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Karnail Singh
Janta College of Pharmacy, Butana, Sonepat, Haryana, India

Kavita Gahlot
School of Pharmaceutical Sciences, IFTM University, Moradabad, Uttar Pradesh, India

Correspondence
Karnail Singh
Janta College of Pharmacy, Butana, Sonepat, Haryana, India

Pharmacognostic evaluation of stem and leaf of *Cicer arietinum* Linn.

Karnail Singh and Kavita Gahlot

Abstract
Objective: To evaluate the pharmacognostic characters of an important medicinal plant *Cicer arietinum* Linn.
Methods: The present paper highlights the morphological and histological characters of stem and leaf of *Cicer arietinum* including ash values, extractive values, fluorescence analysis and preliminary phytochemical screening of the stem and leaf of *Cicer arietinum*.
Result: The characteristics microscopic features of leaves were observed as trichomes, xylem cells, phloem cells, spongy parenchyma and palisade cells. The characteristics microscopic features of stem were observed as cuticle, epidermis, cortex, phloem fibre, phloem parenchyma, covering trichomes, medullary rays, xylem and pith. The morphological features of leaves and stem of *Cicer arietinum* shows its shape, size, colors etc. The other pharmacognostical parameters were also shows a significant and identical result.
Conclusions: This paper may help us proposed parameters to establish the authenticity of *Cicer arietinum* and can possibly help to differentiate the drug from its other species.

Keywords: Leguminosae, pharmacognosy, *Cicer arietinum.*

1. Introduction
Over the last decade there has been a growing interest in drugs of plant origin in contrast to the synthetics that are regarded as unsafe to human and environment (Thomas *et al*., 2001) [1]. *Cicer arietinum* Linn belonging to family Leguminosae is an annual herb which is spread into Southern Europe, India, Egypt and Southern America. It is extensively cultivated in India mainly in Rajasthan, Hyderabad, Patiala, East Punjab, Haryana and Madhya Pradesh (Raghanathan *et al*., 2005) [2]. It needs warm and moist climatic conditions to propagate. Its black gram is native of India but the white species commonly called Kabuli came to India in 18th century from European countries and area like Afghanistan etc. In India it is very often used as a crash diet and it is one of the most widely made recipes in India kitchen due to its good taste and nutritive values. Traditionally it is used for many diseases. The present investigation includes morphological and anatomical evaluation using standard and compendial methods, determination of physicochemical constants and preliminary phytochemical screening of different extracts. In the light of traditional uses and sporadic recent pharmacologic reports, *Cicer arietinum* seems to be a potential crude drug for thorough investigation. Thus the present investigation has been undertaken with an objective to establish Pharmacognostical standards for *Cicer arietinum* stem and leaf, so that authentic plant material could be explored properly for its traditional claims.

2. Materials and Methods
2.1 Collection and authentication of plant material
The Plant *Cicer arietinum* was collected during February to March from different region of Haryana mainly from the Dist. Jind and authenticated through NISCAIR, New Delhi and voucher specimen was preserved for further references. The stems and leafs were separated, washed under running tap water; air dried under shade, coarsely powdered and kept in airtight container until further use.

2.2 Macroscopic and microscopic analysis of stem and leaf
The Macroscopic of the stem and leaf was studied according to the method of Brain & turner (Brain *et al*., 1975a) [3] and Cubero (Cubero. 1987) [4]. For the microscopical studies, cross sections were prepared and stained as per the procedure of Johansen (Johansen. 1940) [5] and Wallis (Wallis. 1997) [6]. The powder microscopy analysis was done according to the method of Brain and Turner (Brain *et al*., 1975b) [7], Dutta (Dutta, 1995) [8] and Kokate (Kokate, 1986a) [9].

2.3 Physicochemical analysis of stem and leaf
Physicochemical analysis of stem and leaf i.e. percentage of ash values and extractive values were performed according to the official methods prescribed in Indian Pharmacopoeia (Indian Pharmacopoeia 1996) [10] and WHO (WHO. 1998) [11]. Fluorescence analysis was carried out according to the method of Chase and Pratt (Chase *et al*. 1949) [12], Kokoski (Kokoski 1958) [13] and Evans (Evans 2002) [14].

2.4 Preliminary phytochemical screening of stem and leaf
Preliminary phytochemical screenings for organic and inorganic elements of both (Stem and Leaf) were carried out by using standard procedures described by Harborne (Harborne 1998) [15] and Khandelwal (Khandelwal 2008) [16].

3. Results and Discussion
3.1 Macroscopic characters of Stem
Cicer arietinum stems are branched, erect or spreading, sometimes shrubby much branched, 0.2-1m tall, glandular pubescent, olive, dark green or bluish green in color. The stem has hairs between the nodes and the hairs on the stem are distributed more of less uniformly. The flowering stem is circular, or with lots of small angles so that it is roughly circular. The *Cicer arietinum* stem branches are classified as primary, secondary, and tertiary branches.

3.1.1 Primary branches
It arises from the ground level as they develop from the plumular shoot as well as the lateral branches of the seedling. They are thick, strong, and woody, and may range from one to eight in number.

3.1.2 Secondary branches
It develops at buds located on the primary branches. They are less vigorous than the primary branches. Their number ranges from 2 to 12. The number of secondary branches determines the total number of leaves, and hence the total photosynthetic area.

3.1.3 Tertiary branches
It arises from the secondary branches. The primary branches form an angle with a vertical axis, ranging from almost a right angle (prostrate habit) to an acute angle (erect). Generally stems are incurved at the top, forming a spreading canopy (Fig. 1).

