Standardization and Safety study of an important Diuretic Unani Drug Abhal (*Juniperus communis*)

Sada Akhtar*, Dr. Abdur Rauf, Dr. Sumbul Rehman and Mohammad Zakir Siddiqui**
*Department of Ilmul Advia, Ajmal Khan Tibbiya College, AMU, Aligarh, 202001
**Department of Biotechnology, Faculty of Natural Sciences, Jamia Millia Islamia, New Delhi, 110025

Abstract

The study was aimed to evaluate physicochemical and safety parameters of the Unani drug Abhal (*Juniperus communis*) in order to ensure its identity, purity and safety of the drug. The plant is being potentially used as diuretic, antidiarrhoeal, anti-inflammatory, astringent and antiseptic and used in the treatment of various disorders. The physicochemical parameters helped in the identification. Phytochemical analysis showed the presence of secondary metabolites and the result of safety study revealed the presence of heavy metal, Lead (Pb) within the permissible limit as per WHO guidelines while aflatoxins, pesticides residue and microbial load were found absent in the test drug. So it could be said that the drug is free from toxicity and can be used safely.

Keywords: Standardization, Safety study, Abhal, *Juniperus communis*.

Introduction

Abhal is the dried ripe fruit of *Juniperus communis* Linn belongs to the family Cupressaceae. In Arabic it is called Jouz ul Abhal (Azam Khan, 2012), Habhhul aaraar, Abhal, Samratul A'ra'r (Ibn Sina, 2014; Usmani, 2008). In Persian it is called Tukhme Rahal (Azam Khan, 2012). In Hindi it is known as Haubera, Devdaar and Moheer (Ibn Sina, 2014; Usmani, 2008; Azam Khan, 2012). It is found in Himalayas from Kumaon Westwards at an altitude of 12500 – 14000 ft. to Temperate and Subarctic Europe, Asia, North Africa and North America. Irvine mentions that they are imported into Patna from Nepal, and used in the treatment of gonorrhoea (Kirtikar and Basu, 1996). It is dense, more or less procumbent shrub, rarely a small tree (Anonymous, 2001). The drug has a pleasant, somewhat terbinthinate odour, and a sweetish taste. They are employed for flavoring gin and food products; they are sometimes used as an article of food (Trease and Evans, 2009). Fruit sub-spherical, berry like, purplish black, occasionally showing a 'bloom', about 0.5-1.0 cm in diameter, apex shows tri radiate mark and depression indicating the suture of three fleshy bracts. At the base there are six small, pointed bracts arranged in whorls, but occasionally 3 or 4 whorls present. Three hard, triangular seeds are embedded in the fleshy mesocarp, each with a woody testa bearing large partly sunk resinous duct. The odour is terbinthine and taste bitter (Anonymous, (a) 2007). In Unani System of Medicine the drug is used for its diuretic, carminative, antiseptic and stimulant properties. It is used in gonorrhoea, leucorrhoea and subcutaneous diseases. Hippocrates used it in certain disorders of the womb, and Dioscorides mentioned its diuretic and digestive properties, and used in cough and pectoral affections (Dymock *et al., 1893;* Afaq *et al.*, 2006).

Material and Methods

Sample Preparation

Abhal (Fruit of *Juniperus communis*) 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/ 38-3). 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-0217/17.

Abhal was grounded to get course powder and then subjected to physicochemical and phytochemical studies to determine various constants. It 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.24241809031M-3011, Sample Date:03/09/18, Date of Report:10/09/18].

Fig. 1: Market sample of Abhal (*Juniperus communis*)

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) (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).

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).

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).

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) (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).

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).

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).

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).

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).

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

Phytochemical Analysis

Qualitative 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).
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).
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).
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).

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).

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).

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).

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).

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).

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).

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).

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).

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).

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).

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).

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).

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) (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) (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 phase. 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) (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).

