Article

Botanical pharmacognosy of *Cichorium intybus* seeds

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Abstract
Cichorium intybus L., also widely known as chicory or kasni, is a perennial herb, native to Europe, northern parts of Africa, and West and Central Asia. It was introduced elsewhere in temperate and semi-arid regions and is widely naturalized in many places for its roots and seeds. In India it is especially cultivated in Gurgaon (Haryana) and Patan (Gujarat) and the seeds are used in Ayurvedic medicines for various ailments. Though the seeds are used in Ayurvedic medicines and being cultivated on a commercial scale, pharmacognostical characteristic features of the seeds or achenes to authenticate the traded material is limited. Botanical pharmacognostical features such as organoleptic, macroscopic and microscopic characters of the seeds were studied. The seeds are angular, faintly pentagonal with 5 conspicuous and up to 9 inconspicuous ridges. In cross section it shows lignified epicarp, single-layered square-shaped testa, and cotyledons with aleurone grains and oil droplets. This study given detailed morphology and anatomy of the traded seeds for their easy identification and botanical authentication.

Keywords *Cichorium intybus*; pharmacognosy; seed; anatomy.

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1 Introduction

Cichorium intybus L. (family: Asteraceae) is an erect perennial herb, commonly called as chicory or Kasni. Although it is native to Europe, it is also distributed in Australia, North America, South America (Al-Snafi, 2016; Das et al., 2016), Chili, and South Africa (Street, et al., 2013). In India, in northwestern Himalayas and plains, chicory is grown along with winter season crops and often found as an escape (Maheshwari, 1963; Hooker, 1882). *Cichorium intybus* is cultivated for roots and seeds, which should not be confused with *C. endivia* cultivated for their green vegetable and fodder. The northwestern Indian plains and Himalayas are the commercial scale cultivation sources of the seeds, where this material is traded in large quantity at Patan (Gujarat) and at Haily Mandi and Pipli Mand (Haryana). Although the wild chicory is perennial, the crop is commercially cultivated as a biennial crop (Kiers, 1999). Chicory has very pronounced history in traditional system of medicine, which is used to treat simple wounds to diabetes (Kowal, 2007). Different parts of the

plant have been used in traditional medicines globally and cultivated for their leaves, roots, and seeds in commercial scales (Street et al., 2017). The leaves are used as green vegetables to prepare salads and soups in some regions of Italy, including Latium and Tuscany (Guarrera, 2003; Pieroni, 2005). Italy, Germany, and Belgium are the top three countries that import cichory in fresh or chilled form (APEDA, 2020). Roasted and ground chicory roots are used as a coffee substitute or additive or supplement, particularly in India and South Africa, where more than 90% of consuming coffee contains chicory (Sastri, 1950; Singh, 1984). Cichory roots are also the commercial source of both fructans (Monti, 2005) and inulin (Frank, 2002). Chicory contains many medicinally important phytochemicals such as carbohydrates, tannins, alkaloids, triterpenoids, flavonoids, fatty acids, and volatile oils (Nandagopal, 2007). Annual usage of this herbal raw material in Indian domestic industry is 840 MT, which is obtained from both cultivated and wild collection sources (Ved, 2007). India exports around 500 MT cichory roots to various countries annually (APEDA, 2020). The seeds are used as a therapeutic raw drug in Ayurvedic medicines (Singh and Chahal, 2018). The seed extract exhibits the highest antioxidant activity compared with leaf and root extract and it also possesses potential liver protective property (Jurgonski et al., 2011). In Ayurveda, the seeds are used in the treatment of various liver diseases (Huseini et al., 2005; Ahmed, 2003; Najmi, 2005). Although the seeds with much therapeutic benefits but, there is a scarcity of information on the botanical pharmacognosy of this important ayurvedic drug. The current study evaluated organoleptic, macroscopic, and microscopic characters of *Cichorium intybus* seed in detail.

2 Materials and Methods

Cichorium intybus plants and seeds were collected from cultivated lands of Haryana and Gujarat states (Table. 1) and confirmed the botanical characters with local floras (Anonymous, 1993). The herbarium with voucher number 107895 was reposited in 'FRLH Herbarium and Raw Drug Repository, Bengaluru, Karnataka, India. The seeds were collected from the authentic plants were used as a reference sample to compare the pharmacognostical characteristics of commercially procured seeds from various suppliers (Table 2). The seeds were rehydrated, fixed in FAA solution followed by dehydration with ethanol : t-butanol series and embedded in paraffin wax, then 10 µ thick sections were taken using Leica RM 2125 rotary microtome (Donald, 1940). The microscopic features were photographed using Leica DM1000 trinocular microscope attached with digital camera under the bright field and polarizer. The seeds were powdered and sieved through 80 mesh, and a pinch of the meshed powder was mounted on a glass slides with a drop of 0.1% aqueous solutions of toluidine blue, iodine and sudan-III stains and observed under microscope to record the powder characterers (WHO, 1998).

