Pharmacognostic evaluation of *Cydonia oblonga* Mill. seeds obtained from North Kashmir

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Abstract:

Background: *Cydonia oblonga* Mill. (Quince) is a well-known plant of Kashmir with immense medical importance. However, meagre information is available on the standardization parameters of the plant (seed in particular) due to existing variability in quince germplasm, yield and fruit quality. Therefore, it is necessary to evaluate the pharmacognostical properties of *Cydonia oblonga* Mill. seeds (*Bihidāna*) obtained from Kashmir to utilize the same for precisely predictable therapeutic benefits. **Methods:** Macroscopical and microscopical characters, physicochemical and phytochemical properties were studied to establish the quality standards. **Results:** The macroscopic, microscopic, physicochemical and phytochemical parameters of *Bihidāna* and extracts revealed various diagnostic characteristics of the species, some of which did not correspond with the previous studies. **Conclusion:** This study provided complete pharmacognostic profile of *Bihidāna* and hence will be useful for correct identification and authentication of the species for future studies.

Keywords: *Bihidāna*, *Cydonia oblonga* Mill. seeds, mucilage, pharmacognosy, physicochemical

Introduction:

Quince (*Cydonia oblonga* Mill. Syn. *Cydonia vulgaris* Pers.), is one of the oldest pome fruit species. Various shreds of evidence suggest the presence of quince domestication in Mesopotamia between 5000-4000 BC which continued up to 500 BC in the ancient Persia and Greek empires. According to Abdollahi (2019), it was the Mesopotamian civilisation of Akkadian (2334-2154 BC) which domesticated the wild quinces for the first time.[1] In ancient

Greek mythology, quinces are believed to symbolize fertility and hence were offered in weddings.[2] The Greeks and Romans preserved quince fruits in honey which led to another name for it i.e., "*melimelum*" which means 'honey apple' or 'golden apple'. Many documents in ancient Greece have mentioned the fruit and plant, including the famous book 'Enquiry into plants' by *Theophrastus* (371–287 BC).[1,3]

The generic name *Cydonia* is derived from the Greek word "*Kydonia of Crete*" (an ancient city located in the North-Western Coast of Crete, Greece) where this plant was abundantly grown since ancient times and the word "*oblonga*" is derived from two Latin words '*ob'* and *'longus'*, "ob" means "obverse/inverse" and "longus" means "long".[2,4] Quince is grown in all the temperate zones of the world.[5] In the first century AD, *Dioscorides* mentioned the medicinal uses of quince fruit and other parts of the plant under the name '*Kudonia*'.[6] Likewise, the medicinal uses of the fruit, seeds, and leaves were appreciated by many eminent physicians of the medieval era including *Zakaria Razi*, *Ibn Abbas Majusi, Ibn Sina* & *Ibn Rushd*. The quince seed is obtained from mature *Cydonia oblonga* Mill. fruit known as *Bamchount* in local language and the seed is popular as *Bihidāna* in Unani medicine. There is great diversity of genotypes of quince. Some authors have considered a larger number of subspecies and varieties based on fruit shape such as pyriform or typical pear-shaped, maliformis or apple-shaped, marmorata or variegated & pyramidalis or pyramid-shaped.[4] This plant is a large perennial shrub bearing a pome fruit that is bright golden-yellow when mature.[7]

Quince seeds are used as medicine in various traditional systems of medicine including Unani Tib where it is described as a *Luabi dawa* (mucilaginous drug) possessing *Bārid wa Raṭab* (cold and moist)[8,9] temperament which makes it: *Mulayyin* (laxative), *Muhallil* (resolvent), *Habis* (static), *Mudammil-i-Qurūḥ* (healing), *Mulattif* (demulcent), and *Muqawwi* (tonic). On the basis of these pharmacological actions, it is therapeutically used for the treatment of *Khushunat-i-Halq* (sore throat)[10,11], *Saḥj-i-Am'a* (excoriation of intestines)[12], *Qula'* (stomatitis) , *Ṣafrawi Bukhār* (bilious fevers), burns & sunburns, *namla* (herpes), sore gums, sore teeth, hyperhidrosis, etc.[13,8] According to modern pharmacological studies, quince seed possesses the following activities viz., anti-diarrheal, anti-microbial, bronchodilator, anti-proliferative and wound healing, anti-cancer and anti-oxidant activity. [14]

The physical, as well as the chemical characteristics of quince fruit, are highly influenced by climatic factors and physical, chemical, biological properties of soil.[14] The

existing variability in quince germplasm has not been exploited much in the geographical region of Jammu & Kashmir. This is the reason why the extent of variability with respect to yield and fruit quality is not available as of yet.[15] Therefore, it is a must to evaluate the macroscopic, microscopic, physicochemical and phytochemical properties of *Bihidāna* obtained from a village in North Kashmir so that further evidence is gathered in the direction of utilising *Bihidāna* as medicine in future as, according to the WHO, the pharmacognostical account of a medicinal plant is the first step towards ascertaining the identity and the degree of purity of a plant material.[16]

Materials and Methods

Collection of Plant Material:

A survey tour to Zaingeer (located at an altitude of 1678m) in Baramulla, J&K was conducted on April 8, 2019, to collect the plant specimen during its flowering stages for future reference and the preparation of herbarium for the authentication of identification. The voucher specimen was deposited in the herbarium of Survey of Medicinal Plants Unit, RRIUM, Srinagar under voucher specimen no. 5860-5862 (Figure 1).