Pesticidal Residue Estimation

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

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	Round
2.	Colour	Brown
3.	Odour	Aromatic
4.	Texture	Wrinkled
5.	Taste	Bitter

Table 2: Physicochemical parameters

S.No.	Parameters	Results
1.	Successive Extractive values	Petroleum ether 4.473±0.466 Diethyl ether 6.22±0.301 Chloroform 2.22±0.183 Alcohol 4.273±0.184 Aqueous 14.14±0.881
2.	Solubility	Alcohol soluble content : 12.643±0.328 Aqueous soluble content : 6.21±0.343
3.	Loss of weight on drying at 105°C	5.74±0.278
4.	Moisture content	6.406±0.103
5.	Ash value	Total Ash: 2.16±0.16 Water soluble: 0.5±0.33 Acid Insoluble Ash: 0.66±0.28
6.	pH values	1 % water solution 6.8±0.033 10 % water solution 5.7±0.033
7.	Crude Fibre content	36.81±1.406

8.	Bulk Density (gm/ml)	Poured bulk density : 0.347±0.005 Tapped bulk density : 0.480±0.005

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
3.		Fehling´s test	+ve
4.	Glycoside	Baker's Yeast Test	+ve
5.	Flavonoids	Mg Ribbon Test	-ve
6.	Tannin	Ferric chloride test	ve
	Protein	Xanthoproteinic test	-ve
7.		Biurette´s test	-ve
	Steroid	Salkowski reaction	+ve
8.		Liebermann-Burchard's Test	+ve
9.	Amino acid	Ninhydrin solution	-ve
10.	Resins	Acetic Anhydride Test	-ve
11.	Phenol	Lead acetate Test	+ve
	Starch	Iodine Test	-ve

Table 4: Fluorescence analysis of Abhal (*Juniperus communis*) in different chemicals & reagents

S. No.	Powdered drug + Reagents	Day light	UV short	UV long
1.	P. drug + Conc. HNO_3	Orange	Dark Green	Black
2.	P. drug + Conc. HCl	Blackish Brown	Black	Black
3.	P. drug + Conc. H_2SO_4	Black	Black	Black
4.	P. drug + Leishman's Stain	Turmeric Yellow	Green	Greenish Black
5.	P. drug + Glacial Acetic acid	Brown	Blackish Green	Dark Brown
6.	P. drug + Formic Acid	Brown	Green	Greenish Black
7.	P. drug + NaOH (10%)	Dark Brown	Greenish Black	Black
8.	P. drug +Dil. HNO_3	Brown	Blackish Green	Dark Brown
9.	P. drug + Dil. H_2SO_4	Brown	Blackish Green	Dark Brown
10.	P. drug +Dil. HCl	Brown	Green	Dark Brown
11.	P. drug + Dragendorff's reagent	Brick Orange	Dark Green	Black
12.	P. drug + Mayers Reagent	Brown	Dark Green	Dark Brown
13.	P. drug +Benedict's reagent	Blackish Brown	Black	Black
14.	P. drug + Fehling reagent	Brown	Blackish Green	Greenish Black
15.	P. drug + KOH (10%) Methanolic	Blackish	Black	Dark Brown

		Brown		
16.	P. drug + $CuSO_4$ (5%)	Brown	Dark Green	Blackish Green
17.	P. drug + Ninhydrin (2%) in Acetone	Black	Greenish Black	Dark Brown
18.	P. drug + Distilled Water	Brown	Green	Dark Brown
19.	P. drug +Lead Acetate (5%)	Brown	Green	Dark Brown

