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## RESEARCH ARTICLE

## Standardization of Khubazi- a fruit of *Malva sylvestris* Linn. – A Unani Drug

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### Abstract

Proper identification & standardization is mandatory to ensure the therapeutic efficacy of herbal drugs used for health ailments. All single drugs and compound formulations should be standardized according to the guidelines of Pharmacopoeia to ascertain their quality standards. Physicochemical and Phytochemical Standardization is considered a prerequisite for the assessment of biological activity or determination of biological standards of the plant material. It provides the analytical characteristics which may prove to be useful in fixing the physicochemical standard for the Unani drugs. Unani Medicine possesses a large number of drugs used in various diseases as mentioned by eminent Unani Physicians based on their own long term experience. But, a doubt always remains regarding the standardization of Unani drugs. Present study was done with an aim to standardize and to assure the quality of an important Unani drug Khubazi (*Malva sylvestris* Linn.) used for various upper respiratory tract infectious diseases.

Khubazi (*Malva sylvestris* L.) belong to the family Malvaceae, its fruits are used in respiratory diseases such as asthma, cough, diphtheria, chronic bronchitis, sore-throat, coryza. An effort has been made to carry out the physicochemical and phytochemical studies of Khubazi. Physicochemical parameters as Extractive Values in different solvents: petroleum ether (3.56%), di-ethyl ether (0.75%), chloroform (2.08%), acetone (0.85%), ethanolic (3.80%), aqueous (11.72%); Solubility: Water (6.46 %) & Alcohol (0.97 %); Moisture content (2.56 %), Total Ash values (14.15%), pH of 1% (6.94) & 10% solution (7.2) and loss on drying (3.84%). Phytochemical Analysis revealed the presence of almost all the phyto-constituents in the test drug sample i.e. alkaloid, flavonoids, glycoside, carbohydrate, tannin, protein, amino acids, starch and resins.

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### INTRODUCTION

The interest in evaluating therapeutic effects of plants has increased, as 80% of world population depends on complementary and alternative medicine for their health care needs (Magee KA, 2005; Duraipandiyani, 2006). The drug Khubazi consists of dried fruits of *Malva sylvestris* Linn. (Family-Malvaceae). It is an erect, branched, nearly glabrous, woody biennial or perennial plant found throughout the plains of India (Anonymous, 2009). Use of the drug 'Khubazi' in Unani system of medicine dates back about a couple of thousands of years. It was mentioned in Unani Classical Book '*Kitabul Hashaish*' of Dioscorides (1st B.C.); this was used by both Greeks and Romans as a medicine on account of its mucilaginous and cooling properties.

The fruit of Khubazi are light brown coloured consist of from 10-12 glabrous wrinkled carpels, each containing one reniform seed; some of it are mature, but around half are in various stages of maturity, a portion of the thin papery

calyx is attached to the fruit, and in a good fresh sample a few deep blue flowers may be found as well as the peduncles and portions of the leaf. Seeds are planted in June that grew freely in Bombay, and produce flowering plants in the rainy season (Dymock, 1890; Khory and Katrak, 1985). Khubazi as a whole is a perennial herb of 0.3-1.2m high with tall, erect, strong, woody and branched stem; leaves on long stalks. 3-7 lobed, reniform at the base, lobes radiating from a common centre, the lobes are shallow with margin scalloped, smooth above and roughly hairy below. Bracteoles are ovate, entire, shorter than the campanulate calyx, corolla 3.8 cm. diameter of purple colour, with veins of deeper tint, much longer than the calyx. It is distributed in India in West temperate Himalayas from the Punjab to Kumaon 2000-8000 ft. - In Bombay, Mysore and Madras; and as a weed of cultivation in Siberia, Caucasus, Europe, North Africa (Kirtikar and Basu, 1987).

#### **MEDICINAL PROPERTIES:-**

*M. sylvestris* is the supreme one among its comrade drugs used in respiratory ailments. It has been praised by the physicians of all times as a ‘panacea’ and is broadly used in a huge number of diseases.