Fig 1: *Cicer arietinum* stem

3.2 Macroscopic characters of Leaf: (Fig 1a).

S. No.	Characters	Properties
1	Shape	Leaves are compound
2	Size	10-20 mm in length and 1.5-2.3 mm in width
3	Colour	Fresh leaves are green and dry ones are yellowish green
4	Surface	Pubescent
5	Base	Rounded
6	Leaf Hair	Leaf is fuzzy or hairy
7	Margin	Serrate
8	Apex	Acuminate (Sharply pointed)
9	Leaf number	7-15
10	Leaf arrangement	One leaf per node along the stem
11	Odour	Characteristic
12	Taste	Sour
13	Leaf variation	The leaf are nearly similar in size, prominence in teeth

Fig 1a: Leaf of Cicer arietinum

3.3 Microscopic characters of Stem
3.3.1 Transverse section
The upper most layer of cuticle is seen, covering trichomes and a single layer of epidermis, which consists of small tangentially elongated rectangular cells with brownish, thick-outer walls and a band of cortex, which consists of 7 to 9 rows of big parenchymatous cells with intercellular spaces. The inner part of cortex contains scattered groups of phloem fibres. The Vascular bundle comprises of xylem and phloem (Fig. 2a). Each phloem bundle is surrounded by a parenchymatous sheath containing calcium oxalate and starch grains (Fig. 2b). Medullary rays pass through both phloem and xylem. Primary xylem was distinct on the inner side of the secondary xylem. The Medullary rays are uni-seriate or multi-seriate parenchymatous cells, narrow in the xylem region and wider in the phloem region. Medullary rays in the phloem region are non-lignified whereas lignified in the xylem region and starch grains are present in few cells (Fig.2c). Pith consists of few rounds to oval thin walled parenchymatous cells (Fig. 2a).

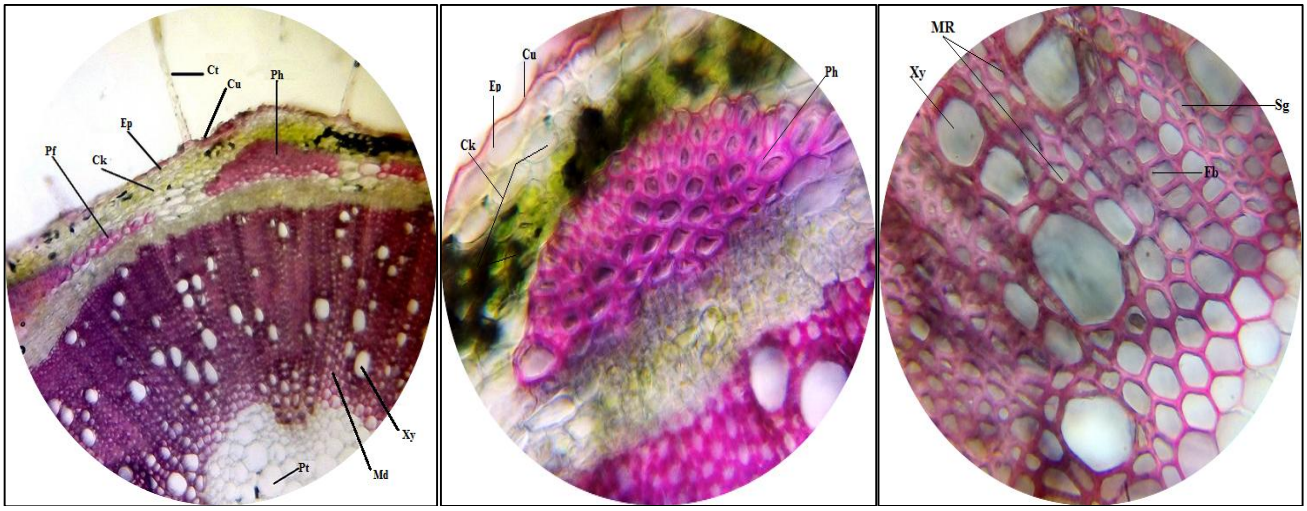

Fig 2a **Fig 2b** **Fig 2c**

Fig 2: "Microscopy of stem of *Cicer arietinum*" a; Transverse section consists of Cuticle, Epidermis, Cortex, Phloem fibre, Phloem, Covering Trichomes, Medullary rays, Xylem and Pith (100x) b; Transverse section contains, Cuticle, Epidermis, Cortex and Phloem (400x) c; Transverse section consists, Medullary rays, Xylem, Fibres and starch grains (400x)

Abbreviations: Cu- Cuticle, Ck-Cortex, Fb-Fibres, MR-Medullary rays, Ct- Covering Trichomes, Pf-Phloem fibre, Ph-Phloem, Pt-Pith, Sg-Starch grains, Xy- xylem, Ep- Epidermis.

3.4: Powder characteristic of stem

Presence of crystals of calcium oxalate, cork cell, tracheids, cortex, unicellular (Covering) Trichomes, stone cells, spiral vessel, bordered pits, xylem vessel are present. (Fig. 3).