For the collection of seeds, another survey tour was conducted on October 17, 2019. At that time the plants were in the fruiting stage. The mature fruits were collected from the same plants from which specimens in the form of branches along with flowers were earlier collected during the first survey. Seeds were separated manually, shade-dried, and stored in a cool and dry place.

Equipment:

Microscope (*Magnus* made), Weighing Balance (*Denver* made), ultrasonic bath sonicator (*PCi ultrasonics* made), pH meter (pH testr 30, *Bio-matrix* Technologies), Hot Air Oven (*IMPACT* instruments company) centrifuge (*Remi* made), falcon tubes (15ml).

Reagents and chemicals:

Merck and *Rankem* made chemicals were mostly used.

Preparation of extracts:

Aqueous extract: Aqueous extraction of quince seeds was done according to the procedure followed by Sekachaei (2017) with slight modification. A total of 100 gm clean seed was soaked in 2.5 litre distilled water for 24 hours (water: seed ratio 25:1 v/w). The mixture was

then placed in an ultrasonic water bath (i.e., 0.61-12.39 minutes). In order to separate seeds from resultant gum, the slurry was centrifuged at the rate of 4,000 RPM for 10 minutes. The supernatant was dried in a hot air oven at 70 degrees until dried. The extract was powdered in mortar and pestle.[17]

Hydroalcoholic extract: Hydroalcoholic extraction was done by using the conventional cold maceration method. However, the ratio of water/alcohol: seed was taken according to Sekachaei AD, 2017. A total of 100gm crushed seed was soaked in 2.5 litre distilled water+ ethanol in the ratio 1:1 for 24 hours. This was filtered through a muslin cloth. The extract was dried in hot air oven at 70 degrees. The dried extract was later powdered in porcelain mortar and pestle.[17]

Parameters for Drug Standardization

Macroscopic evaluation

The macroscopic evaluation involved the detailed study of the visual appearance and sensory profile of the crude drug sample. It helps to differentiate between related species having similar appearance.[18]

Microscopic evaluation

The seeds of *Cydonia oblonga* Mill. were powdered and sliced and then boiled in chloral hydrate solution for few minutes. A little quantity of powder was taken onto a microscopic slide, evenly spread with the help of a brush, stained with phloroglucinol solution and a drop of concentrated HCl then a few drops of glycerine (10%) was added to it. The slides were covered with a cover slip and observed under a microscope for various microscopic characters.

Physico-chemical evaluation:

The physico-chemical parameters included loss on drying, extractive values, ash value, fluorescent analysis, etc which were carried out as per standard operating procedures.

Determination of Moisture Content (Loss on Drying):

About 10 g of the drug that had not been dried previously was placed in a dish which was tared evaporated. Then the drug was dried at 105°C for 5 hours, and weighed. The drying and weighing continued at one hour interval until the difference between two successive weighing corresponded to not more than 0.25 per cent. Constant weight is reached when two

consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccator, show not more than 0.01 g difference.[19, 20]

Determination of ash value

Total ash: Accurately weighed 5 gm of the powdered drug was incinerated in a silica crucible dish at a temperature not exceeding 450℃ until free from carbon, then cooled and weighed. The filtrate was collected on an ash less filter paper. The residue and filter paper were incinerated, evaporated to dryness, and again ignited at a temperature not exceeding 450℃. The percentage of ash to the air-dried drug was calculated.

Acid-Insoluble Ash: To the crucible containing total ash, 25 ml of dilute hydrochloric acid was added. The insoluble matter was collected on an ash less filter paper (Whatman 41) and washed with hot water until the filtrate became neutral. The filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot-plate and ignited to constant weight. The residue was allowed to cool in a suitable desiccator for 30 minutes and weighed without delay. Now the content of acid-insoluble ash was calculated with reference to the air-dried drug.

Water Soluble Ash: The ash was boiled for 5 minutes with 25 ml of water; insoluble matter in a crucible was collected on an ash less filter paper, then washed with hot water, and ignited for 15 minutes at a temperature not exceeding 450℃. The weight of the insoluble matter was subtracted from the weight of the total ash (the difference in weight represents the water-soluble ash). The percentage of water-soluble ash with reference to the air-dried drug was calculated.[19]

Determination of pH at 1% solution

An accurately weighed 1 gm powdered drug was dissolved in accurately measured 100ml of distilled water, filtered and the pH of the filtrate was checked with a standardised pH meter.[21]

Determination of extractive values[20]

Alcohol Soluble Extractive:

Cold extraction: A total of 5g drug was first dried in shade thereafter it was coarsely powdered and macerated in 100 ml of specified strength of alcohol in a closed flask for 24 hours. Later, it was filtered and evaporated to get constant weight. The percentage of alcohol-soluble extractive with reference to the air-dried drug was calculated.

Hot extraction: A total of 5g shade-dried drug was coarsely powdered then macerated with 100 ml of specified strength of alcohol in a closed flask for 24 hours. The next day the solution was heated on a water bath. Later, it was filtered and evaporated to dryness as of to attain constant weight. The percentage of alcohol-soluble extractive with reference to the air-dried drug was calculated.

Water soluble Extractive:

Cold extraction: A total of 5 g of shade-dried drug was coarsely powdered then macerated with 100 ml of distilled water for 24 hours. Frequent agitation was done for first 6 hours then it was allowed to stand. Later, it was filtered and evaporated to dryness as of to attain constant weight. The percentage of water-soluble extractive with reference to the air-dried drug was calculated.