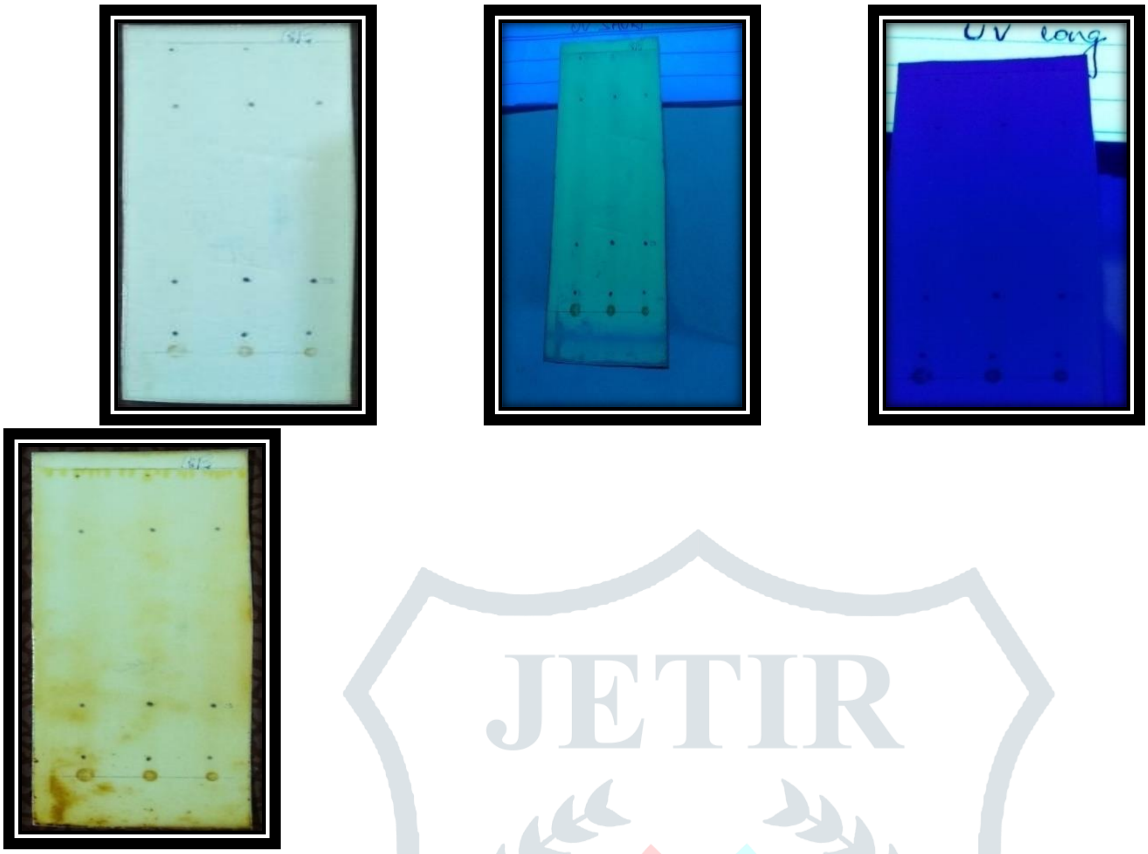
Table 5: Fluorescence analysis of Successive extracts of Abhal (*Juniperus communis*)

S.No.	**Extracts**	**Day Light**	**UV Short**	**UV Long**
1.	Petroleum Ether	Light Green	Green	Light Brown
2.	Diethyl Ether	Golden Green	Green	Light Green
3.	Chloroform	Light Green	Dark Green	Brown
4.	Alcohol	Brown	Dark Green	Black
5.	Aqueous	Reddish Brown	Black	Black

Table 6: TLC of Abhal (*Juniperus communis*)

Extract	**Solvent System**	**Treatment**	**No. of spots**	**R_f Value & colour of spots**
Alcohol	n-Butanol: Acetic acid: Water (5:1:4)	Day Light	1	0.22(Green)
		U V Short	3	0.05(Light brown) 0.22(Brown) 0.78(Black)
		U V Long	2	0.05(Light brown) 0.78(Orange)
		Iodine Vapours	4	0.05(Light Green) 0.22(Light Green) 0.78(Light Green) 0.98(Light Green)
Chloroform	Chloroform: Methanol (2:1)	Day Light	1	0.97(Green)
		UV Short	1	0.97(Brown)
		U V Long	1	0.97(Orange)
		Iodine Vapours	1	0.97(Brownish Green)
Aqueous Extract	n-Butanol: Acetic acid: Water (5:1:4)	Day Light	2	0.24 (Brown) 0.97 (Brown)
		U V Short	2	0.24 (Brown) 0.97 (Brown)
		U V Long	2	0.24 (Light Brown) 0.97 (Light Brown)
		Iodine Vapours	2	0.24 (Dark Brown) 0.97 (Dark Brown)

