The Pharma Innovation Journal 2019; 8(4): 65-72

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(4): 65-72
© 2019 TPI
www.thepharmajournal.com
Received: 09-02-2019
Accepted: 13-03-2019

Sada Akhtar
Department of Ilmul Advia, Ajmal Khan Tibbiya College, AMU, Aligarh, Uttar Pradesh, India

Dr. Abdur Rauf
Department of Ilmul Advia, Ajmal Khan Tibbiya College, AMU, Aligarh, Uttar Pradesh, India

Dr. Sumbul Rehman
Department of Ilmul Advia, Ajmal Khan Tibbiya College, AMU, Aligarh, Uttar Pradesh, India

Mohammad Zakir Siddiqui
Department of Biotechnology, Faculty of Natural Sciences, Jamia Millia Islamia, New Delhi, India

Correspondence
Sada Akhtar
Department of Ilmul Advia, Ajmal Khan Tibbiya College, AMU, Aligarh, Uttar Pradesh, India

Physicochemical standardization and safety evaluation of Mocharas (Gum of *Bombax malabaricum*): A unani drug

Sada Akhtar, Dr. Abdur Rauf, Dr. Sumbul Rehman and Mohammad Zakir Siddiqui

Abstract
Global herbal resources have a great potential as natural drugs and are of great importance, they are very often procured and processed without any scientific evaluation and launched into the market without any mandatory safety and physicochemical study. Therefore, an attempt has been made on a well-known drug of Unani medicine Mocharas (gum of *Bombax malabaricum*) by evaluation of Physicochemical parameters (Extractive values, Solubility, Moisture content, Ash Values, pH of 1% & 10% solution, Loss of weight on drying, Bulk Density (Poured & Tapped densities), Crude fibre content, TLC & Fluorescence analysis) and toxicological parameters like heavy metals, aflatoxins, microbial load determination and pesticide residue.

Keywords: standardization, mocharas, *Bombax malabaricum*, safety study

Introduction
Mocharas is the gum of the tree *Bombax malabaricum* DC (SYN. *B. ceiba* Linn; *Salmalia malabarica* DC Schott & Endl.) that belongs to the family Bombaceae. It is widely distributed throughout the Tropical Eastern Himalaya and the hotter forest regions of India to Burma and Ceylon (Hooker, 1982; Nadkarni, 1954; Khory and Katrak, 1985) [18, 22, 20]. It also occurs in Assam and Tripura and in the Andamans where taller trees are found (Anonymous, 2004) [6]. The gum is sometimes known as *Supari ka phul* (*Areca catechu*) in allusion to the fact that children masticate the blunt thorns of *Bombax malabaricum*; the flower buds are known as *Semargulla* (Dymock *et al.* 1890) [14]. In classical Unani texts Mocharas is known by *Samagh-ul-Mocharas, Samagh-e-Seembhal, Burag, Bargubanka* and *Supari ka Phul* (Anonymous, 2006; Ghani, 2010; Azam Khan, 2014) [8, 17, 9]. It is large deciduous tree, remarkable height and covered with hard conical prickles. Mocharas is not a normal juice but the product of diseased action, which consists in the proliferation of the cells of the bark; the gum only exudes from the bark, which has been so injured by decay or by insects; incisions in the healthy bark produce nothing. On incisions into the diseased bark, a number of small cavities are seen, which contain a jelly like substance, some granular matter and starch (Khory and Katrak, 1985) [20]. The gum of *Bombax malabaricum* is amorphous, opaque, solid, brick-red to black in colour. The pieces are irregular and of different sizes varying from 2.5 to 5.0 cm long. The surface is quite smooth and shiny and sometimes a small portion of bark is attached. The odour is slightly pungent and taste is acrid, fracture is hard, difficult to break but brittle (Anonymous, 1997). The whole tree of Mocharas like its root, leaves, flowers, stem bark and gum is used for its medicinal properties but in Unani system of medicine its gum is mainly used for its several properties like astringent, demulcent, styptic, aphrodisiac, spermatogenic, tonic, repellent, avaricious semen viscositive and used in diarrhoea, pulmonary tuberculosis, leucorrhoea (Nadkarni, 1954; Dey, 1973; Khory and Katrak, 1985) [22, 13, 20]. It is used for menorrhagia and dysentry (Anonymous, 1997). The bark gum contains catechutannic acid. The gum (2gm) mixed with cow's milk (30ml) is reported to cure bleeding piles (Anonymous, 2000) [5].

Material and Methods
Mocharas (Gum of *Bombax malabaricum* DC) was procured from the local market of Aligarh. The identity was confirmed with the help of literatures available and Pharmacognosy Section, Department of Ilmul Advia, A. K. Tibbiya College, Aligarh Muslim University, Aligarh. The sample was further authenticated by National Institute of Science Communication and

Information Resources (NISCAIR), New Delhi (NISCAIR /RHMD/ Consult/ 2017/ 3089-38-4). The specimen of the test drug was submitted to Mawalid-e-Salasa Museum of the Department for future reference with the voucher No of SC-0215/17.