Hot extraction: A total of 5 g of shade-dried drug was coarsely powdered then macerated with 100 ml of distilled water in for 24 hours. Frequent agitation was done for first 6 hours then it was allowed to stand. The next day the solution was heated on a water bath. Later, it was filtered and evaporated to dryness as of to attain constant weight. The percentage of water-soluble extractive with reference to the air-dried drug was calculated.

Fluorescence analysis

The fluorescence character of the seed powder (40 N mesh) was studied both in daylight and UV light (254 and 366nm) and after treatment with different reagents like sodium hydroxide, hydrochloric acid, sulphuric acid, picric acid, ethanol etc.[22]

Phyto-chemical evaluation:

Phyto-chemical screening of aqueous and hydro alcoholic extracts of the test drug was evaluated for different active constituents.[23, 24]

Test for carbohydrate

Molisch Test: A few drops of alcoholic α-naphthol solution were added to 2 mL of aqueous solution of the extract. Thereafter, a few drops of concentrated sulphuric acid were allowed to run down along the side of the test tube to form a layer without shaking. The junction was observed for a purple or violet colour ring as an indicator of presence of carbohydrates.

Fehling's Test for Reducing Sugar: Fehling's A solution and Fehling's B solution were mixed in equal volume (1ml each) in a test tube. The mixed solution was made to boil for a

minute. Then equal amount (2 ml) of extract solution was added to it. Appearance of brick red precipitate confirmed the presence of reducing sugar.

Selivanoff's test (for ketones): To the extract solution, crystals of resorcinol were added and an equal volume of concentrated hydrochloric acid was added too. Then the solution was heated on water bath. Appearance of rose colour confirmed the presence of carbohydrates (ketones).

Iodine test: About 3 ml of test solution and few drops of dilute iodine were mixed. Appearance of blue color which disappeared on heating and reappeared on cooling indicated the presence of starch.

Test for tannin

Lead acetate: Extracts were treated with a few drops of lead acetate solution. Formation of white precipitate indicated the presence of tannins.

Ferric Chloride Test: The aqueous solution of extract was dissolved in distilled water and filtered. Equal volume of 5% ferric chloride solution were added to the filtrate. A greenish black precipitate was formed which confirmed the presence of tannins.

Test for glycoside

Borntrager's test: Test material was boil with 1ml sulphuric acid for 5 minutes. The solution was filtered when hot and then allowed to cool. Then equal volume of chloroform was added and shaken. The organic layer (layer of chloroform) was separated and mixed with half the volume of dilute ammonia. A rose pink color at the ammoniac layer indicated the presence of anthraquinone glycoside.

Keller-Kiliani Test: A solution of glacial acetic acid (4ml) with a drop of 2% Ferric chloride mixture was mixed with the 10 ml aqueous plant extract and 1 ml concentrated sulphuric acid. A brown ring formed between the layers showed the presence of cardiac steroidal glycosides.

Test for cyanogenic glycoside

Grignard reagent or sodium picrate test: A filter paper strip was soaked in 10 % picric acid, then in 10% sodium carbonate and dried. Moistened powdered drug was placed in a conical flask. The flask was corked and the filter paper placed in the slit between the cork and flask neck. Presence of cyanogenic glycoside is indicated when the filter paper turns brick red.

Same procedure was done on the aqueous and hydroalcoholic extracts of the test drug separately.

Test for Saponin

Froth test: In a test tube 2 ml of test solution was placed in water. Test tube was shaken vigorously and observed for the formation of froth which become stable.

Test for phytosterols

Salkowski Test: Extract (5 ml) was mixed with chloroform (2 ml), and concentrated sulphuric acid (3 ml) was carefully added to form a layer. Reddish brown colour observed at the interface indicated presence of terpenoids.

Liebermann Burchard's Test: 2 ml of acetic acid and 2 ml of chloroform were added with whole aqueous plant crude extract. The mixture was then cooled, then concentrated H_2SO_4 was added. Green colour showed the entity of aglycone, steroidal part of glycosides.

Test for flavonoids

Shinoda Test: A 5ml of 95% ethanol, 3drops of HCl and 0.5 gm of zinc turnings were added to the extract. Pink color formation indicated the presence of flavonoids.

Sodium hydroxide: To the residue of Shinoda test, addition of increasing quantity of NaOH show yellow coloration which decolorises on addition of acid.[23]

Test for Alkaloid:

Dragendorff's Test: Small quantity of concentrated extract was treated with few drops of diluted hydrochloric acid and filtered With Dragendorff's reagent (solution of potassium bismuth iodide) formed orange brown precipitate.

Hager's Test: With Hager's reagent (saturated picric acid solution) formed yellow precipitate.

Test for proteins and amino acids

Biuret Test: Extract was treated with 1 ml 10% sodium hydroxide solution separately and heated. A drop of 0.7% copper sulphate solution to the above mixtures was added. If there was no purplish violet colour, it indicated the absence of proteins.

Ninhydrin test: Ninhydrin reagent (0.25% w/v) was added to the test drug extracts and the solution was boiled for few minutes. Formation of blue colour indicated the presence of amino acids.

Results and observations

Macroscopic evaluation:

The organoleptic characteristics of *Bihidāna* viz., shape, size, odour, colour, taste are illustrated in (Table 1 & Figure 1).

Microscopic evaluation:

The microscopic evaluation of powdered and transverse section (T.S) of seeds of *Cydonia oblonga* Mill. was carried out. The various microscopic features of both samples have been shown in photomicrographs (Figure 2).