All parts of the plant are mucilaginous and cooling; febrifuge; good for blepharitis and all inflammatory conditions; internally good for sore throat, chronic bronchitis, jaundice and enlargement of the spleen; useful in strangury, urinary discharges and scorpion sting. It is used in pulmonary diseases and all inflammatory conditions of intestine and urinary bladder (Anonymous, 2009) also used in affections of the mucous membrane of the pulmonary tract and of the urinary bladder (Kirtikar and Basu, 1987). In tenesmus it is employed as a clyster; in external inflammations as a poultice. as laxative, spasmolytic, expectorant, anti tussive, anti oxident anti diarrheal and highly recommended for acne and skin care (Lillian, *et. al.*2010). It is also used in constipation, cough, horseness, diuretic, dysentery, burning micturation, as anti inflammatory, its enema is very beneficial in burning conditions of rectum, and uterus and used in wound healing (Ghani, 2011; Ibn-e-Baitar 1999).

No work has been reported regarding standardization of this drug so far. Keeping in mind the medicinal importance of this plant in Indian System of Medicine specifically Unani System of Medicine a physico-chemical and phytochemical study of Khubazi was carried out following various parameters.

#### **Material and Method**

**Collection of plant material:** The drug samples of Khubazi fruits (Figure-1) were collected from local market of Aligarh city (U.P) and were properly identified by botanical literature available and then confirmed by Prof. S. H. Afaq Pharmacognosy Section of Department of Ilmu Advia, Aligarh Muslim University, Aligarh. Voucher specimens were preserved in the herbarium of Medicinal Plant Lab in the Department of Ilmu Advia, Faculty of Unani Medicine, Aligarh Muslim University, Aligarh (Voucher No. SC-0142/14).

**Physico-chemical parameters:** The dried powder of the fruits of Khubazi was used for physico-chemical analysis. First the organoleptic characters were identified followed by various physico-chemical studies like total ash, acid insoluble ash, water soluble ash, alcohol and water soluble matter, moisture content, successive extractive values using soxhlet extraction method, bulk density, crude fiber content and pH studies were carried out as per guidelines of W.H.O. (Anonymous, 1998) and Govt. of India (Anonymous, 2008). Qualitative analysis of the drug was conducted to identify the organic chemical constituents present in the drug (Overtone, 1963; Harborne, 1973). Thin layer chromatographic analysis was conducted (Stahl, 1969; Harborne, 1973) on precoated silica gel 60F<sub>254</sub> TLC plates. The plates were visualized in Day light, in short UV and Long UV. They were also derivatised using iodine vapors.

#### **OBSERVATIONS**

**A. Organoleptic characters:**

The powder of the fruits of Khubazi was brownish Yellow with characteristic odour with slightly bitter taste (Summarized in Table-1).

**B. Physico-chemical constants:**

The analytical values of different physico-chemical constants were determined (Table-2).

**C. Qualitative analysis of phyto-chemical constituents of drug:**

The phytochemicals present in the drug were identified on the basis of different chemical tests given for various plant constituents, results have been summarized in Table-3.

**D. FTAR Analysis:**

Fluorescence analysis of the successive extract was studied under day light as well as Ultra Violet (short and long wave length), (table-4). FTAR Analysis was also done of the powdered drug after reacting them with various chemical reagents (Table-5).

**E. Thin layer chromatographic (TLC) profile:**

TLC analysis of successive extracts was carried out using different solvent systems and visualizing agents and  $R_f$  values were calculated to standardize the drug for its identity and purity (Fig. 2 & 3; Table-6).

**RESULTS AND DISCUSSION**

The present study is an attempt to ascertain the pharmacopoeial standards for the standardization of *Malva sylvestris* Khubazi the quality, identity, purity and strength of the powder has been undertaken as a tool to bring out several features like ash standards, solubility in alcohol and water, successive extractive values, and qualitative screening of physicochemical, total alkaloids, total flavonoids, phenol, nitrogen, fatty matter, Sterol/Terpenes, Protein and Carbohydrates. Characterization of an herbal drug is essential for the quality control to check the presence of adulterants as a single drug remedy or its polyherbal Unani formulation.