The powder of gum was subjected to physicochemical and phytochemical studies to determine various constants. Powder of gum was also studied to evaluate the presence of microbial load, pesticides residue, aflatoxins estimation and heavy metal analysis at Delhi Test House, Azadpur, Delhi-110033 (India) [QR-0302, Report No.24251809031M-4011,Sample Date:03/09/18, Date of Report:10/09/18].

Fig 1: Market Sample of Mocharas (*Bombax malabaricum*)

Evaluation of organoleptic characteristics

It includes the evaluation of herbal drugs by size, shape, colour, odour, taste and particular characteristics like touch, texture etc (Bijauliya, *et al.,* 2017) [11] (Table 1).

Physicochemical studies

The Physicochemical study included the determination of successive extractive values of the test drug in different solvents, alcohol and water soluble contents, moisture content, ash values, loss of weight on drying, pH values, crude fibre content and bulk density (Table 2).

Successive extractive values

The successive extractive values of the test drug in different organic solvents viz. petroleum ether, diethyl ether, chloroform, alcohol and distilled water were determined using a Soxhlet's apparatus. The heat was applied for six hours for each solvent on a water bath/ heating mantle. The extracts were filtered and after evaporation of the solvents; the extractive values were determined with reference to the weight of air dried drug. The procedures was repeated three times and the mean value for each extract was calculated (Anonymous, 1968) [3].

Water and alcohol soluble contents

5 gm of the air dried powdered drug was taken with 100 ml of distilled water, in a glass stoppered conical flask for 24 hours. The mixture was carefully shaken frequently for 6 hours and then allowed standing for 18 hours. It was filtered and the whole filtrate was evaporated to dryness on a water bath at 105ºC to constant weight, cooled in desiccator for 30 minutes and weighed. The percentage of water soluble matter was calculated with reference to the amount of air dried drug. The percentage of alcohol soluble matter was determined as above by using alcohol in place of water (Anonymous, 1968) [3].

Loss of weight on drying

10 gm of drug was taken, spread uniformly and thin layered in a shallow petridish. It was heated at a regulated temperature of 105 ºC, cooled in a desiccator and weighed. The process was repeated many times till two consecutive weights were found constant. The percentage of loss in weight was calculated with respect to initial weight (Jenkins *et. al.,* 2008) [19].

Moisture Content

The toluene distillation method (Dean and Stark Method) was used for the determination of moisture content. 10 gm of drug was taken in the flask and 75ml of distilled toluene was added to it. The level of toluene should be above the drug level in the flask. The drug was submerged in toluene then it was distilled for sufficient time. The volume of water collected in receiver tube (graduated in ml) was noted and the percentage of moisture was calculated with reference to the weight of the air dried drug taken for the process. This process was repeated for three times and the mean value was calculated (Jenkins *et. al.,* 2008) [19] (Table 8).

Ash Values

Total Ash

2 gm of drug was incinerated in a silica crucible of constant weight at a temperature not exceeding 800ºC in a muffle furnace until free from carbon, cooled and weighed, the percentage of ash was calculated by subtracting the weight of crucible from the weight of crucible + ash. The percentage of total ash was calculated with reference to the weight of drug taken (Anonymous, 1968) [3].

Water Soluble Ash

The obtained ash was boiled with 25ml of distilled water for 10 min. The insoluble matter was collected in an ash less filter paper; (Whatman No. 42), washed with hot water and ignited in crucible, at a temperature not more than 800 ºC, the weight of insoluble ash was subtracted from the weight of total ash, giving the weight of water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug taken (Anonymous, 1968) [3].

Acid Insoluble Ash

The total ash was boiled with 25 ml of 10% hydrochloric acid for 10 min. The insoluble matter was collected on ash less filter paper (Whatman No. 42), washed with hot water and ignited in crucible at a temperature not exceeding 800ºC till constant weight. The percentage of acid-insoluble ash was calculated with reference to the weight of drug taken (Anonymous, 1968) [3].

pH Value

Determination of pH was carried out by a digital pH meter (model no. HI96107, HANNA Instruments). The instrument was standardized by using buffer solution of 4.0, 7.0, and 9.20 to ascertain the accuracy of the instrument prior to the experiment. The pH value of 1% and 10% aqueous solution of powder drug was measured (Anonymous, 1968) [3].

Crude Fibre Content

10gm of the powdered drug material was refluxed with 150ml of petroleum ether (60-80º C). After that 200ml of boiling

sulphuric acid (1.25%) was added. (Sulphuric acid was prepared by diluting 51ml of N H_2SO_4 to 200ml at 25ºC). The mixture of acid and drug was cooled and then heated to boiling, the flame being adjusted for slow steady boiling for 30minutes. Acid insoluble residue was collected on a filter paper to remove acid, the residue was then put back into the flask and refluxed with 200ml of boiling 1.25% Sodium hydroxide solution (70ml 1 N NaOH diluted to exactly 200ml), the mixture was again boiled for 30mins, filtered and washed with hot water to remove all the alkali, finally the residue was dried at 100º C until of constant weight, which gives the value of crude fibre (Jenkins *et. al.,* 2008) [19].

