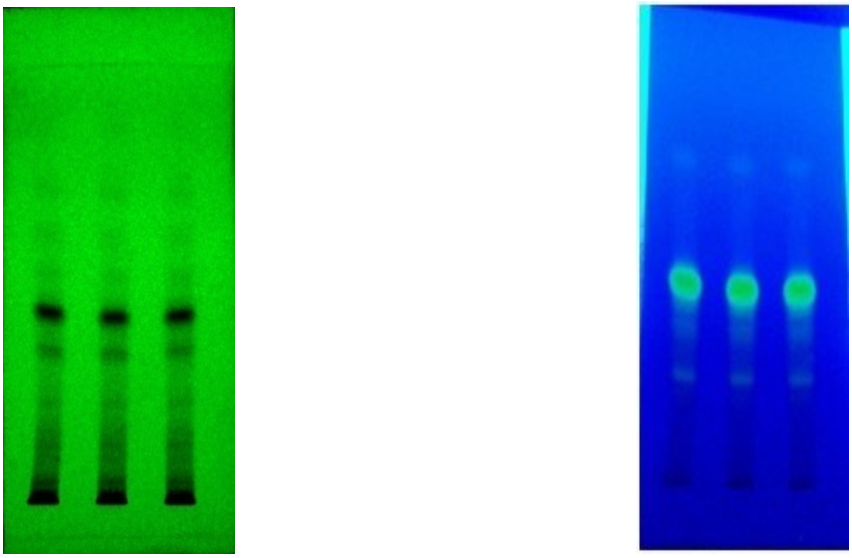
(A) **(B)**

Figure 7: 3D view of petroleum ether extract at 254 nm (A) and 366 nm (B)



(A) **(B)**

Figure 8: Chromatogram of petroleum ether extract at 254 nm (A) and 366 nm (B)



(A) **(B)**

CONCLUSION

Majority of physicians currently prescribes many herbal remedies to the patients without considering its quality and standards. This practice gives rise unwanted effects and diminished therapeutic effects, ultimately leads loss of confident of the patients towards herbal medicines. As the usage of these herbal medicines is increased, issues regarding their quality, safety, and efficacy have raised up. The purpose of standardization of herbal products is obviously to ensure the therapeutic efficacy and reduce unwanted effects. Therefore, maintaining the quality of these herbal products is an essential factor.

Qurse mafasil is an important drug, which is commonly used in rheumatological conditions in Unani medicine. It is a timely need to standardize the herbal formulation to determine the quality of the drug. Hence, efforts have been made to provide scientific data on standardization of *QM* tablet. The physicochemical and chromatographic studies of this herbal formulation would help to identify and determine its quality, purity and nature of its adulteration. Further, these data could be utilized to standardize this valuable herbal formulation for further studies.

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