**OBSERVATIONS:****Table-1: Organoleptic Characters of *M. sylvestris* (Khubazi)**

S.No.	Organoleptic parameters	Observations
1.	Colour	Brownish Yellow
2.	Smell	Characteristic
3.	Taste	Slightly bitter

**Table 2: Physicochemical study of Powder of *M. sylvestris* (Khubazi)**

S.No.	Parameters	Percentage (w/w)*
<b>1</b>	<b>Ash value</b>	
	Total ash	14.15
	Acid insoluble ash	2.52
	Water soluble ash	5.54
<b>2</b>	<b>Soluble Part</b>	
	Ethanol soluble	0.97
	Aqueous soluble	6.46
<b>3</b>	<b>Successive Extractive Values</b>	
	Pet. Ether	3.56
	Di-ethyl ether	0.75
	Chloroform	2.08
	Acetone	0.85
	Alcohol	3.80
	Aqueous	11.72
<b>4</b>	<b>Moisture content</b>	2.56
<b>5</b>	<b>Loss on Drying</b>	3.84
<b>6</b>	<b>pH values</b>	
	1% water solution	6.94
	10% water solution	7.2
<b>7</b>	<b>Crude fibre</b>	25.49
<b>8</b>	<b>Bulk Density</b>	0.56

\*Note: Values are average of three experiments.

**Table 3: Preliminary Screening of major Phyto-chemicals of *M. sylvestris* (Khubazi)**

S.No	Chemical Constituent	Tests/Reagent	Inference
1	Alkaloids	Dragendorff's reagent	+
		Wagner's reagent	+
		Mayer's reagent	+
2	Carbohydrate	Molisch's Test	+
		Fehling's Test	+
		Benedict Test	+
3	Flavonoids	Mg ribbon and Dil.Hcl	+
4	Glycosides	NaOH Test	+
5	Tannins/Phenols	Ferric Chloride Test	+
		Liebermann's Test	+
		Lead Acetate Test	+
6	Proteins	Xanthoproteic Test	+
		Biuret Test	+
7	Starch	Iodine Test	+
8	Saponins	Frothing with NaHCO <sub>3</sub>	+
9	Steroid/Terpenes	Salkowski Reaction	+
10	Amino Acids	Ninhydrin Solution	-
11	Resin	Acetic Anhydride test	+

Indications: '-' Absence and '+' presence of constituent.

**Table 4 FTAR Analysis of *M. sylvestris* (Khubazi)**

	Extract	Day Light	UV Long	UV Short
1.	<b>Pet. Ether</b>	Yellowish	Purple	Green
2.	<b>Di-ethyl ether</b>	Greenish	Dark Brown	Green
3.	<b>Chloroform</b>	Light Green	Black	Green
4.	<b>Acetone</b>	Brown	Bluish	Greenish
5.	<b>Alcohol</b>	Brownish	Black	Dark Green
6.	<b>Aqueous</b>	Brown	Black	Dark Green

**Table-5 Fluorescence Analysis of *M. sylvestris* with different chemical reagents**

S. No.	Powdered drug + Chemical Reagent	Day light	UV short	UV long
1.	Powdered drug + Conc. HNO <sub>3</sub>	Pale	Light Green	Dark Green
2.	Powdered drug + Conc. Hcl	Brown	Green	Black
3.	Powdered drug + Conc. H <sub>2</sub> SO <sub>4</sub>	Brown	Green	Dark Green
4.	Powdered drug + 2% Iodine solution	Brown	Green	Black
5.	Powdered drug + Glacial Acetic acid + HNO <sub>3</sub>	Brown	Light Green	Dark Green
6.	Powdered drug + Glacial acetic acid	Brown	Green	Black
7.	Powdered drug + NaOH (10%)	Brown	Dark Green	Black
8.	Powdered drug +Dil. HNO <sub>3</sub>	Brown	Light Green	Black
9.	Powdered drug + Dil. H <sub>2</sub> SO <sub>4</sub>	Brown	Light Green	Dark Green
10.	Powdered drug +Dil. Hcl	Brown	Green	Dark Green
11.	Powdered drug + Dragendorff's	Golden	Green	Black
12.	Powdered drug + Wagner's Reagent	Golden	Green	Black
13.	Powdered drug + Benedict's reagent	Greenish	Light Green	Black
14.	Powdered drug + Fehling reagent	Bluish	Brown	Black
15.	Powdered drug + KOH (10%) Methanolic	Brown	Green	Black
16.	Powdered drug + CuSO <sub>4</sub> (5%)	White	Light Green	Black
17.	Powdered drug + Ninhydrin (2%) in Acetone	Brown	Green	Black
18.	Powdered drug + Picric Acid	Yellow	Green	Black
19.	Powdered drug + Lead Acetate (5%)	Brown	Light Green	Black