Bulk Density

Poured Bulk Density

It was determined by pouring sample of drug (50 gm drug was taken) into a graduated cylinder and the volume occupied by the drug was measured (Table 11).

Tapped Bulk Density

It was determined by measuring the volume occupied by the sample of known mass (50gm) into a graduated cylinder after subjecting to prescribed amplitude and frequency of tapping over a prescribed period of time (15 minutes) using digital tapped Densitometer.

Bulk Density is calculated by the formulae:-

Bulk Density = Mass/Volume in gm/ml

Qualitative phytochemical analysis

The qualitative analysis of different chemical constituents, present in test drugs was carried out according to the scheme proposed by Bhattacharjee and Das (1969) [10].

The powder of the test drugs was extracted with petroleum ether (bp.60-80 ºC). The petroleum ether extract (I) was tested for free phenols, alkaloids and sterols/terpenes. A part of this extract was saponified and portion (II) was tested for fatty acids, whereas, unsaponified portion (III) was tested again for phenols, and sterols/terpenes for confirmation. The defatted marc was divided into two portions. One portion was extracted with hot water and the other with ethanol (70%). The aqueous (IV) and ethanolic (V) extracts were tested for alkaloids, flavonoids, saponins, sugars and tannins. Aqueous extract was extracted with ether and ether soluble portion (VI) was tested again for alkaloids, sterols/terpenes, whereas, water-soluble portion (VII) was tested for glycosides. The water-soluble portion was again hydrolyzed with 5% hydrochloric acid and extracted with chloroform. The aglycone portion (VIII) was tested for insoluble hydrochloride of alkaloid. Chloroform soluble portion (IX) was tested for alkaloids and sterols/terpenes, whereas; water-soluble fraction (X) was tested for alkaloids. One part of this water-soluble portion was basified with any alkali (ammonia) and extracted with immiscible solvent (ether). The solvent soluble part (XI) was again tested for alkaloids (Table 3).

Test for Alkaloids

A drop of Dragendroff's reagent in the extract was added. The brown precipitate showed the presence of alkaloids (Afaq *et al*., 1994) [1].

Hager's test: Few drops of Hager's reagent were added in 1 ml of alcoholic test solution. The presence of yellow colour precipitate indicated the presence of Alkaloids (Afaq *et al*., 1994) [1].

Wagner's test: Few drops of Wagner's reagent were added in 1 ml of alcoholic test solution dissolved with 2 ml of dil. HCl. The presence of yellow brown colour precipitates indicated the presence of Alkaloids (Afaq *et al*., 1994) [1].

Test for Carbohydrate / Sugars

Fehling's Test

In the aqueous extract, a mixture of equal parts of Fehling's solution A and B previously mixed was added and heated. A brick red precipitate of cuprous oxide indicated the presence of reducing sugars.

Molisch's test

In an aqueous solution, α-napthol was added. Afterwards, concentrated sulphuric acid was gently poured. A brown colour ring at the junction of the two solutions indicated the presence of the sugar (Afaq *et al*., 1994) [1].

Test for flavonoids

A piece of Magnesium ribbon was added to the ethanolic extract of the drug followed by drop wise addition of concentrated Hcl. Colour ranging from orange pink to red is a confirmatory test for flavonoids (Fransworth, 1966) [15].

Test for glycosides

The test solution was filtered and sugar was removed by fermentation with baker's yeast. The acid was removed by precipitation with magnesium oxide or barium hydroxide. The remaining ethanolic extract containing the glycosides was subsequently detected by the following methods:

a. The hydrolysis of the solution was done with concentrated sulphuric acid and after the hydrolysis, sugar was determined with the help of Fehling's solutions.

b. The Molisch's test was done for sugar using α-napthol and concentrated sulphuric acid (Afaq *et al.,* 1994) [1].

Test for Tannin

Ferric chloride solution was added in the aqueous extract of the drug. A bluish-black colour, which disappeared on addition of dilute sulphuric acid followed by a yellowish brown precipitate, showed the presence of tannin (Afaq *et al*., 1994) [1].

Test for Proteins

Biuret's test

In 1 ml of hot aq. extract of the drug 5-8 drops of 10 W/V solution hydroxide solution was added followed by 1 or 2 drops of 3 % W/V copper sulphate solution. A red or violet colour was obtained (Anonymous, 1987) [4].

Millon's reaction

To the test solution, Millon's reagent was mixed and white coloured precipitate showed the presence of proteins (Afaq *et al*., 1994) [1].

Test for Starch

0.015 gm of Iodine and 0.015 gm of Potassium Iodide was added in 5 ml of distilled water, 2 ml of iodine solution formed was added to 2 ml of aqueous test solution, the presence of blue colour indicated the presence of starch (Ali, 2010) [2].