Fig. 2: TLC of Alcoholic extract of Abhal



Day Light
Iodine vapour

UV Short

UV Long

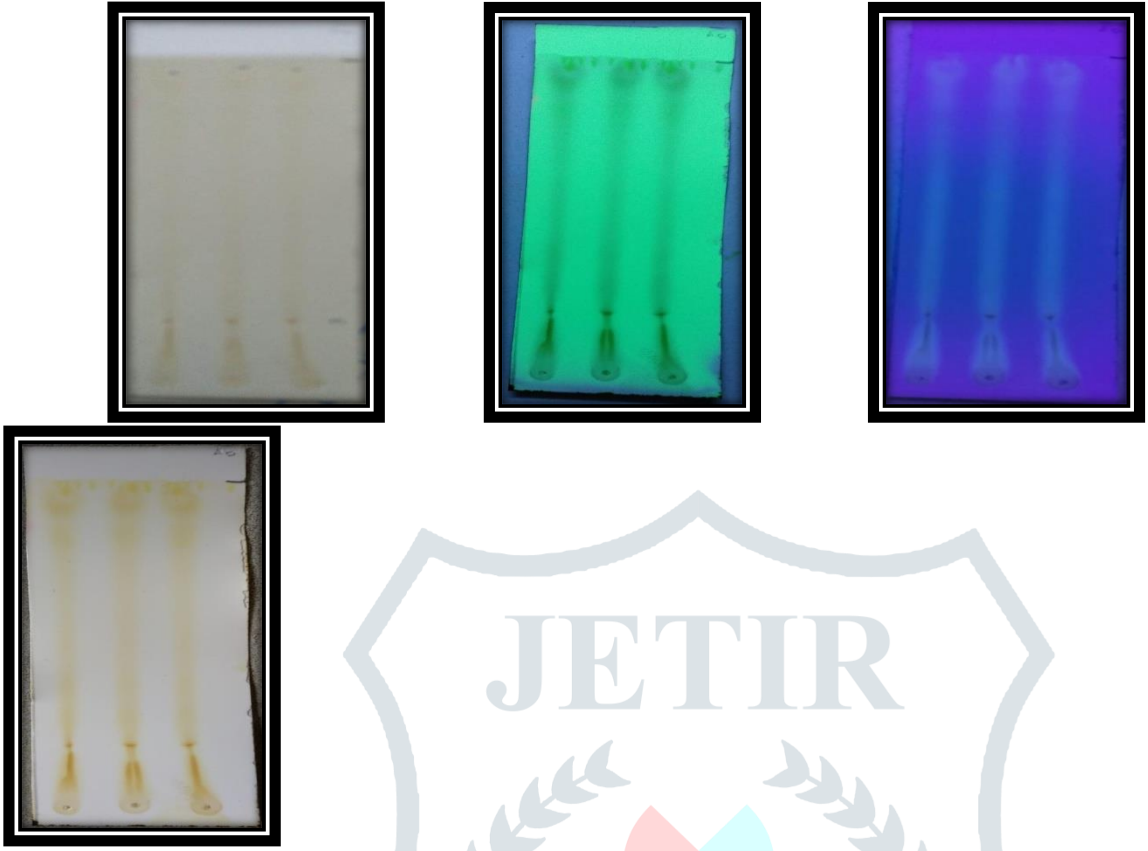
Fig. 3: TLC of Chloroform extract of Abhal

Day Light
Iodine vapour

UV Short

UV Long

Fig. 4: TLC of Aqueous extract of Abhal



Day Light UV Short UV Long Iodine vapour

Table 7: Microbial Load in Abhal (*Juniperus communis*)

S.No.	Tests	Result	Permissible limit
1.	Total Bacterial Count	1480	Not more than 1x10^{5}cfu/gm
2.	Total Yeast & Mould	80	Not more than 1 x10^3 cfu/gm

