

Pharmacognostical standardization of *Cicer arietinum* (seeds), a traditional antidiabetic drugs

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Abstract

Purpose: In herbal pharmaceuticals there are many plant drugs which are being used since long time as antidiabetic agents. The ultimate objective of the pharmacognostic investigation is to identify of the genuine crude drug and determination of the extent of adulteration or substitution, if any. Establishment of pharmacognostic profile will assist in standardization for quality, purity and sample identification.

Method: Pharmacognostic standardizations of the antidiabetic herbal drugs such as seeds of *Cicer arietinum* was carried out to by using macroscopical and microscopical characters, physiochemical studies, phytochemical studies and chromatographic fingerprint profiles.

Results: The study of antidiabetic herbal drugs has revealed total characters of these valuable drugs in detail, covering morphological and microscopical features which differentiate the drugs from its adulterants and looks like materials. Physiochemical studies were conducted to check quality and purity of the herbal drugs. The study of phytochemical screening and chemical testing determined the possible existence of natural chemical compounds and their chemical type by color reactions. Chromatographic fingerprints showed complete chemical profiles of multiple compounds present in the drugs.

Conclusion: Data generated can be utilized to determine correct identity, purity of plant part, adulterants and for authentication and quality control of raw materials used for the development of herbal formulations.

Keywords: Antidiabetic plants, *Cicer arietinum* seeds, standardizations, Pharmacognostic evaluation, Physiochemical studies

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INTRODUCTION

Since antiquity, diabetes mellitus has been treated with medicinal plants. Numerous physiologically active compounds can be found in abundance in fruits and vegetables. This action is performed by variety of foodstuffs which are consumed daily from vegetables origin such as kerala, jamun, methi, onion, garlic and dhania.^[1,2] All these herbal drugs and many others have powerful antidiabetic action which has been scientifically proved now.^[3,4] In order to obtain good results from these plant drugs, authenticity of the sample must be necessary.

The present study pertains to detailed pharmacognostical standardization of antidiabetic herbal drug namely: *Cicer arietinum* (seeds) which is used to treat and prevent several pathological diseases in Ayurvedic and Unani medicines.⁵⁻⁹

MATERIALS AND METHODS

Punica granatum flowers and *Cicer arietinum* seeds were bought from Delhi's Khari-Baoli local market. Dr. M.P. Sharma, Taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi, identified the samples. The

voucher specimens were placed in the Faculty of Pharmacy's Pharmacognosy and Phytochemistry Research Laboratory at Jamia Hamdard in New Delhi.

Morphological studies

The drug's shape, size, color, odor, and fracture were all examined morphologically.¹⁰⁻¹³

Microscopic research

The technique outlined by O'Brien et al., 1964¹⁴ was used to conduct the microscopic investigations. The Nikon Labphot 2 microscopic device was used to take microphotographs at various magnifications. Crystals, starch grains, and lignified cells were studied using polarized light.^{15,16}

Physio-chemical studies

Different physio-chemical values such as alcohol soluble matter, water soluble matter, successive extractives, ash values and pH were determined.¹⁷⁻²⁴

Phytochemical studies

According to the described method, chemical tests were used in the preliminary phytochemical screening for a variety of

secondary metabolites, including sterols, tannins, glycosides, alkaloids, carbohydrates, phenolic compounds, and saponins.^[25-30] Behavior of each powdered drug was observed on treatment with different chemical reagents. Most of these tests were based on color indication of the powder drugs with specific substance. To see the color responses, a fluorescence analysis study of powdered drug material with various reagents under UV light was conducted.^[22-23]

Chromatographic fingerprint study

The drug's high performance thin layer (HPTLC) chromatographic fingerprint profile was developed using Stahl's TLC approach. To determine the kind and estimated quantity of chemicals present, HPTLC analysis was performed on alcoholic extracts made from dried and coarsely powdered *C. arietinum* seeds.^[31-32]

Using a CAMAG Linomat V applying apparatus, the extracts (5 µl each) were applied to separate TLC plates (precoated silica gel G F254 aluminum sheets, 10 cm x 10 cm) with an 8 mm bandwidth. Using a previously saturated twin trough chamber (CAMAG), the chromatograms of all three plant extracts were created up to an 80 mm distance at room temperature in various solvent systems. To find the ideal wavelength displaying the greatest number of compounds, the generated chromatograms were scanned at various wavelengths using the CAMAG TLC scanner III and a deuterium and tungston lamp in absorbance mode.^[33-34]

RESULTS AND DISCUSSION

Macroscopical characters

C. arietinum: *Shape:* round, slightly pressed at one end; *Surface:* rough, *Color:* brown covering with prominent, triangular beak which had concavity with the funicle. *Size:* 5-10 mm; *Texture:* not easily breakable (Fig. 1A).