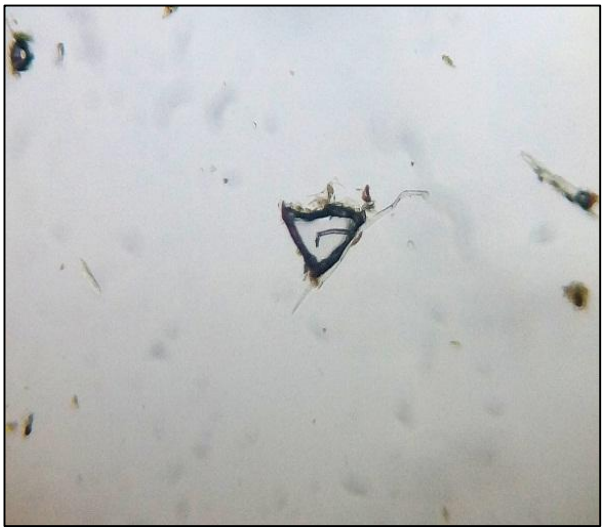

Fig 3A: Calcium oxalate

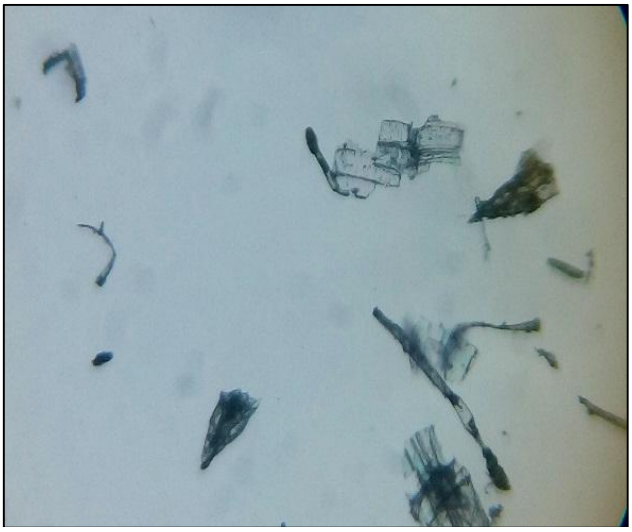

Fig 3B: Cork cell

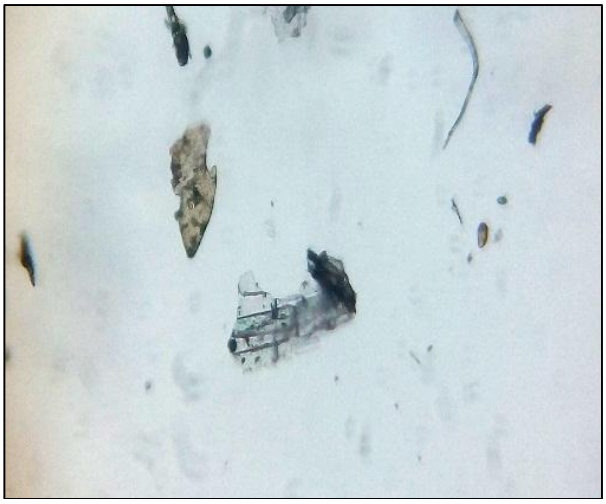

Fig 3C: Tracheids

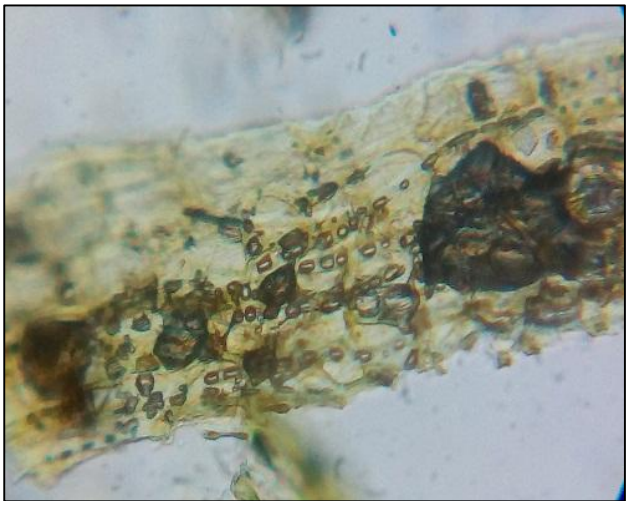

Fig 3D$_1$: Cortex

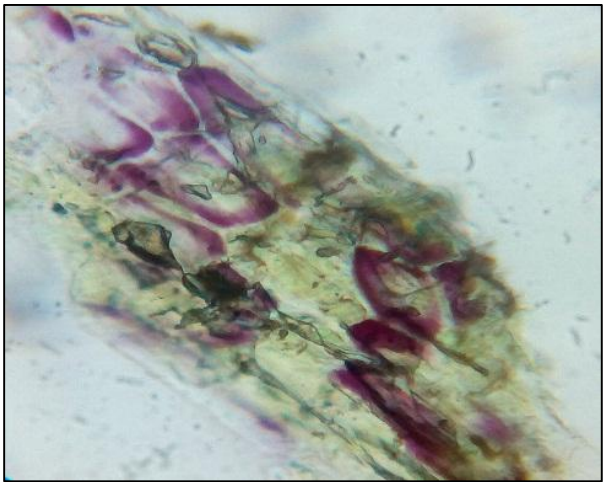

Fig 3D$_2$: Cortex

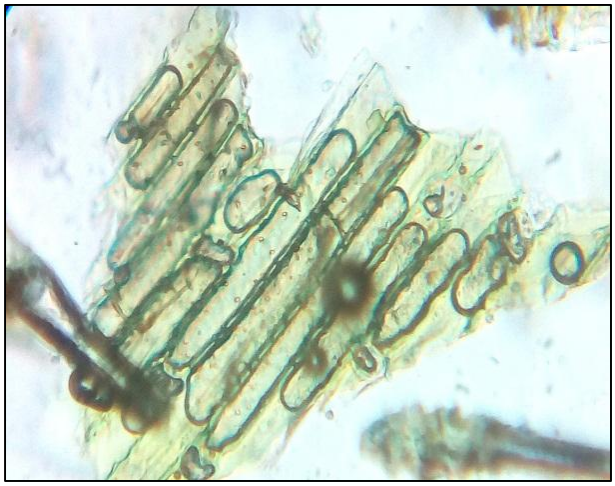

Fig 3D$_3$: Cortex

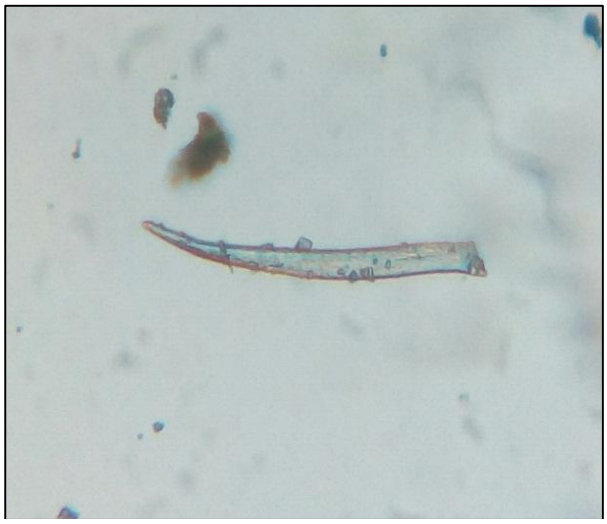

Fig 3E: Unicellular (Covering) Trichomes

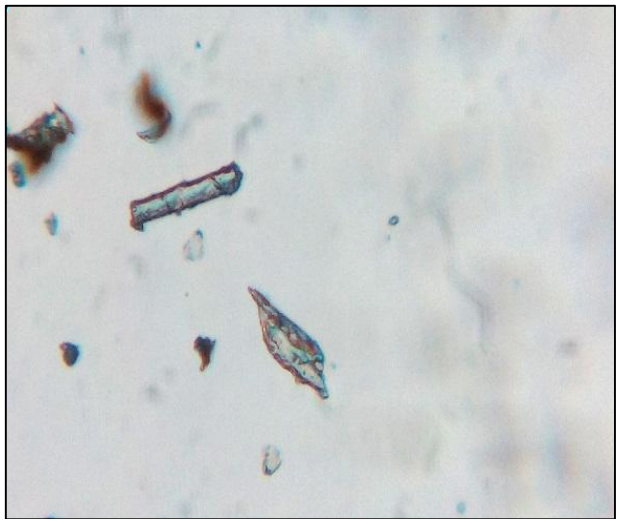