Table 8: Test for specific pathogens in Abhal (*Juniperus communis*)

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 Abhal (*Juniperus communis*)

S.No.	Heavy Metals	Result (mg/kg)	LOQ(mg/kg)	Permissible limit (mg/kg)	Method
1.	Lead (Pb)	7.6	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 Abhal (*Juniperus communis*)

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 Abhal (*Juniperus communis*)

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	Not Detected	0.04	3.0	GCMSMS

	sulphate)				
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

Physicochemical study 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 physicochemical characters of a drug is necessary to ensure its authenticity and genuine quality. Extractive value is the amount of the extract that a drug yields in a solvent is often an approximate measure of the amount of certain constituents that drug contains. Therefore, for establishing the standards of any drug these extractive values play an important role in the test drugs, as the adulterated or exhausted drug material will give different values rather than the extractive percentage of the genuine one. Percentage of solubility is considered as an index of purity. Percentage of loss of weight on drying at 105º C indicates the loss of volatile substances along with water is parameter of purity of drug. The presence of excess moisture is conducive to the promotion of mould and bacterial growth and subsequently to deterioration and spoilage of the drug. Ash value is an important parameter for the detection of impurities and adulteration of the drug. Ash is due to the presence of inorganic matter present in berries. Acid-insoluble ash indicates the presence of more siliceous matter in the drug. pH value of the drug is also an important parameter. The drug in opposite pH is unionized and absorbed rapidly from the stomach. Bulk density is also considered as an important physical characteristic of herbal drugs and helps in their identification. Crude fibre consists largely of cellulose and lignin (97%) plus some mineral matter. It represents only 60 to 80% of cellulose and 4 to 6% of lignin. It is useful as a measure of nutritive value of animal feeds and also in the analysis of various foods and food products to detect adulteration, quality and quantity. Qualitative phytochemical analysis of the drug (Abhal) was also carried out as the therapeutic properties of the crude drugs are mainly due to physiologically active chemical constituents present in the drugs, and the lower percentage of chemical constituents may cause lesser therapeutic values. Thin layer chromatography is one of the important parameters used for detecting the adulteration for judging the quality of the drugs. The resolution of different kinds of chemical components are separated by using TLC and calculating the Rf values after detecting the spots in order to standardize the drug for its identity, purity and strength. The exhausted or deteriorated drugs may lose the components and the number of spots appeared might be less. Fluorescence analysis further confirmed the identity, purity and quality of the drug sample. Microbial load determination is essential as the unscientific methods of collection, storage, transportation and congenital climatic conditions can render the herbs prone to microbial and fungal infestations. Heavy metals have a tendency to accumulate in the food chain and have low excretion rates through the kidneys which could result in damaging effects on humans even at low concentrations. Consumption of Aflatoxin (B_1, B_2, G_1, G_2) contaminated foods and medicines cause Aflatoxicosis. So the determination of Aflatoxins in herbs was done to ensure safety. Pesticides such as organochlorines (OCs), organophosphorus (OP) and their preparations are found in the crude drugs and cause toxicity.

Conclusion

Various physiochemical, TLC and safety parameters of the drug sample were studied. The organoleptic characterization of the Abhal (*Juniperus communis*) would help in its identification. Physicochemical study of the drug sample determined a comprehensive range of the physicochemical constants as per the standard procedures. Also the safety study revealed that the drug is free from toxins and is safe for oral administration. Studies exhibited a set of diagnostic characters, which will help to identify the drug in dried condition.

These parameters will be useful in authentification and identifying the adulterants and quality control of Abhal (*Juniperus communis*).

References

1. Afaq, S.H., Tajuddin and Siddiqui, M.M.H. Standardization of Herbal Drugs. Department of Ilmul Advia, Faculty of Unani Medicine, Aligarh Muslim University press, Aligarh, 1994, 66-70, 143-146.
2. Afaq, S.H. and Tajuddin. Pharmacognosy of Selected Unani Medicinal Plants. Department of Ilmul Advia, Faculty of Unani Medicine, Published by Aligarh Muslim University Press, Aligarh, 2006, 9-13.