Physicochemical evaluation:

Loss on drying:

The moisture content of the seed of *Cydonia oblonga* Mill. was estimated as 7.8%. (Table 2)

Ash value:

The total ash value, water soluble ash value, and acid insoluble ash of *Bihidāna* were calculated as 3.4%w/w, 0.4%w/w, and 0.2%w/w respectively. (Table 3)

pH determination:

The pH of 1% solution of uncrushed seed and powdered seed as measured using pH paper was found to be in-between 7 & 8 for uncrushed seed and in-between 6 & 7 for powdered seed, likewise, the values on pH meter were 7.3 for uncrushed seed and 6.7 for powdered seed. (Table 4 and Figure 3)

Extractive values:

In hot extraction, the extractive value of *Bihidāna* in water and alcohol was found to be 15.6% & 4.4%, respectively. In cold extraction, the extractive value of *Bihidāna* in both water and alcohol was found to be 9.2%. (Table 5).

Fluorescence analysis:

The fluorescence analysis under daylight, UV short and UV long of the powdered seeds of *Cydonia oblonga* Mill. has been given in (Table 6).

Aqueous extraction and Hydro-alcoholic extraction:

The percentage of extractive value obtained by following the method of extraction given by Sekachaei (2017) was found as 12.5% for aqueous and 10% for hydroalcoholic.[17] (Table 7)

Phytochemical evaluation:

The phytochemical analysis showed that both the extracts i.e., aqueous and hydroalcoholic tested positive for Molish test, Fehlings test, lead acetate test, NaOH test, Biurets test, and Ninhydrin test. Both the extracts tested negative in Iodine test, Selvinoffs test, ferric chloride test, Salkowski test, Liebermann Burchards test, Keller Killiani test, Borntragers test, Sodium Pictrate test, Dragendroff's test, and Hagers test. The test for saponins i.e., foam test also came negative for *Cydonia oblonga* Mill. seeds (Table 8)

Table 1- Macroscopic characters of *Cydonia oblonga* Mill. seeds

S. No.	Parameter	Character
1	Colour	Golden brown
2	Odour	Bitter almonds
3	Taste	Bland sweet
4	Size	6-8mm long, 3-4mm wide
5	Shape	Plano-convex

Table 2 - Loss on drying (LOD)

S No.	Wt. of drug (g)	Wt. of petri dish + drug before drying A (g)	Wt. of petri dish + drug after drying B (g)	A-B (g)	LOD %w/w	%of average mean (w/w)
1	5	51.45	51.05	0.40	8	**7.8**
2	5	47.06	46.68	0.38	7.6	
3	5	50.56	50.17	0.39	7.8	

Table 3- Ash values

Total ash	Wt. of drug (g)	Wt. of empty crucible (g)	Wt. of empty crucible + total ash (g)	Wt. of total ash (g)	Total ash value (%w/w)
	5	39.15	39.32	0.17	3.4
Water soluble ash	Wt. of drug (g)	Wt. of empty crucible (g)	Wt. of empty crucible + water insoluble ash with ashless filter paper (g)	Wt. of total water insoluble ash - wt. of total ash (g)	Total ash value (%w/w)
	5	27.52	27.67	0.02	0.4
Acid insoluble ash	Wt. of drug (g)	Wt. of empty crucible (g)	Wt. of empty crucible + acid insoluble ash (g) with ashless filter paper	Wt. of total acid insoluble ash (g)	Total ash value (%w/w)
	5	39.15	39.16	0.01	0.2

Table 4 -pH value

pH of 1 % solution of uncrushed seed	On pH paper	In-between 7 & 8
	On pH meter	7.3
pH of 1 % solution of powdered seed	On pH paper	In-between 6 & 7
	On pH meter	6.7

Table 5 - Extractive values

Extractive values	**Solvent**	**Value**
Cold extractive value	Water	9.2%
	Alcohol	9.2%
Hot extractive value	Water	15.6%
	Alcohol	4.4%

Table 6 - Fluorescence analysis

Drug treatment	Visible light	Short wave	Long wave
Powdered drug as such	Creamy brown	White to light green	Light purple
Powdered drug + water	Off white with brown particles	Light green	Light purple
Powdered drug + conc. Hcl	Straw colored	Greenish	Dull green
Powdered drug + dil. Hcl	Off white	Light green	Light purple
Powdered drug + (0.5N) 10% H_2SO_4	Milky white	Light green	Dull purple
Powdered drug + 10%NaOH	Light green	Light green	Grey
Powdered drug + picric acid	Yellow	Yellow green	Brown
Powdered drug + methanol	Hazy	Transparent (tinted green)	Transparent (tinted purple)
Powdered drug + Acetic acid	Off white	Off white	Off white
Powdered drug + Chloroform	Translucent white	Light green	Light grey
Powdered drug + pet. Ether	Translucent white	Green tinted	White purple tinted
Powdered drug + Benzene	Translucent	Tinted green	Grey
Powdered drug + Ferric chloride	Brownish orange	Light green	Dark brown
Powdered drug + Ammonia solution	Milky green	Milky green	Grey

Table 7- Aqueous and Hydroalcoholic extracts[17]

S No.	Extracts	Method of extraction	Nature of extract	%yield w/w
1	Aqueous	Cold maceration	Solid brittle	12.5%
2	Hydroalcoholic (1:1)	Cold maceration	Solid brittle	10%