Microscopical Characters

C. arietinum seeds

A transverse section of seed showed wide and tall beak-like funicular part which had spongy parenchymatous cells and a central vertical spindle shaped structure called 'tracheid-bar' (Fig 2A). The tracheid bar was surrounded by soft lobed parenchyma cells. The bar consisted of horizontally elongated tracheids with reticulate pits (Fig 2B, 2C).

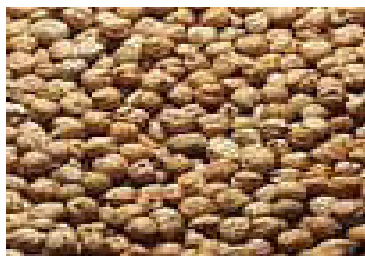


Fig 1: A: Seeds of the *C. arietinum*

Seed coat

Seed coat was 500 µm thick and mainly consisted of epidermal layer of narrowly elongated, compact columnar cells which were tall, thin walled and uneven in height. The surface of the seed coat had wide conical or wide mound of projections (Fig 2D).

Fig 2A: Vertical section of the seed through terminal beak and tracheid bar Fig 2B: Tracheid bar and the spongy parenchyma: entire view

Fig 2C: Tracheid bar: a sector enlarged Fig 2D: Transverse section of seed coat Fig 2E: A portion of the cells of the cotyledons

Fig 2F: Starch grains of the cotyledon cells: under light microscope (bright field) Fig 2G: Starch grains of the cotyledon cells: under polarized light microscope Fig 2H: Seed coat- a segment with palisade cells of the outer part Fig 2I: Osteosclereids Fig 2J: Starch grains in the seed powder (stained with IKI) Fig 2K: Starch grains in the seed powder (stained with IKI) Cot: Cotyledons; Fu: Funiculus; SC: Seed coat; SP: Spongy parenchyma; TB: Tracheid bar; Tr: Tracheids; IEp: Inner epidermis of the cotyledon; ISc: Inner paranchymatous seed coat; OSc: Outer seed coat of columnar cells; OS: Osteosclereids of the seed coat; VS: Vascular strand; SG: Starch grains; SC: Seed coat segment; SG: Starch grains; ESG: Elliptical starch grains; CSG: Circular starch grains; Pa: Parenchyma cells with granular content.

Inner to the palisade of epidermis, was a continuous, wide layer of osteosclereids. The osteosclereids had narrow cylindrical middle portion and dilated ends resembling the shape of the 'bone'. The inner part of the seed coat was wide and paranchymatous. The cells were circular, thin walled and more or less loosely arranged. No specific inclusions were seen in the seed coat cells.

Cotyledons

The cotyledons had thin outer and inner epidermal layers of small rectangular thick-walled cells. The ground tissue consisted of large, circular, thin-walled parenchyma cells with wide intercellular spaces (Fig 2E).

The cells of cotyledons were heavily loaded with starch grains (Fig 2F, 2G). The grains were of simple-type. They were either circular or spindle shaped. The circular starch grains had X-shaped polarimark while, the spindle shaped starch grains had Y-shaped polarimark when the grains were seen under the polarized light microscope (Fig 2H).

The powder exhibited the following elements as seen under the microscope:

Fragments of seed coat

Fragments of seed coat with thick conical segments consisted of elongated, thin columnar cells and osteosclereids (Fig 2I).

Osteosclereids

The osteosclereids were bone-shaped cells with narrow middle part and swollen ends. They were thick walled and lignified (Fig 2J).