**Table-6 Thin Layer Chromatography of *M. sylvestris* (Khubazi)**

Treatment	Mobile phase:	No of spots	Rf value and colour of spots
<b>Petroleum Ether Extract</b>			
Day Light	Petroleum ether : Di-ethyl ether (4:1)	1	0.63 (Brown)
UV Short		1	0.63 (Bluish)
Iodine Vapour		1	0.63 (Yellowish)
<b>Aqueous Extract</b>			
Day Light	Butanol: Acetic acid: Water (5:1:4)	1	0.83 (Greenish)
UV Short		3	0.083 (Bluish), 0.60(Purple), 0.83 (Purple)
UV Long		3	0.083 (white), 0.60 (white), 0.83 (white), 0.85(white)
Iodine Vapour		2	0.83 (Pale Yellow), 0.60 (Pale Yellow)
<b>Alcoholic Extract</b>			
Day Light	Chloroform:Methanol (9:1)	1	0.83 (Pale yellow)
UV Short		2	0.57 (Purple) , 0.83(Purple)
UV Long		2	0.57 (white), 0.83(white)
Iodine Vapour		1	0.83(Pale Yellow)



**Fig. 1 Market Sample of Khubazi (*Malva sylvestris* Linn.)**



**Day Light**

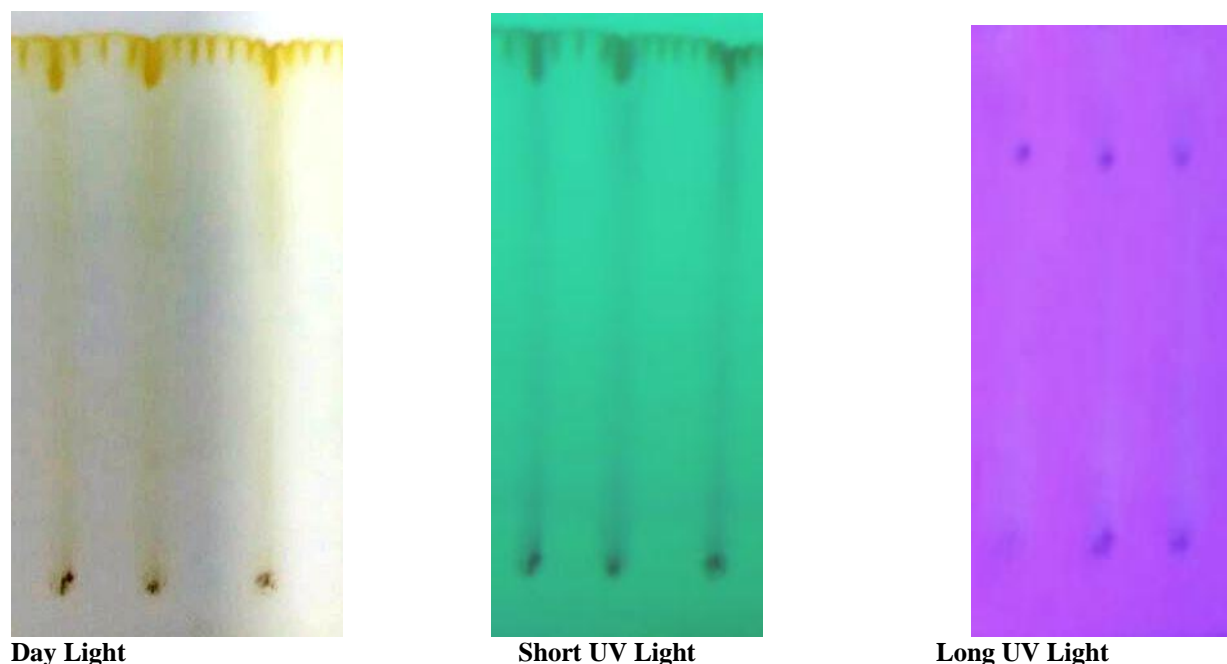


**Short UV Light**



**Long UV Light**

**Fig. 2 TLC of Aqueous extract of Khubazi**



**Fig. 3 TLC of Petroleum ether extract of Khubazi**

## CONCLUSION

Physico-chemical standardization is of prime importance in quality control of Unani drugs. As the efficacy of many drugs mainly depends upon its physical and chemical properties therefore, the determination of physico-chemical characters for the authenticity of a drug is necessary before studying any medicinal property. Present study has screened out various parameters required for the standardization of Khubazi that may be used as a future reference for its identification. However their may be variation in the quantitative assessment of phyto-constituents according in samples obtained from different regions due to various atmospheric factors as well as storage and drying conditions.

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