Test for Phenol

i. Ferric chloride solution was added in 2 ml of ethanolic or

aqueous test solution. Blue or green colour indicated the presence of phenols (Finar, 1973) [16].

ii. 5-8 drops of 1% aqueous solution of Lead acetate was added to aqueous or ethanolic test solution. The presence of yellow coloured precipitate indicated the presence of phenols

iii. Libermann's Test: 2ml of ethanolic or aqueous test solution was dissolved with 0.5 ml of 70 % H_2SO_4 followed by the addition of few drops of aqueous sodium nitrite solution (0.5%). Red colour on dilution indicated the presence of phenols (Brewster and Mc Even, 1971) [12].

Test for Sterol/Terpenes

Salkowski reaction: In the test solution of chloroform 2 ml sulphuric acid (concentrated) was mixed from the side of the test tube. The colour of the ring at the junction of the two layers was observed. A red colour ring indicated the presence of the sterols/terpenes (Afaq *et al*., 1994) [1].

Test for Amino Acids

The ethanolic extract was mixed with ninhydrin solution (0.1% in acetone). After heating gently on water bath for few minutes it gave a blue to red-violet colour that indicated the presence of amino acids (Brewster and Mc Even, 1971) [12].

Test for Resin

The test solution was gently heated and acetic anhydride was added in it. After cooling, one drop of sulphuric acid was mixed. A purplish red colour that rapidly changed to violet indicated the presence of the resins (Afaq *et al.*, 1994) [1].

Fluorescence Analysis

Fluorescence Analysis of powdered drugs

Fluorescence analysis of the powdered drugs was done for identification. The powdered drugs were treated with different chemicals and observed in daylight and under ultra violet light. The changes in colours were noted (Nagulan and Kumar, 2016) [23] (Table 4).

Fluorescence Analysis of the successive extracts of drug sample

Successive extracts of all the drug samples viz. Petroleum ether, diethyl ether, chloroform, ethyl acetate, acetone, ethanol and aqueous extracts were observed in day light and UV light (Nagulan and Kumar, 2016) [23] (Table 5).

Thin Layer Chromatography (TLC)

Thin layer chromatography of different extracts of the drugs was carried out on T.L.C precoated aluminium plates (silica gel 60 of F_{254}, layer thickness 0.25 mm) by taking various mobile phases. After the development of the plates, they were sprayed with different reagents and examined under day, UV lights (UV short and UV long) and Iodine vapours to detect the spots representing various constituents. The R*f* values of the spots were calculated using following formulae (Anonymous, 1968, Afaq *et al*., 1994) [3. 1] (Table 6).

R*f* value = Distance travelled by the spot / Distance travelled by the solvent

Safety studies

Microbiological determination tests

Total viable aerobic count (TVC)

For detection of the antibacterial activity of the test drug, the total viable aerobic count (TVC) of the test drug was carried out, determined, as specified in the test procedure, using following methods:

Pre-treatment of the test drug

Depending on the nature of the compound sample used, it was dissolved using a suitable method and any antimicrobial property present in the sample was eliminated by dilution or neutralization. Buffered Sodium Chloride-Peptone Solution, pH 7.0 (MM1275-500G Himedia Labs, Mumbai, India) was used for dilute the test sample.

Plate Count for bacteria: 1 ml of the pretreated test sample was added to about 15 ml of the liquefied casein-soybean digest agar in a petridish of 90 mm diameter at a temperature not exceeding 45 ^{0}C. Alternatively the test sample was spreaded on the surface of the solidified medium. Two dishes were prepared with the same dilution, they were inverted and incubated at 30-35^{0}C for 48-72 hrs, unless a more reliable count was obtained in a short period of time. The number of colonies so formed was counted and the results were calculated using the plates with the largest number of colonies, up to a maximum of 300.

Plate Count for fungi: 1 ml of the pretreated test sample was added to about 15 ml of the liquefied Sabouraud glucose agar with antibiotics in a petridish of 90 mm diameter at a temperature not exceeding 45^{0}C. Alternatively the test sample was spreaded on the surface of the solidified medium. Two dishes were prepared with the same dilution; they were inverted and incubated at 20 - 25^{0}C for 5 days, unless a more reliable count was obtained in a short period of time. The number of colonies so formed was counted and the results were calculated using the plates with not more than 100 colonies.

Heavy Metals Determination

Heavy metals including Lead, Mercury, Arsenic and Cadmium were determined in the test sample using Atomic Absorption Spectroscopy (AAS).