Fig 3F: Stone cells & Spiral vessel

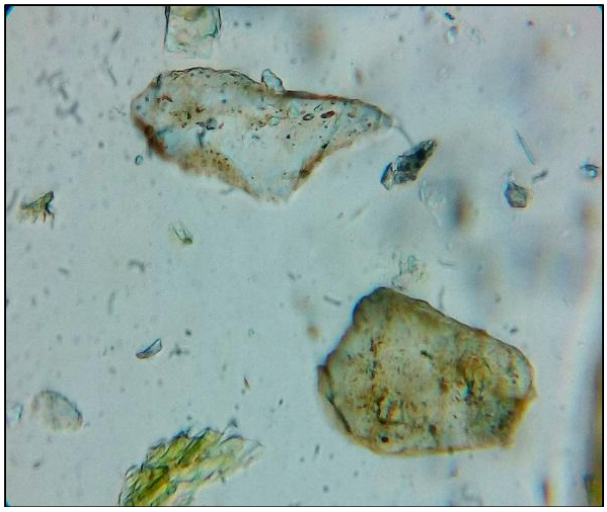

Fig 3G: Bordered pits

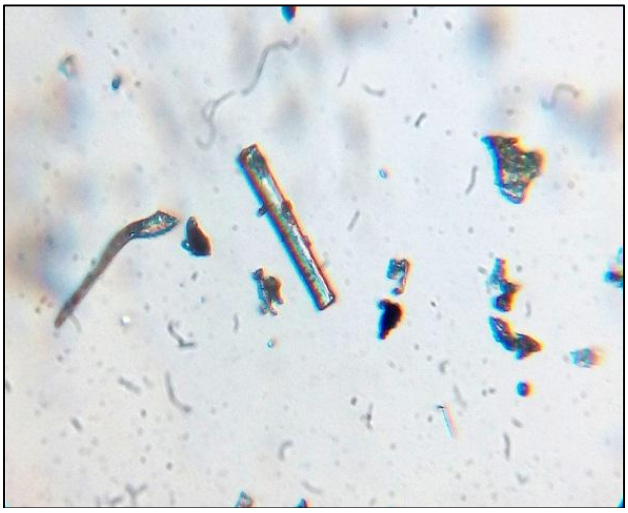

Fig 3H: Xylem vessel

Fig 3: "Powder microscopy of stem of Cicer arietinum"

3.5 Microscopic characters of Leaf

3.5.1 Transverse Section of Leaf (Midrib)

Structurally the herbaceous leaf consists of Upper epidermis, Lower epidermis, covering Trichomes, Vascular bundle consist of xylem and phloem, Collenchyma, Sclerenchyma, Spongy parenchyma and Palisade cell (Fig. 4a, 4b & 4c).