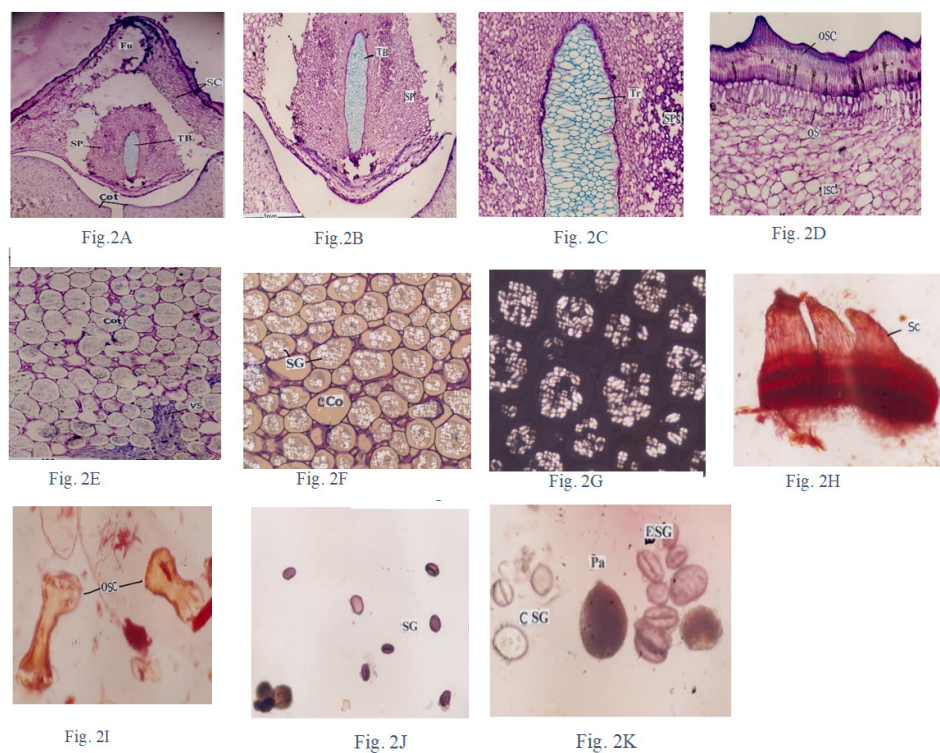
Fig 2: Microscopic characters of *C. arietinum* seeds

Table 1: Physiochemical studies of the crude drugs

S.No.	Parameters	<i>C. arietinum</i>
1	Alcohol soluble Extractive values (%w/w)	10.44
2	Water soluble Extractive values (%w/w)	10.56
3	pH values (1% aqueous solution)	7.04
4	pH values (10% aqueous solution)	6.84
5	Total Ash values (%w/w)	3.62
6	Acid insoluble ash (%w/w)	1.40
7	Water soluble ash (%w/w)	2.09
8	Petroleum ether Successive extractive values (%w/w)	3.34
9	Chloroform Successive extractive values (%w/w)	3.95
10	Ethanol Successive extractive values (%w/w)	5.21
11	Aqueous Successive extractive values (%w/w)	8.54

Table 2: Preliminary phytochemical screening of crude drugs

S.No.	Chemical constituents	<i>C. arietinum</i>
1	Alkaloids	-
2	Carbohydrates	+
3	Glycosides	+
4	Phenolic compounds	+
5	Flavonoids	+
6	Proteins and amino acids	+
7	Saponins	+
8	Sterols	+
9	Acidic compounds	-
10	Mucilage	+
11	Fixed oils/fats	+
12	Resin	+

+ Present; - Absent

Starch grains

Abundant starch grains were found in the powder. When stained with IKI, they appeared purple or dark. Some of them were circular and others were elongated with median longitudinal dark line (Fig 2K).

Physiochemical studies

Physiochemical studies were done in order to check quality and purity of herbal drugs.

The results are tabulated in Table 1.

Phytochemical studies

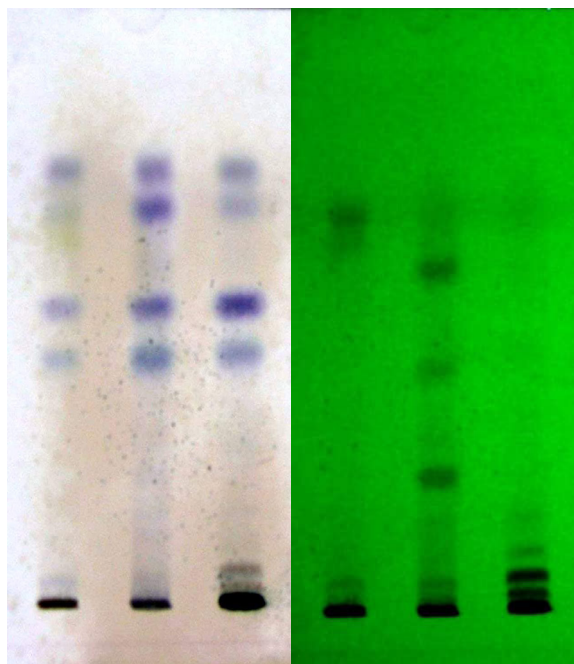
The study of phytochemical screening and chemical testing were carried out for determination of the possible existence of natural chemical compounds and their chemical type by color reactions. The results are recorded in Table 2, 3 & 4. These data can be used for determining correct identity and purity of plants part and detection of adulteration as well.