Aflatoxin Estimation

The test for the determination of aflatoxins B_1, B_2, G_1 and G_2 was carried out using LC-MS/MS. 2 gm of test drug was blended at high speed with 20 ml of 60% acetonitrile/water for two minutes. The blended sample was centrifuged for ten minutes using 1600 rpm (av.), supernatant was retained and diluted with 2 ml of filtrate with 48 ml of phosphate buffered saline (PBS, pH 7.4) to give a solvent concentration of 2.5% or less; methanol/water was prepared by taking 2 ml of sample and diluted with 14 ml of PBS (pH 7.4) to give a solvent concentration of 10% or less. The sample diluent was passed through the immunoaffinity column at a flow rate of 5 ml/ min. The column was then washed by passing 20 ml of distilled water through the column at the flow rate of approximately 5 ml/ min and dried by rapidly passing air through the column. 1.5 ml of distilled water was added to the sample elute. 500 µl of sample was injected onto the LCMS-MS (LC- Perkin, MS Applied Bio System, Model No.2000, Mobile Phase). A- Water 100%, B-ACN 100%, Column oven temperature = 30, Column ZORBAX Rx c18, narrow base 2.1×150 mm - 5 micron, Flow = 0.750 ml). The aflatoxin concentration was quantified by comparing sample peak heights or areas to the total aflatoxin standard (R-Biopharm) (Lohar, 2007) [21].

Pesticidal Residue Estimation

The test for the assessment of specific pesticide residues like Organochloride compounds, Organophosphorus compounds and Pyrethroids compound was conducted using GC-MS/MS (Ramkrishanan *et al.*, 2015) [24].

Observation and Results

The Organoleptic evaluation carried out has been given below in table 1:

Table 1: Organoleptic characters

S. No.	Organoleptic characters	Observations
1.	Appearance	Irregular pieces
2.	Colour	Reddish brown
3.	Odour	Pungent
4.	Texture	Brittle & Rough
5.	Taste	Astringent & slightly bitter

Table 2: Physicochemical parameters

S. No.	Parameters	Results	
1.	Successive Extractive values	Petroleum ether	0.123±0.014
		Di ethyl ether	0.416±0.286
		Chloroform	0.491±0.066
		Alcohol	6.84±0.745
		Aqueous	58.636±0.401
2.	Solubility	Alcohol soluble content :	7.726±0.414
		Aqueous soluble content :	11.89±0.170
3.	Loss of weight on drying at 105ºC		6.70±0.563
4.	Moisture content		7.90±0.19
5.	Ash value	Total Ash:	2.26±0.14
		Water soluble:	0.83±0.16
		Acid Insoluble Ash:	0.5±0.15
6.	pH values	1 % water solution	7.2 ± 0.033
		10 % water solution	6.2 ± 0.033
7.	Crude Fibre content	2.51±0.335	
8.	Bulk Density (gm/ml)	Poured bulk density :	0.819±0.006
		Tapped bulk density :	1.078±0.011

Table 3: Phytochemical analysis of Mocharas (Gum of *Bombax malabaricum*)

S. No.	Chemical constituents	Tests/reagent	Inference
1.	Alkaloid	Dragendroff´s reagent	-ve
	Hager´s test	-ve	
	Mayer´s reagent	-ve	
2.	Carbohydrate	Molisch´s Test	-ve
	Fehling´s test	-ve	
3.	Glycoside	Baker's Yeast Test	+ve
4.	Flavonoids	Mg Ribbon Test	+ve
5.	Tannin	Ferric chloride test	+ve
6.	Protein	Xanthoproteinic test	-ve
	Biurette´s test	-ve	
7.	Steroid	Salkowski reaction	+ve
	Liebermann-Burchard's Test	+ve	
8.	Amino acid	Ninhydrin solution	-ve
9.	Resins	Acetic Anhydride Test	-ve
10.	Phenol	Lead acetate Test	+ve
11.	Saponins	Frothing with $NaHCO_3$	+ve
12.	Starch	Iodine Test	-ve

Table 4: Fluorescence analysis of Mocharas (Gum of *Bombax malabaricum*)

S. No.	Powdered drug + Reagents	Day light	UV short	UV long
1.	P. drug + Conc. HNO_3	Orange	Green	Black
2.	P. drug + Conc. HCl	Black	Black	Black
3.	P. drug + Conc. H_2SO_4	Black	Black	Black
4.	P. drug + Leishmans Stain	Orange	Green	Black
5.	P. drug + Formic Acid	Orange	Green	Black
6.	P. drug + Glacial acetic acid	Light brick red	Light Green	Light Brown
7.	P. drug + NaOH *(10%)*	Blackish Red	Black	Black
8.	P. drug +Dil. HNO_3	Light Orange	Light Green	Dark Brown
9.	P. drug + Dil. H_2SO_4	Light Orange	Light Green	Black
10.	P. drug +Dil. HCl	Peach	Light Green	Dark Brown
11.	P.drug + Dragendorff's reagent	Orange	Blackish Green	Black
12.	P. drug + Mayer's reagent	Yellowish Orange	Light Green	Black
13.	P. drug +Benedict's reagent	Brown	Brown	Black

14.	P. drug + Fehling reagent	Light Brown	Light Green	Black
15.	P.drug+KOH *(10%)* Methanolic	Brown	Green	Black
16.	P. drug + $CuSO_4$ *(5%)*	Greenish	Light Green	Brown
17.	P. drug + Ninhydrin *(2%)* in Acetone	Light Brown	Green	Black
18.	P. drug + Distilled Water	Peach	Light Green	Black
19.	P. drug +Lead Acetate *(5%)*	Brick red	Greenish	Black