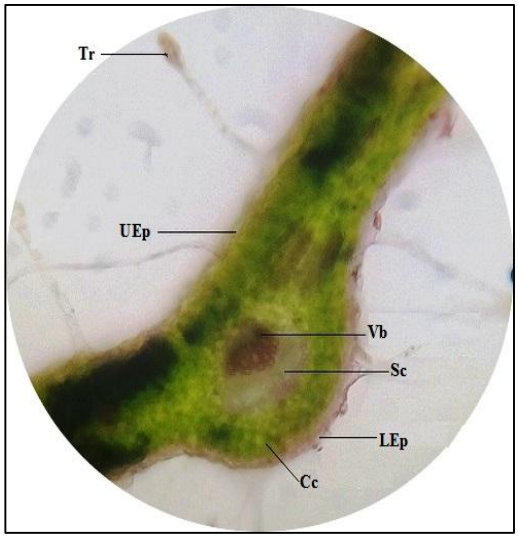

Fig 4a

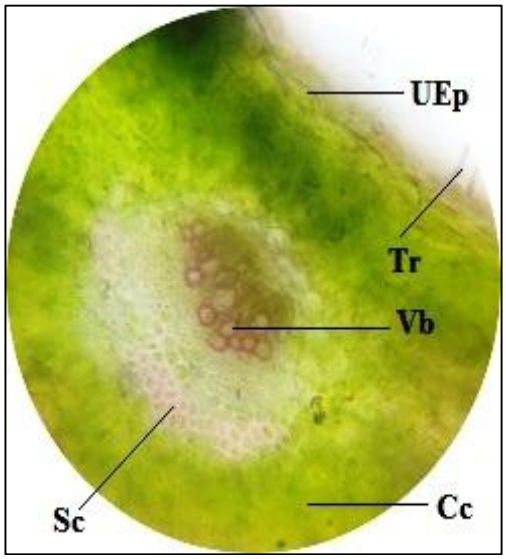

Fig 4b

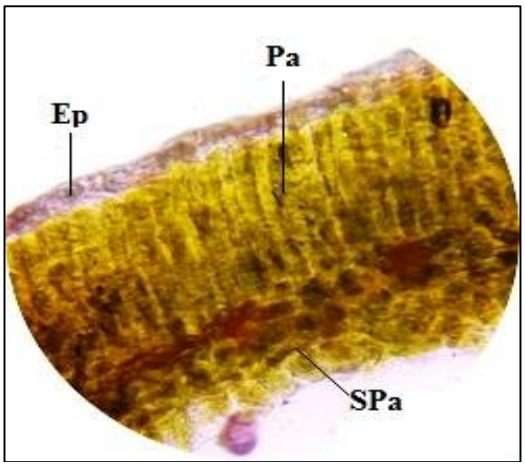

Fig 4c

3.5.2 Transverse Section of Leaf (Petiole)

In the transverse section of *Cicer arietinum* leaf of petiole part, it consists of cuticle, epidermis, trichomes, phloem and collenchyma (Fig. 4d & 4e).

Fig 4d

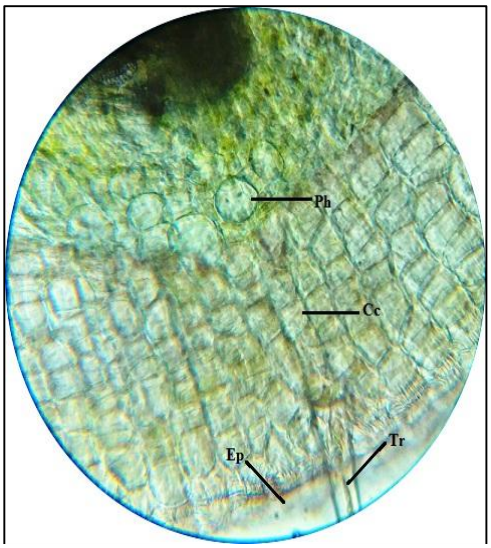

Fig 4e

3.5.3 Transverse Section of Leaf (Apex)

In the transverse section of apex part of *Cicer arietinum* leaf, cuticle, epidermis, Trichomes, Stomata, Palisade cell, crystal sheath were observed (Fig. 4f & 4g).

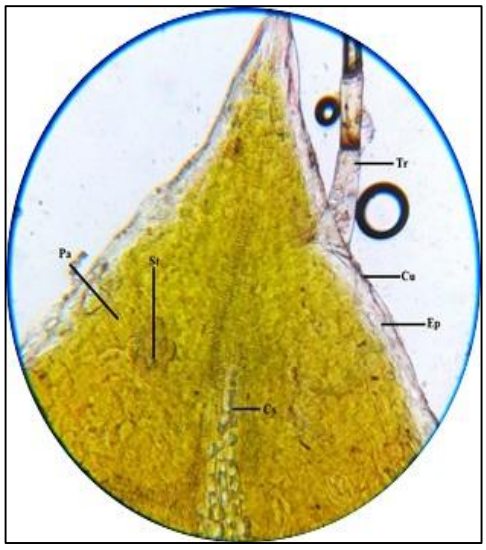

Fig 4f

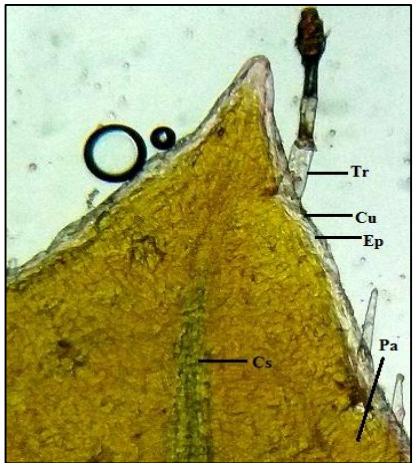

Fig 4g

3.5.4 Transverse Section of Leaf (Margin)

In the transverse section of margin part of *Cicer arietinum* leaf cuticle, epidermis, Stomata, Palisade cell, crystal sheath were observed (Fig. 4h).

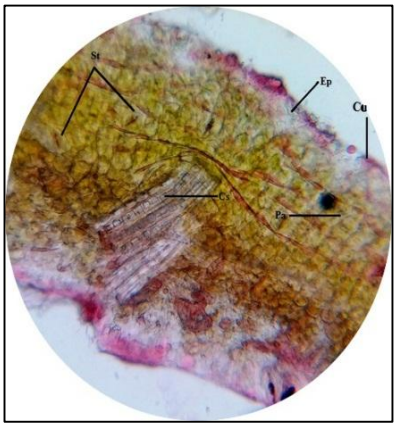

Fig 4h

Abbreviations Used

Tr.- Trichomes, UEp.- Upper Epidermis, LEp.- Lower Epidermis, Vb.- Vascular bundle, Cu.- Cuticle, Cs.- Crystal Sheath, Ph.- Phloem, Sc.- Sclerenchyma, Cc.- Collenchymas, Spa.- Spongy parenchyma, Pa.- Palisade cell.