Table 8- Phytochemical screening of aqueous and hydroalcoholic extracts

Test	Inference	Aqueous	Hydro alcoholic
CARBOHYDRATES			
Molish's test	Violet ring	+ve	+ve
Fehling's test	Brick red	+ve	+ve
Iodine test	Blue color	-ve	-ve
Selvinoff's test	Rose pink	-ve	-ve
TANNINS			
Lead acetate	White ppt	+ve	+ve
Ferric chloride	Black color	-ve	-ve
FLAVONOIDS			
Shinoda test	Pink color	-ve	-ve
NaOH	Colourless	+ve	+ve
PHYTOSTEROLS			
Salkowski test	Yellow brown ring at junction	-ve	-ve
Liebermann birchards test	Green color	-ve	-ve
PROTEINS			
Biuret test	Violet color	+ve	+ve
Ninhydrin test	Blue color	+ve	+ve
GLYCOSIDES			
Keller kiliani test	Brown ring at junction	-ve	-ve
Borntrager's test	Rose pink	-ve	-ve
Sodium pictrate test (Sodium pictrate test was positive in crude powdered drug)	Brick red	-ve	-ve
ALKALOIDS			
Dragendroffs test	Orange ppt.	-ve	-ve
Hagers test	Yellow ppt.	-ve	-ve
SAPONINS			
Foam test	Foaming	-ve	-ve

Figure 1- Photograph of herbarium specimen of *Cydonia oblonga* Mill.

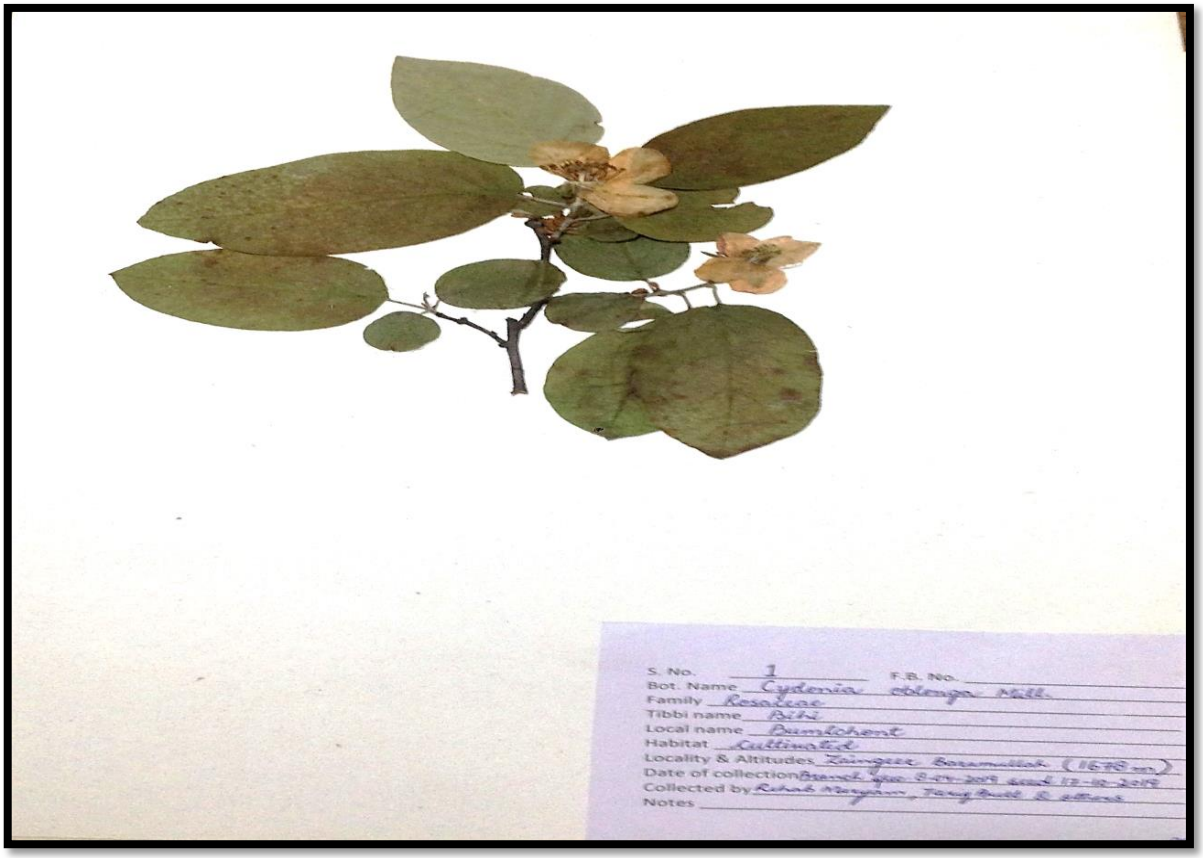


Figure 2- Showing a) The Flower; b) whole plant; c) fruits after harvesting and d) seeds of *Cydonia oblonga* Mill. after separating from the fruit.