3. Ali, M. Textbook of Pharmacognosy. Edn 2, CBS Publishers & Distributors PVT. LTD., Daryaganj, New Delhi, 2010, 71.
4. Anonymous. British Pharmacopoeia. General Medical Council, The Pharmaceutical Press 17, Bloomsbury Square London, 1968, 1208, 1276-1277, 1286-1288.
5. Anonymous Physicochemical Standards of Unani Formulations. Central Council for Research in Unani Medicine, New Delhi, 1987, Vol. 2, 291.
6. Anonymous. The Wealth of India- A Dictionary of Indian Raw Materials and Industrial Products. National Institute of Science Communication, Council of Scientific & Industrial Research, Dr. K S Krishnan Marg, New Delhi, 2001, Vol. 5, 306-308.
7. Anonymous. The Unani Pharmacopoeia of India. Central Council for Research in Unani Medicine, New Delhi, Part-I Vol. 4, 2007, 5-6.
8. Azam Khan. Muheet-e-Azam. Urdu Translation, Central Council for Research in Unani Medicine, New Delhi, Vol. 1, 2012, 242-244.
9. Bhattacharjee, A.K. and Das, A.K. Phytochemical screening of some Indian Plants. Journal of Crude Drug Research, Vol. 9, 1969, 1408-1412.
10. Bijauliya, R.K., Alok, S., Chanchal, D.K. and Kumar, M. A comprehensive review on Standardization of Herbal Drugs. International Journal of Pharmaceutical Sciences and Research, 2017, 8(9), 3663-3667.
11. Brewster, R.C. and Evan, M.C. Organic Chemistry. Edn 3, Prentice Hall of India, New Delhi, 1971, 604.
12. Dymock, W., Warden, C.J.H. and Hooper, D. Pharmacographia Indica. The Institute of Health and Tibbi Research, Hamdard National Foundation, Pakistan, Vol. 3, 1893, 371-373.
13. Evans, W.C. Trease and Evans Pharmacognosy. EDN 16, Saunders Elsevier Ltd., Edinburg London New York Philadelphia St Louis Sydney Toronto, 2009, 278.
14. Farnsworth, N.R. Biological and Phytochemical Screening of Plants. Jounal of Pharmaceutical Sciences, 1966, 55(3), 225.
15. Finar, I.L. Organic Chemistry. Published by Dorling Kindersley PVT.LTD, 1973, 1(6), 701.
16. Ibn Sina. Al-Qanun fi'l Tibb. Idara Kitab-ul-Shifa, Darya Ganj, New Delhi, Vol. 2, 2014, 73.
17. Jenkins, G.I., Kenevel, A.M. and Digangi, F.E. Quantitative Pharmaceutical Chemistry. Edn 6, CBS Publishers & Distributors PVT. LTD., Daryaganj, New Delhi, 2008, 229-230, 239-242.
18. Kirtikar, K.R. and Basu, B.D. Indian Medicinal Plants. Edn 2, International Book Distributors, 9/3 Rajpur Road, Dehradun, Vol. 3, 1996, 2380-2382.
19. Lohar, D.R. Protocol for testing of Ayurvedic, Siddha and Unani medicine pharmacopoeial laboratory for Indian medicine, Ghaziabad, 2007, 77-89.
20. Nagulan, S. and Kumar, S.R. Phytochemical, Physicochemical, and Fluorescence Analysis of Leaf Extract of *Syzygium calophyllifolium* Walp. Asian Journal of Pharmaceutical and Clinical Research, 2016, 9(1), 275-278.
21. Ramkrishanan, G., Gayathri, V., Sathia, S., Parameswari, R.P., and Saravana, C.B. Physicochemical and Phytochemical Standardization of Thraatchathi chooranam-A polyherbal formulation. Journal of Pharmaceutical Science & Research, 2015, 7(6), 305-313.
22. Usmani, M.I. Tanqeehul Mufradat. Ibn Sina Tibbiya College, Azamgarh, 2009, 19-20.