3.6 Quantitative Microscopy

Anisocytic type stomata were observed in leaf part of *Cicer arietinum* (Fig. 5a). Stomatal number and stomatal index was also found out in that study (Fig. 5b).

Fig 5a

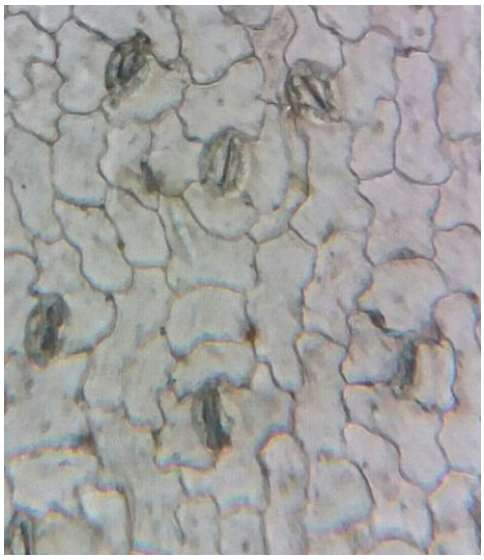

Fig 5b

3.6.1 Determination of Stomatal Number and Stomatal Index

Stomatal number is the average number of stomata per square millimeter of epidermis. The percentage proportion of the ultimate divisions of the epidermis of a leaf which can be converted into stomata is termed as stomatal index. Stomatal index can be calculated by using following equation.

$$I = S / E + S \times 100$$

Where,

I = stomatal index

S = number of stomata per mm^2

E = number of ordinary epidermal cells per mm^2

A piece of leaf was cleaned and peeled out by means of forceps. It was kept on slide and mounted in glycerin water. Camera lucida was attached and drawing board was placed for drawing the cells. A square of 1 mm by means of stage micrometer was drawn on it. The slide with cleared leaf was placed on the stage and the epidermal cells and stomata were traced. The number of stomata and the number of epidermal cells in each field were counted and the stomatal Index counted with the help of above given formula and result is given in table no. 1.

3.6.2 Determination of vein-islet and vein-let termination number

Vein islet is the minute area of photosynthetic tissue encircled by the ultimate division of the conducting strands. Vein termination number is the number of veinlet terminations per mm of leaf surface. A piece of the leaf was cleared by boiling in chloral hydrate solution and camera lucida and drawings board were arranged and 1 mm line was drawn with help of stage mm. A square was constructed on this line in the centre of the field. The slide was placed on the stage. The veins included within the square were traced off, completing the outline of those islets which overlap two adjustment side of the square. The average number of vein islet from the four adjoining square, to get the value for one square mm was calculated (Fig. 5c). The number of veinlet termination present within the square was counted and the average number of veinlet termination number from the four adjoining square to get the value for 1 square mm was found known as vein termination number and result is given in table no. 1.

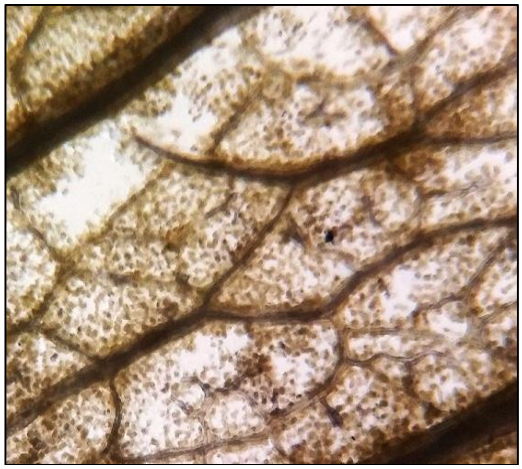

Fig 5c: Vein-islet and vein-let termination number

3.6.3 Determination of palisade ratio

A piece of the leaf was boiled in chloral hydrate and was placed under microscope. Camera lucida and drawing board were arranged and the outline of four cells of the epidermis was traced using 4 mm objective. Then, palisade layer was focused down and sufficient cells for covering the tracing of the epidermal cells were traced off. The outline of those palisade cells which were intersected by the epidermal walls was completed. The palisade cells under the four epidermal cells (including cells which are more than half and excluding cells which are less than half within the area of epidermal cells) were counted. The average number of cells beneath epidermal cells was calculated known as palisade ratio and result is given in table no. 1.

Table 1: Quantitative microscopy of *Cicer arietinum* Leaf

S. No.	Parameters	Number
1	Stomatal Number	06
2	Stomatal Index	9.37 %
3	vein-islet number	15
4	Vein-termination number	9
5	palisade ratio	5.5

3.7 Powder Microscopy

Presence of crystals of Starch grains, calcium oxalate crystal, unicellular trichomes, crystal sheath, Epidermal Cell with stomata and Multicellular trichomes are present (Fig. 6a-6g).