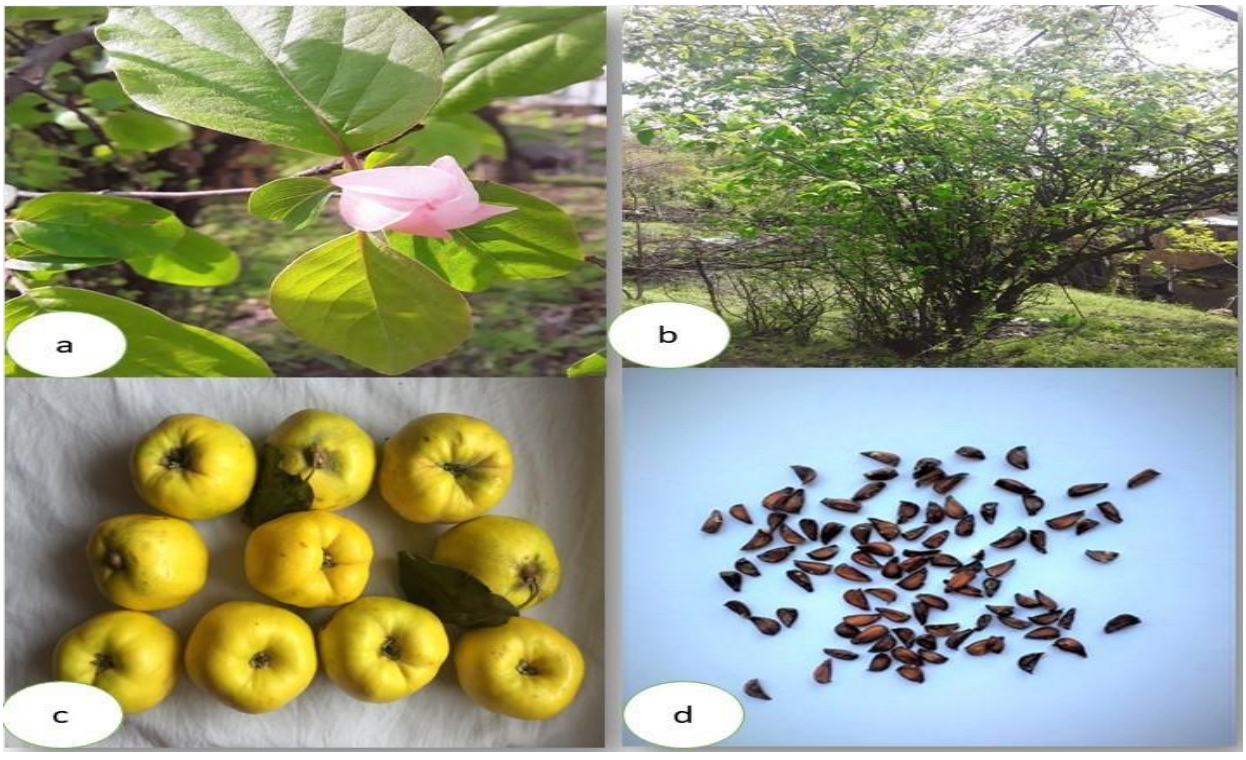
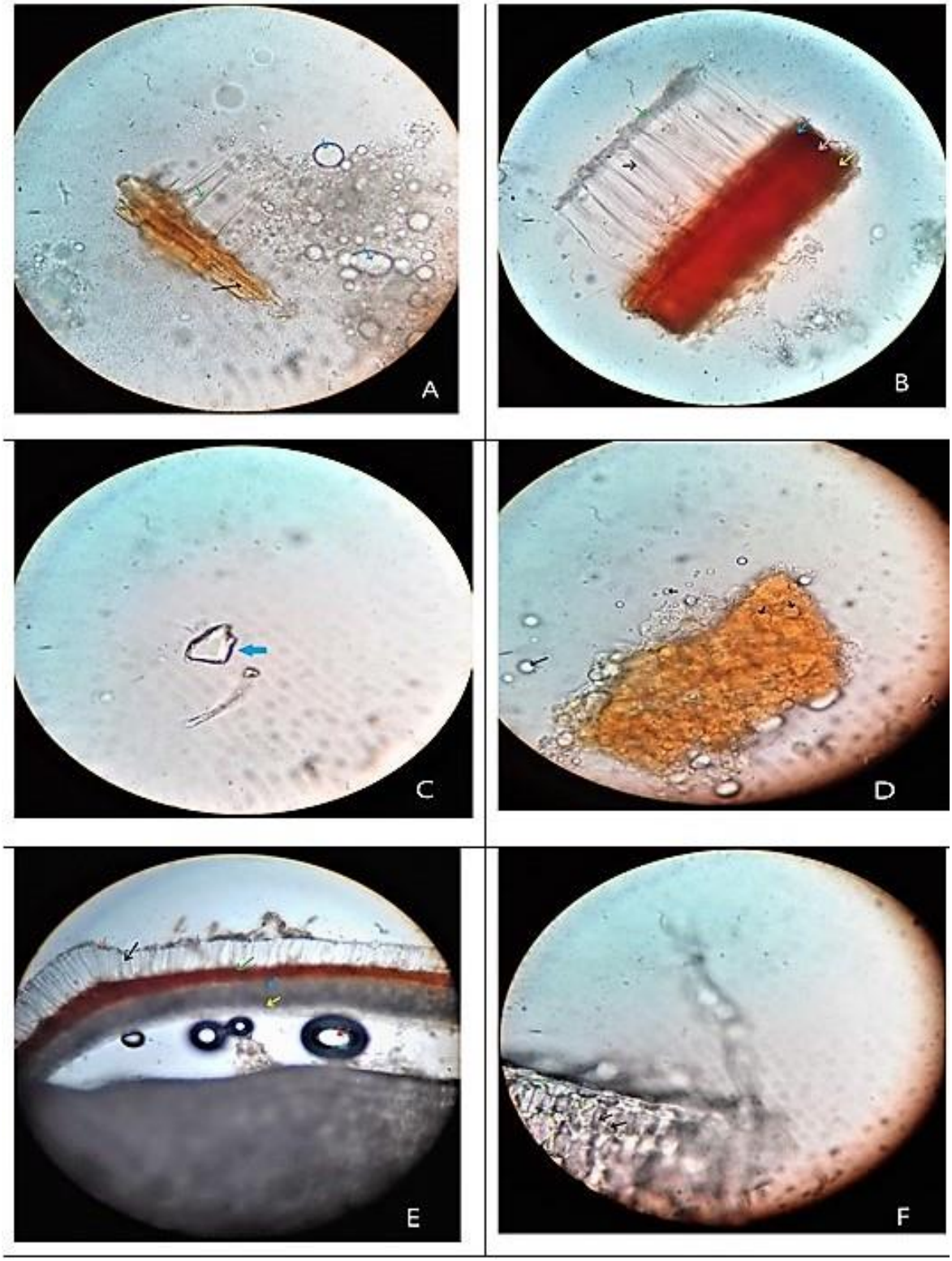