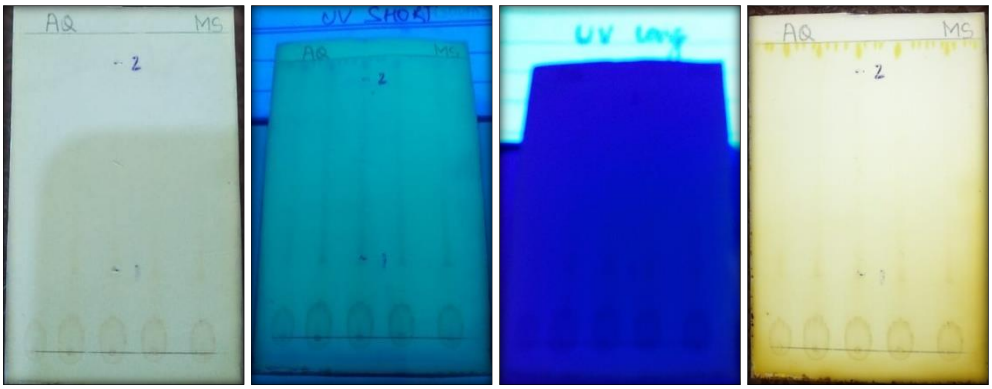
P. drug = Powdered drug

Table 5: Fluorescence analysis of successive extracts of Mocharas *(Bombax malabaricum*)

S.No.	Extracts	Day Light	UV Short	UV Long
1.	Petroleum Ether	Muddy	Muddy	Muddy
2.	Diethyl Ether	Muddy	Muddy	Muddy
3.	Chloroform	-	-	-
4.	Alcohol	Brick Red	Black	Black
5.	Aqueous	Reddish Brown	Black	Black

Table 6: TLC profile of Mocharas (Gum of *Bombax malabaricum*)

Extract	Solvent system	Treatment	Number of spots	Rf Value & colour of spots
Aqueous	n-butanol:acetic acid:distilled water (4:1:5)	Day Light	2	0.25 (Light brown) 0.9 (Yellow)
		UV Short	2	0.25 (Brown) 0.9 (Brown)
		UV long	1	0.25 (Light Brown)
		Iodine Vapours	2	0.25 (Light brown) 0.9 (Yellow)



Day Light UV Short UV Long Iodine Vapours

Fig 2: TLC of aqueous extract of Mocharas (Gum of *Bombax malabaricum*)

Table 7: Microbial Load in Mocharas (Gum of *Bombax malabaricum*)

S. No.	Tests	Result	Permissible limit
1.	Total Bacterial Count	1690	Not more than $1x10^5$cfu/gm
2.	Total Yeast & Mould	130	Not more than 1 $x10^3$ cfu/gm

Table 8: Test for specific pathogens in Mocharas (Gum of *Bombax malabaricum*)

S. No.	Pathogens (/gm)	Result (gm)	Permissible limit as per API
1.	*Escherichia coli*	Absent	Absent
2.	*Salmonella*	Absent	Absent
3.	*Staphylococcus aureus*	Absent	Absent
4.	*Pseudomonas aeruginosa*	Absent	Absent

Table 9: Heavy Metals in Mocharas (Gum of *Bombax malabaricum*)

S. No.	Heavy Metals	Result *(mg/kg)*	LOQ*(mg/kg)*	Permissible limit *(mg/kg)*	Method
1.	Lead *(Pb)*	Not detected	2.50	Not more than 10	AAS
2.	Mercury *(Hg)*	Not detected	0.5	Not more than 1	AAS
3.	Arsenic *(As)*	Not detected	1.25	Not more than 3	AAS
4.	Cadmium *(Cd)*	Not detected	0.25	Not more than 0.3	AAS

LOQ = Limit of Quantification
BLQ = Below the limit of quantification
AAS = Atomic Absorption spectroscopy

Table 10: Aflatoxins in Mocharas (Gum of *Bombax malabaricum*)

S. No.	Aflatoxins (mg/kg)	Result	LOQ	Permissible limit (mg/kg)	Method
1.	Aflatoxin B_1	Not detected	0.001	Not more than 0.5	LCMSMS
2.	Aflatoxin G_1	Not detected	0.001	Not more than 0.5	LCMSMS
3.	Aflatoxin B_2	Not detected	0.001	Not more than 0.1	LCMSMS
4.	Aflatoxin G_2	Not detected	0.001	Not more than 0.1	LCMSMS

LOQ = Limit of Quantification
BLQ = Below the limit of quantification
LCMS/MS = Liquid Chromatography Mass Spectrometry

Table 11: Pesticidal residue in Mocharas (Gum of *Bombax malabaricum*)