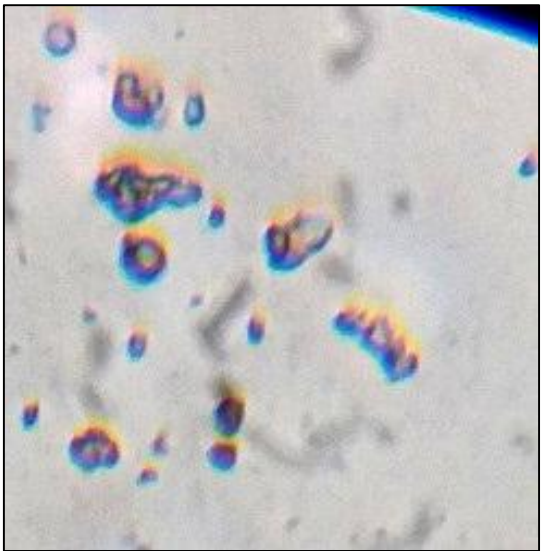

Fig 6a: Starch grains

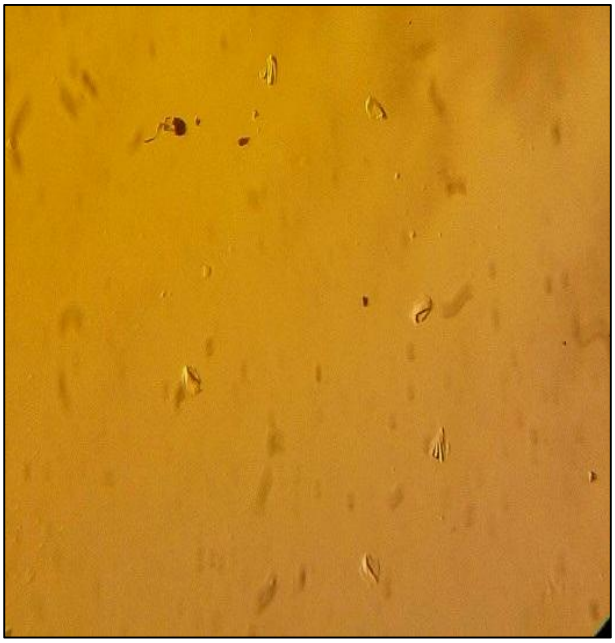

Fig 6b: Calcium oxalate crystal

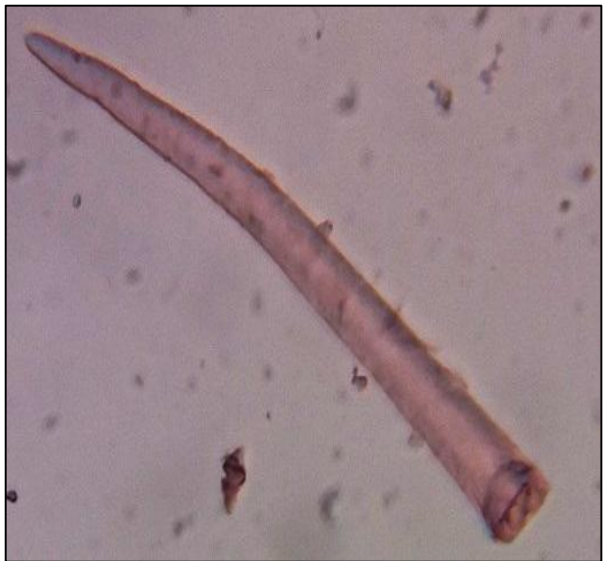

Fig 6c: Unicellular (Covering) Trichomes

Fig 6d: Crystal Sheath

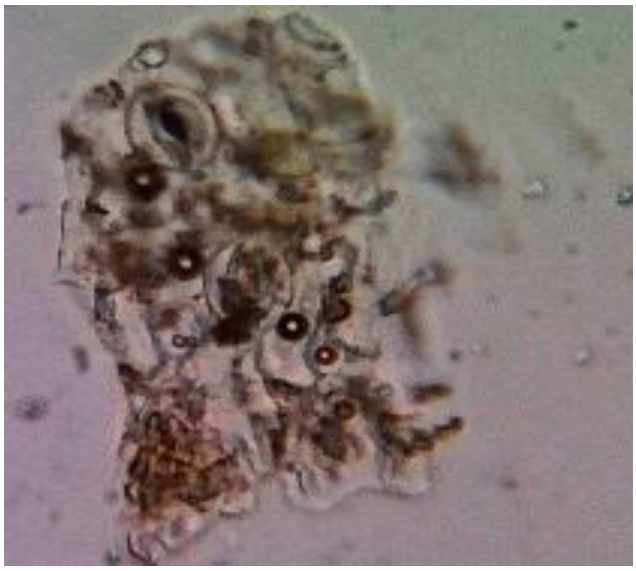

Fig 6e: Epidermal Cell with Stomata

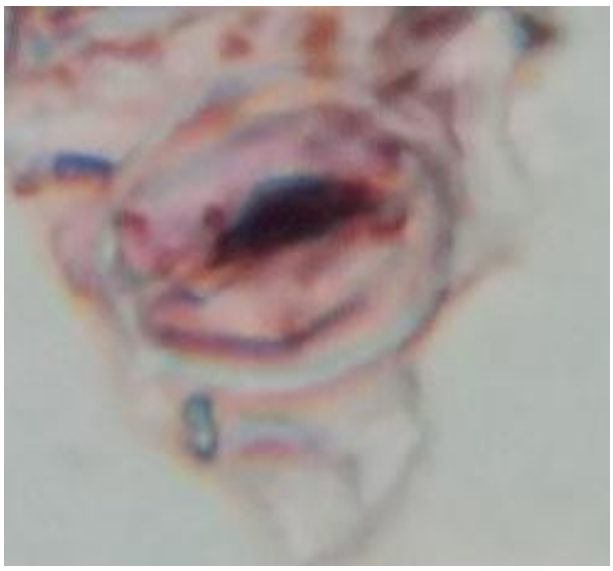

Fig 6f: Stomata

Fig 6g: Multicellular (Glandular) Trichomes

4. Preliminary phytochemical screening

Preliminary phytochemical screening of stem and leaf of *Cicer arietinum* for organic and inorganic elements were carried out by using standard procedures. The result of organic elements revealed the presence of alkaloids, glycosides, carbohydrates, saponin, amino acid, tannins, protein and flavonoids (Table 2) and the result of inorganic elements show the presence of iron, calcium, Magnesium, phosphate, sulphate, and chloride (Table 3).