Table 3: Color observations of the powdered drugs

S. No.	Treatment with reagents	<i>C. arietinum</i>
1	Powder as such	Yellowish brown
2	Water	Cream
3	Petroleum ether	Yellowish brown
4	Chloroform	Yellowish brown
5	Methanol	Dark yellow
6	Conc. HCl	Dark mustard
7	Conc. HNO ₃	Yellowish brown
8	Conc. H ₂ SO ₄	Dark brown
9	Glacial acetic acid	Brownish yellow
10	Picric acid	Yellow
11	50% HCl	Yellowish brown
12	50% HNO ₃	Yellowish brown
13	50% H ₂ SO ₄	Yellowish brown
14	5% KOH	Dark yellow
15	5% FeCl ₃	Brownish yellow
16	1N NaOH in water	Yellowish brown
17	1N NaOH in methanol	Yellow

Table 4: Fluorescence analysis of the powdered drugs under ultra violet radiation

S.No.	Treatment with reagents	<i>C. arietinum</i>	
		UV 254 nm	UV 366 nm
1	Powder as such	Cream	Whitish brown
2	Powder treated with conc. HCl	Fluorescent yellow	Whitish green
3	Powder treated with conc. HNO ₃	Light fluorescent	Dark brown
4	Powder treated with conc. H ₂ SO ₄	Dark green	Light yellowish green
5	Powder treated with glacial acetic acid	Light fluorescent green	Light cream
6	Powder treated with picric acid	Light fluorescent green	Black
7	Powder treated with 50% HCl	Light fluorescent green	Creamish brown
8	Powder treated with 50% HNO ₃	Light fluorescent green	Dark brown
9	Powder treated with 50% H ₂ SO ₄	Creamish green	Yellowish white
10	Powder treated with 5% KOH	Light fluorescent	Brownish cream
11	Powder treated with 5% FeCl ₃	Light fluorescent green	Black
12	Powder treated with 1N NaOH in water	Light fluorescent green	Brownish cream
13	Powder treated with 1N NaOH in methanol	Fluorescent yellow	Creamish brown

**Fig 5:** HPTLC plate of ethanolic extracts of *C. arietinum* seeds upon derivatization with anisaldehyde in sulphuric acid at UV-254 and 366 nm

Chromatographic fingerprint study

Alcoholic extracts of drugs were analyzed by HPTLC for development of fingerprints. HPTLC fingerprint gives full profile of multiple compounds present in a drug. The chromatograms obtained after development in different solvent system followed by scanning depicted the presence of number of substances in the extracts. The extracts showed the presence of 20 (*C. arietinum*; spray) spots (Fig.5), with different R_f values as given in Table 5. The data generated can serve as finger prints of drugs for their identification and purity analysis.

Table 5: Fingerprint profile of crude drugs

S.No.	Plant Extract	Solvent system	Number of spots	R_f values	Visualizing agent
1	<i>C. arietinum</i> seeds ethanolic extract	Toluene: ethyl acetate methanol: Glacial acetic acid (6.5 :3: 1: 0.5)	20 (spray)	0.09, 0.14, 0.19, 0.26, 0.34, 0.40, 0.45, 0.49, 0.52, 0.57, 0.60, 0.65, 0.74, 0.77, 0.82, 0.86	Anisaldehyde in sulphuric acid

CONCLUSION

The results of present investigations reveal several diagnostics features of *C. arietinum* seeds on the basis of which identification of the crude drug can be ascertained easily. The data generated can be used for determining of correct identity of the drugs and detection of adulterants as well. Thus, the standardization parameters which were performed are very useful for proper identification of an authentic sample. All these studies can serve for the recognition of characteristic features of the drug in all respects which is not only helpful for the utilization of these plants as herbal medicine or drug against different ailments but also useful in manufacturing as raw material or in prescription medicine. As a result, it is directive towards the search of drugs from natural origin and to utilize them and their main pharmaceuticals manufacturing as useful raw material and active agents.

Fruits and vegetables are an invaluable source of many biologically active substances. This action is performed by variety of foodstuffs which are consumed daily from vegetables origin such as kerala, jamun, methi, onion, garlic and dhania.^[1,2] All these herbal drugs and many others have powerful antidiabetic action which has been scientifically proved now.^[3,4] In order to obtain good results from these plant drugs, authenticity of the sample must be necessary.

The present study pertains to detailed pharmacognostical standardization of antidiabetic herbal drug *C. arietinum* seeds which is used to treat and prevent several pathological diseases in Ayurvedic and Unani medicines.^[5-9]

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