S. No.	Pesticide Residue (mg/kg)	Result	LOQ	Permissible limit (mg/kg)	Method
1.	Alachlor	Not Detected	0.02	0.02	GCMSMS
2.	Aldrin &Dieldrin	Not Detected	0.04	0.05	GCMSMS
3.	Azinophos-methyl	Not Detected	0.04	1.0	GCMSMS
4.	Bromopropylate	Not Detected	0.08	3.0	GCMSMS
5.	Chlordane	Not Detected	0.04	0.05	GCMSMS
6.	Chlorfenvinphos	Not Detected	0.04	0.5	GCMSMS
7.	Chlorpyrifos	Not Detected	0.04	0.2	GCMSMS
8.	Chlorpyrifos-methyl	Not Detected	0.04	0.1	GCMSMS
9.	Cypermethrin	Not Detected	0.10	1.0	GCMSMS
10.	DDT *(*Sum of pp-DDT, pp-DDE and pp-TDE	Not Detected	0.04	1.0	GCMSMS
11.	Deltamethrin	Not Detected	0.10	0.5	GCMSMS
12.	Diazinon	Not Detected	0.04	0.5	GCMSMS
13.	Dichlorvos	Not Detected	0.04	1.0	GCMSMS
14.	Dithiocarbamates	Not Detected	0.01	2.0	UV-VIS Spectrophotometry
15.	Endosulfan (Sum of Isomer and Endosulfan sulphate*)*	Not Detected	0.04	3.0	GCMSMS
16.	Endrin	Not Detected	0.04	0.05	GCMSMS
17.	Ethion	Not Detected	0.04	2.0	GCMSMS
18.	Fenitrothion	Not Detected	0.04	0.05	GCMSMS
19.	Fenvalerate	Not Detected	0.10	1.5	GCMSMS
20.	Fonofos	Not Detected	0.04	0.05	GCMSMS
21.	Heptachlor (Sum of Heptacholar& Heptachlor epoxide)	Not Detected	0.04	0.05	GCMSMS
22.	Hexachlorobenzene	Not Detected	0.04	0.1	GCMSMS
23.	Hexachlorocyclohexane isomer (other than ý)	Not Detected	0.04	0.3	GCMSMS
24.	Lindane *(*ý-Hexachlorocylohexane	Not Detected	0.04	0.6	GCMSMS
25.	Malathion	Not Detected	0.04	1.0	GCMSMS
26.	Methidathion	Not Detected	0.04	0.2	GCMSMS
27.	Parathion	Not Detected	0.04	0.5	GCMSMS
28.	Parathion Methyl	Not Detected	0.04	0.2	GCMSMS
29.	Permethrin	Not Detected	0.04	1.0	GCMSMS
30.	Phosalone	Not Detected	0.04	0.1	LCMSMS
31.	Piperonylbutoxide	Not Detected	0.04	3.0	LCMSMS
32.	Primiphos Methyl	Not Detected	0.04	4.0	LCMSMS
33.	Pyrethrins	Not Detected	0.10	3.0	GCMSMS
34.	Quintozen (Sum of Quintozene, pentachloroaniline and methyl pentachlorophenyl sulphide)	Not Detected	0.10	1.0	LCMSMS

DDT = Dichloro diphenyl trichloroethane
DDE = Dichloro diphenyl dichloroethylene
GCMS/MS = Gas Chromatography Mass Spectrometry
LCMS/MS = Liquid Chromatography Mass Spectrometry

Discussion

Proper identification and standardisation is mandatory to ensure the therapeutic efficacy of herbal drugs used for health ailments. Standardisation is an essential tool to ensure identity, purity and quality of herbal drugs. Preceding parameters were used for the physicochemical study of Mocharas (gum of *Bombax malabaricum*). For establishing the standards of any drug the extractive values play an important role, as the adulterated or exhausted drug material will give different values rather than the extractive percentage of the genuine one. Percentage of Solubility is also considered as an index of purity, as alcohol can dissolve almost all substances including glycosides, resins, alkaloids etc. The ash value determination give the amount of inorganic content of drug which determines adulteration of drug. The moisture content of the drug is variable because mostly herbal drugs are hygroscopic and excessive moisture content provides suitable medium for the growth of different type of micro-organisms like bacteria and fungi which leads to deterioration of drug. The pH provides quantitative indication of the acidity and alkalinity of a solution. Qualitative phytochemical analysis was also carried out for the determination of the presence of alkaloids, flavonoids, glycosides, tannins, phenols, resins, sterols/terpenes, sugars, starch, amino acid, proteins and saponins. Thin layer chromatography is one of the important parameters used for judging the quality and

purity of the drug. The resolution of different kinds of chemical components are separated by using TLC and calculating the R_f values after detecting the spots in order to standardize the drug for its identity, purity and strength. Safety study revealed that heavy metals namely lead, mercury, cadmium and arsenic were found to be absent in the test drug, indicating the gum is safe to utilize as drug. The aflatoxins B_1, B_2, G_1 and G_2 which cause severe hepatotoxicity were absent in the drug sample revealing that they are free from toxins and are safe for internal use. Further, as they are free from aflatoxins so it could be said that the shelf life of the drug would increase. The various pesticide residues were found to be absent. The total microbial load was found to be many folds lower than their permissible limit.