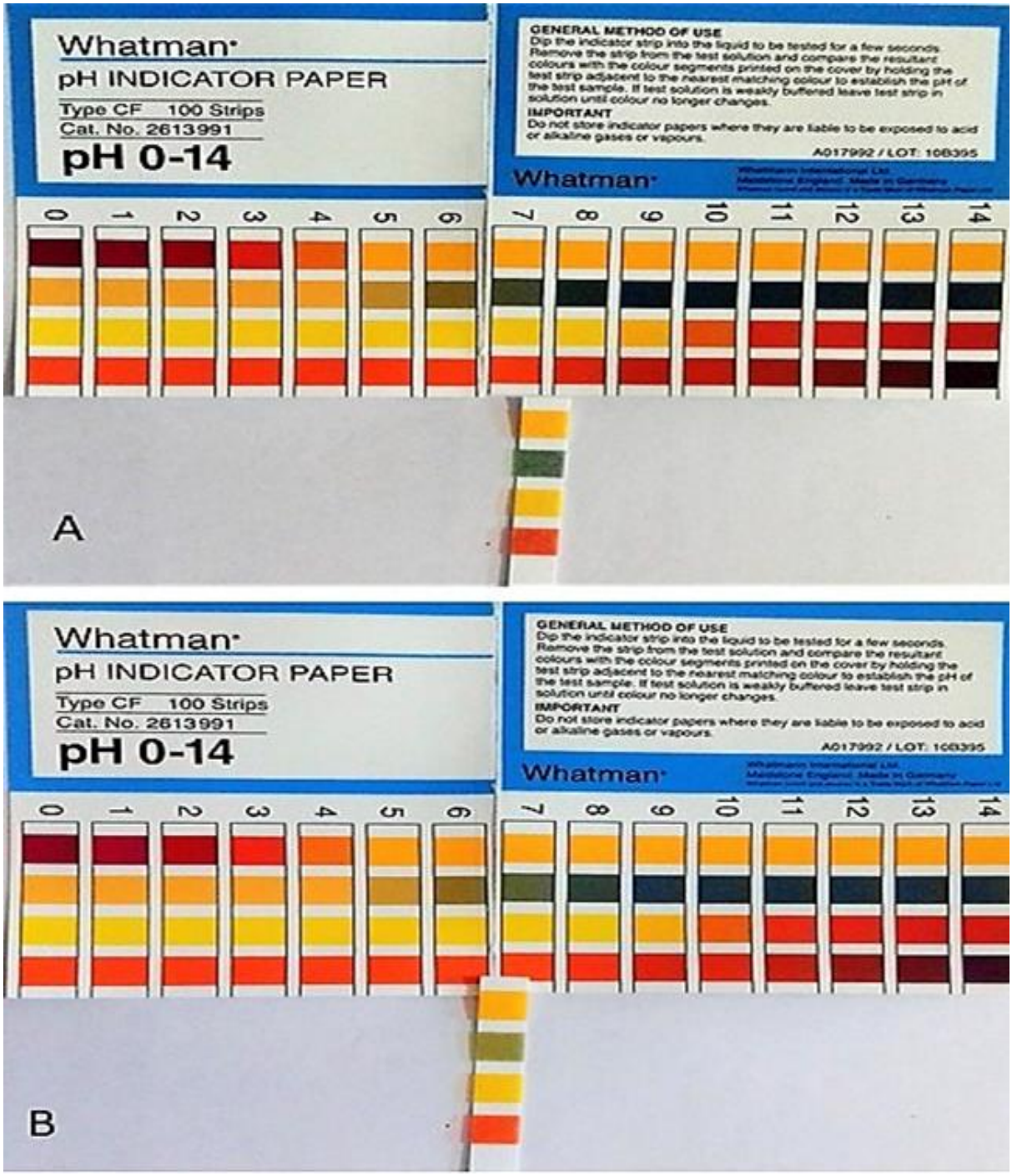