Table 2: Preliminary Phytochemical Screening of Different Extracts of *Cicer arietinum* Stem and Leaf (Organic elements)

Phytoconstituents	Hydroalcoholic Extract		Chloroform extract		Alcohol extract		Pet. Ether extract		Water extract		Hexane extract	
	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf
Alkaloids												
Mayer's test	–	+	–	+	–	+	–	–	–	+	–	–
Dragendroff's Test	–	+	–	+	–	+	–	–	–	+	–	–
Wagner's test	–	+	–	+	–	+	–	–	–	+	–	–
Hager's test	–	-	–	+	–	–	–	–	–	–	–	–
Glycosides												
Legal's Test	–	–	–	–	–	–	–	–	–	–	–	–
Keller-Killiani test	–	–	+	+	–	–	–	–	–	–	–	–
Carbohydrates	+	+	+	–	+	+	–	–	+	–	+	–
Amino acid	+	+	–	–	+	+	–	–	+	+	–	–
Protein												
Biuret test	+	+	–	–	+	+	–	–	+	–	–	–
Xanthoprotein test	+	+	–	–	+	+	–	–	+	–	–	–
Million's test	+	+	+	–	+	+	–	–	+	+	–	–
Saponin	–	–	–	–	–	–	–	–	+	+	–	–
Tannin												
Ferric chloride test	–	+	–	–	–	+	–	–	–	+	–	–
Lead acetate test	–	+	–	–	–	+	–	–	–	–	–	–
Steroid and Triterpenoids												
Liebermann-Burchard test	–	–	–	–	–	–	–	–	–	–	–	+
Salkowski's test	–	–	–	–	–	–	–	–	–	–	–	–
Flavonoids	+	+	–	–	–	+	–	+	–	+	+	+

(+) Sign indicates presence, (–) Sign indicates absence

Table 3: Inorganic Constituents of Stem and Leaf Powder of *Cicer arietinum.*

Elements	Results	
	Stem	Leaf
Calcium	—	+
Magnesium	—	+
Sodium	—	+
Potassium	+	+
Iron	+	+
Zinc	+	+
Sulphate	—	−
Phosphate	+	−
Chloride	+	−
Carbonate	—	−
Nitrates	—	−

(-) Not present, (+) present

5. Physicochemical studies

Ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The percentage of total ash, acid insoluble ash and water soluble ash are carried out in (Table 4). Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The water soluble, alcohol soluble, chloroform soluble and petroleum ether soluble extractive values have been tabulated in (Table 5). The results of fluorescence analysis of the stem powder are presented in (Table 6).

Table 4: Physicochemical parameters of Stem and Leaf of Cicer arietinum

Parameters	Values (%w/w)	
	Stem	Leaf
Alcohol soluble extractive	18.4 %	15.81 %
Water soluble extractive	13.6 %	19.62 %
Chloroform soluble extractive	11.2 %	6.21 %
Petroleum ether soluble extractive	6.4 %	5.34 %
Moisture content (LOD)	6.66 %	8.33 %
Total ash	6.5 %	7.52 %
Acid insoluble ash	4.5 %	3.23 %
Water soluble ash	5.5 %	4.31 %

Table 5: Fluorescence Analysis of the Stem Powder of *Cicer arietinum* Linn.

Treatment	Visible light	UV 254 nm (short)	UV 365 nm (long)
Power as such	Yellowish brown	Light Green	Black
Powder + 10% Picric acid	Yellowish brown	Green	Blackish brown
Powder + 5% Iodine sol^{n}.	Brown	Blackish green	Dark brown
Powder + 5% NaOH	Brown	Dark green	Black
Powder + Conc. NH_3	Greenish black	Green	Dark brown
Powder + Dil. HCl	Blackish brown	Light green	Brownish green
Powder + Conc. HCl	Light Green	Dark green	Dark brown
Powder + Dil. H_2SO_4	Brown	Blackish green	Black
Powder + Conc. H_2SO_4	Reddish brown	Green	Reddish brown
Powder + Dil. HNO_3	Brownish black	Dark green	Blackish brown
Powder + Conc. HNO_3	Light brown	Blackish green	Dark brown
Powder + $FeCl_3$ Solution	Greenish yellow	Brown	Black

Table 6: Fluorescence Analysis of the Leaf Powder of *Cicer arietinum* Linn.

Treatment	Visible light	UV 254 nm (short)	UV 365 nm (long)
Power as such	Yellowish green	Light Green	Brown
Powder + 10% Picric acid	Chocolate brown	Dark green	Black
Powder + 5% Iodine sol^{n}	Greenish brown	Black	Reddish brown
Powder + 5% NaOH	Blackish brown	Green	Black
Powder + Conc. NH_3	Black	Dark green	Brown
Powder + Dil. HCl	Reddish brown	Blackish green	Blackish brown
Powder + Conc. HCl	Light brown	Green	Black
Powder + Dil. H_2SO_4	Yellowish brown	Blackish green	Black
Powder + Conc. H_2SO_4	Dark brown	Dark green	Blackish brown
Powder + Dil. HNO_3	Orange brown	Dark green	Brown
Powder + Conc. HNO_3	Yellowish orange	Light green	Reddish brown
Powder + $FeCl_3$ Solution	Dark brown	Brownish green	Black

Conclusion

Various Pharmacognostic standards including qualitative and quantitative microscopic characters, Fluorescence, various physicochemical parameters and phytochemical screening of various extracts were used to substantiate standardization data on *Cicer arietinum* stem and leaf. This study would be useful for preparation of a monograph and selecting the authentic plant material for exploring its phytochemical and pharmacological potential.

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