Conclusion

Physicochemical constants and phytochemical constituents present in the drug varied not only from plant to plant but also among different plants of same species depending upon various factors like collection, drying, storage and different atmospheric conditions. Physicochemical and phytochemical study helped in the identification and purification of the test drug and proved that the test sample was genuine and of Mocharas. Safety study revealed that the drug sample is free from heavy metals, aflatoxins, pesticide residue and microbial load and is safe for treating diseases. Further, the study revealed a set of diagnostic parameters for the specific drug which will be useful in identification and control to adulterations of the raw drug.

References

1. Afaq SH, Tajuddin, Siddiqui MMH. Standardization of Herbal Drug. Department of Ilmul Advia, Faculty of Unani Medicine, Aligarh Muslim University Press, Aligarh. 1994; 66-70:143-146.
2. Ali M. Textbook of Pharmacognosy. Edn 2, CBS Publishers & Distributors Pvt. Ltd, Daryaganj, New Delhi, 2010, 71.
3. Anonymous. British Pharmacopoeia. General Medical Council, The Pharmaceutical Press 17, Bloomsbury Square London. 1968; 1208(1276-1277):1286-1288.
4. Anonymous. Physicochemical Standards of Unani Formulations. Central Council for Research in Unani Medicine, New Delhi. 1987; 2:291.
5. Anonymous. The Wealth of India- A Dictionary of Indian Raw Materials and Industrial Products., National Institute of Science Communication, Council of Scientific & Industrial Research, New Delhi. 2000; 1:145.
6. Anonymous. Reviews on Indian Medicinal Plants. Indian Council of Medicinal Research, New Delhi. 2004; 4:319-326.
7. Anonymous. Quality Standards of Indian Medicinal Plants. Indian Council of Medicinal Research, New Delhi. 2005; 2:184-192.
8. Anonymous. Qarabadeen-e-Sarkaari. Central Council for Research in Unani Medicine, New Delhi. 2006; 2:131.
9. Azam Khan. Muheet-e-Azam (Urdu Translation). Central Council for Research in Unani Medicine, New Delhi. 2014; 3:252-254.
10. Bhattacharjee AK, Das AK. Phytochemical screening of some Indian Plants. Journal of Crude Drug Research. 1969; 9:1408-1412.
11. Bijauliya RK, Alok S, Chanchal DK, Kumar M. A comprehensive review on Standardization of Herbal Drugs. International Journal of Pharmaceutical Sciences and Research. 2017; 8(9):3663-3667.
12. Brewster RC, Evan MC. Organic Chemistry. Prentice Hall of India, New Delhi, Edn 3, 1971, 604.
13. Dey KL, Bahadur R. The Indigenous Drugs of India, Published by Pama Primlane, The Chronica Botanica, New Delhi, 1973, 48-49.
14. Dymock W, Warden CJH, Hooper D. Pharmacographia Indica, The Institute Of Health and Tibbi Research, Hamdard National Foundation, Pakistan. 1890; 1:215-218.
15. Farnsworth NR. Biological and Phytochemical Screening of Plants. Jounal of Pharmaceutical Sciences. 1966; 55(3):225.
16. Finar IL. Organic Chemistry. Published by Dorling Kindersley Pvt. Ltd. 1973; 1(6):701.
17. Ghani HN. Khzainul Advia. Idara Kitab-ush-Shifa, Darya Ganj, New Delhi, 2010, 1268.
18. Hooker JD. Flora of British India. Bishen Singh Mahendra Pal Singh, 23 A, New Connaught Place, Dehradun. 1982; 1:349.
19. Jenkins GI, Kenevel AM, Digangi FE. Quantitative Pharmaceutical Chemistry. CBS Publishers & Distributors PVT. LTD., Daryaganj, New Delhi, Edn 6, 2008; 229-230:239-242.
20. Khory HK, Katrak NN. Materia Medica of India and Their Therapeutics. Neeraj Publishing House, Delhi, Edn 3, 1985, 103-104.
21. Lohar DR. Protocol for testing of Ayurvedic, Siddha and Unani medicine pharmacopoeial laboratory for Indian medicine, Ghaziabad, 2007, 77-89.
22. Nadkarni KM. Indian Materia Medica. Popular Book Depot, Bombay, Dhootapapeshwar Prakashan Ltd. Panvel, 1954; 1(3):207-209.
23. Nagulan S, Kumar SR. Phytochemical, Physicochemical, and Fluorescence Analysis of Leaf Extract of *Syzygium calophyllifolium* Walp. Asian Journal of Pharmaceutical and Clinical Research. 2016; 9(1):275-278.
24. Ramkrishanan G, Gayathri V, Sathia S, Parameswari RP, Saravana CB. Physicochemical and Phytochemical Standardization of Thraatchathi chooranam-A polyherbal formulation. Journal of Pharmaceutical Science & Research. 2015; 7(6):305-313.