Figure 3- Microscopic images of *Cydonia Oblonga* Mill. seed- **Figure A –** black arrow → cuticle ; green arrow →fragments of columnar epithelial cells ; blue arrow →oil globules in endosperm. **Figure B-** transverse section of seed coat; green arrow→ cuticle ; black arrow→ columnar epithelial layer of cells ; blue arrow→ outer sclereids ;pink arrow→ middle sclereids ; yellow arrow→ inner layer. **Figure C –** blue arrow→ showing prismatic calcium



oxalate crystal**. Figure D**- Powdered microscopy; black arrows→ showing oil globules of endosperm. **Figure E-** Transverse section of seed coat (Testa) **;** brown arrow → cuticle; black arrow → columnar epidermal layer of cells; green arrow → outer sclereids; blue arrow → middle sclereids; yellow arrow→ inner sclereids; red arrow→ oil globule . **Figure F –** black arrows showing sclereids of Testa.

Figure 4- pH determination by using pH paper. A- pH of whole seed of *Cydonia oblonga* mill. B- pH of powdered seed of *Cydonia oblonga* Mill



Discussion

The pharmacognostic evaluation of Cydonia oblonga Mill. seeds obtained from North Kashmir was conducted to provide a comprehensive understanding of the botanical and chemical characteristics of this plant species. Pharmacognostic evaluation plays a crucial role in the recognition, authentication, and standardization of herbal medicines, ensuring their quality, safety, and efficacy. The study focused on various aspects of pharmacognostic evaluation, including macroscopic and microscopic analysis, physicochemical parameters, and preliminary phytochemical screening. Macroscopic analysis involved the examination of visible external features such as size, shape, color, and surface texture of the seeds. The seeds were observed to be oblong in shape, with a characteristic color and texture. The macroscopic characters of *Bihidāna* were perceived as golden-brown colour, bland-sweet taste, plano-convex shape, and smell of bitter almonds. These characteristic features were almost same as mentioned in previous records.[25] These macroscopic features serve as essential diagnostic criteria for the identification and differentiation of Cydonia oblonga Mill. seeds from other plant species. Microscopic analysis involved the examination of thin sections of the seeds under a microscope. This analysis provided insights into the cellular structure, arrangement of various tissues, and presence of characteristic cellular elements. It helps in confirming the identity of the seeds and detecting any adulteration or contamination. Quince has all the characteristics of pear except that the fruit has many seeds which are enveloped in a thick mucilage.[26] The microscopic study of powdered and transverse section (T.S) of *Bihidāna* was also carried out to evaluate the microscopic features such as cellular structure, presence of crystals, oil globules etc. In the result, all the three layers of testa were found in the transverse section of seed coat of *Bihidāna*. In powdered microscopy, the prismatic calcium oxalate crystals could be seen. Some features were found to be same as mentioned in the reference book of standardisation of Unani drugs published by CCRUM, New Delhi. [27] In physicochemical standardisation, various parameters such as moisture content, ash value, pH, extractive values, and florescence analysis were carried out. The moisture content is an important parameter to standardize a crude drug since the excessive moisture content may spoil the sample. In our study, the moisture content of *Bihidāna* was found to be slightly raised with reference to the book of standardisation of Unani drugs published by the CCRUM, New Delhi.[27] In our study, quince fruit was collected in the month of October from North Kashmir and the study was carried out on fresh seeds after shade-drying during winter season. So, the slight variation in the value of moisture content is predictable. The ash content

is the residue remaining after incineration of crude drug which includes 'physiological ash' derived from the plant tissues and 'non-physiological ash' derived from the environmental contaminations such as soil, sand etc. The water-soluble ash is used to assess the quantity of inorganic compounds present in the crude drug and the acid-insoluble ash indicates the presence of contaminants of earthy material especially silica.[21] In our study, the total ash, water soluble ash and acid insoluble ash values were found to be 3.4%, 0.4%, and 0.2% respectively. The total ash value did not exceed from the previous study carried out on this drug .[27] Also, our results may be more reliable since the drug collection was done from its natural source directly, and chances of earthy contamination are less.

The pH value of 1% solution of *Bihidāna* was determined using pH paper and pH meter. The book of standardisation of Unani drugs published by the CCRUM, New Delhi mentions that due to highly viscous nature of the drug, pH at 1% could not be found, [27] however, our study followed the procedure by Sunitha (2020) which made it possible to estimate the pH.[21] The extractive value of a crude drug in a particular solvent provides preliminary information about the quality of that particular drug as the extraction takes out the soluble constituents from the crude drug. Extractive value ascertains whether the plant material has been adulterated with exhausted plant material or not. The extractive value of our test drug was found to be 15.6% (hot extraction water), and 4.4% (hot extraction alcohol). Mukherjee (2002) writes, 'if the extractive value is found to be lower than the one specified in the official compendia, then poor quality materials may be detected at an early stage of herbal manufacturing.' According to the book on standardization of Unani drugs published by the CCRUM, the extractive values of Bihidāna are reported as 55.30% (water) and 5.38% (alcohol), which are significantly higher than the values observed in the plant sample obtained from North Kashmir. It is important to note that the extraction methods employed in the CCRUM publication are successive, unlike the non-successive extraction method used in our study. This distinction in extraction techniques may account for the variation in values.

In our phytochemical analysis, both the aqueous and hydroalcoholic extracts of Bihidāna were qualitatively assessed for various compounds, including carbohydrates, proteins, steroids, flavonoids, tannins, glycosides, and alkaloids. The results revealed the presence of carbohydrates, proteins, tannins, and flavonoids in both extracts. However, steroids, alkaloids, and saponins were absent in both extracts. Additionally, while cyanogenic glycosides were detected in the crude powdered form of the drug, they were not present in the extracts. Literature review has indicated the presence of diverse phytoconstituents in quince

seeds, which include organic acids such as citric, ascorbic, malic, quinic, shikimic, and fumaric acids. Flavones constitute a major portion of the phytochemical constituents, accounting for approximately 63% to 66%. Quince seed mucilage has been reported as a combination of cellulose and water-soluble polysaccharides. The presence of uronic acid has also been confirmed in quince seed mucilage. [28]

Conclusion

The present study presents a comprehensive pharmacognostic profile of Cydonia oblonga Mill. seeds acquired from the North Kashmir region. The findings of this study have significant implications in facilitating the identification and authentication of this particular species. Consequently, this research endeavor has made accessible vital information that is imperative for substantiating the medicinal value and efficacy of Cydonia oblonga Mill. seeds.

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