Table 1 Geographical locations of cultivation fields from where the samples of *C. intybus* seeds were collected.

Sl. No.	Latitude	Longitude	Elevation (m)
1	28.3220	76.6495	214.2579
2	28.3525	76.7429	222.1887
3	28.2870	76.6622	218.5837
4	28.3367	76.6737	224.8324
5	28.3038	76.6472	214.2579
6	28.2567	76.7340	226.5147
7	28.3200	76.7147	228.9180
8	28.3326	76.7258	227.4760
9	28.3458	76.7595	225.9467
10	28.3619	76.6431	222.8447

11	23.3604	72.4129	91.8329
12	23.3245	72.4288	88.6320
13	23.3134	72.4338	87.4121
14	23.3123	72.4343	87.9226
15	23.1806	72.5673	74.6217
16	23.1825	72.5650	73.1057
17	23.1861	72.5606	72.9676
18	23.2390	72.4913	77.0871
19	23.1904	72.5547	75.3572

Table 2 Collection sources of commercially traded samples of *C. intybus* seeds.

Sl. No.	Names of the trades
1	Sri Vinayaka Enterprises, Patan, Gujarat
2	Gopi Krishna Seed Corporation, Haily Mandi, Gurgaon, Haryana
3	R.S. International, New Delhi
4	Vivan Herbals and Healthcare, Ahmedabad, Gujarat
5	Nathimal Rugan Mal, New Delhi
6	Harshita Enterprises, New Delhi

3 Results

3.1 Organoleptic and macroscopic characters

The seeds are obconical, in various shades of creamish to brown to darker; 2-4 mm long and 1-2 mm wide in tips; and slightly bent and angled, with longitudinal ridges (Fig. 1). The base is narrow and acuminate but the ends are slightly flat. Both the base and the apices are truncate. Vertically, seeds are angular, faintly pentagonal to ovoid, with 5 conspicuous and up to 9 inconspicuous ridges. The seeds do not have any characteristic or notable aroma. The surface is rough with many papilla hairs surrounding the broad tip. The tip or upper portion of the seed is broad, crowned with a ring of short chaffy scales that form pappus. The sterile or immature achenes are as large as the mature ones; look slightly shriveled with more wrinkles; and usually pale or straw colored. The pappus in the tips is obtuse, biseriate, 0.5 to 0.7 × 0.1 to 0.3 mm in size and has persistent scale like appearances. The commercial samples procured from various suppliers were similar to the seeds collected from the botanically authentic plants; however, they were admixed with negligible quantity of other floral parts like tepals and young, white to pale colored, shrivelled and smaller-sized seeds. The seeds that were imported were used for harvesting roots too, which do not differ from the locally collected seeds.

3.2 Microscopical characters

The cross-section of the seeds showed circular or elliptic or polygonal outline with 5 conspicuous ridges (Fig. 2). It showed outer pericarp followed by seed testa, endosperm, and innermost core, which was occupied by cotyledons (Fig. 3-4). The thickness of the seeds at the ridges varied between 0.6 and 1.0 mm and 0.4 and 0.6 mm at furrows. The pericarp was lignified and differentiated into outer epicarp, middle mesocarp, and inner endocarp. The epicarp was single-layered, made of square-shaped, thick-walled cells covered with cuticle and showed appressed rows of achenial trichomes. At the anterior tip of the seed, the pappus hairs were about 80-130 µ long, and most of them were filled with dark contents (Fig. 5). The mesocarp consisted of 3-7 layers of cells at groves and 6-13 layers at ridges, and it was thick-walled, circular or elliptical or polygonal, lignified, sclerenchymatous with a various-sized lumen. Almost each cell had 10-15 µ thick prismatic calcium oxalates

(Fig. 6). The mesocarp was followed by a single layer of thick-walled, lignified endocarp layer. The seed testa was square-shaped, 13-35µ thick with thin walls around. Inside testa, there were a few layers of small-sized, elongated, thin-walled, almost crushed cells, which was the endosperm. The outermost layer of endosperm had aleurone grains. The cross-section of cotyledon was slightly hull shaped in outline (Fig. 7), and showed 1-3 layers of elongated, compactly arranged palisade cells, with aleurone grains and oil droplets in the flattened surface or the adaxial surface. The semi-arch side or the outer side or the abaxial surface had polygonal spongy cells, often with aleurone grains and oil droplets. The vascular bundles were traversed in many places. There were no notable differences with respect to the anatomy among the seeds collected from different geography and various suppliers. There were no differences with respect to organoleptic, macroscopy and microscopy of the seeds among the geography and various market samples.

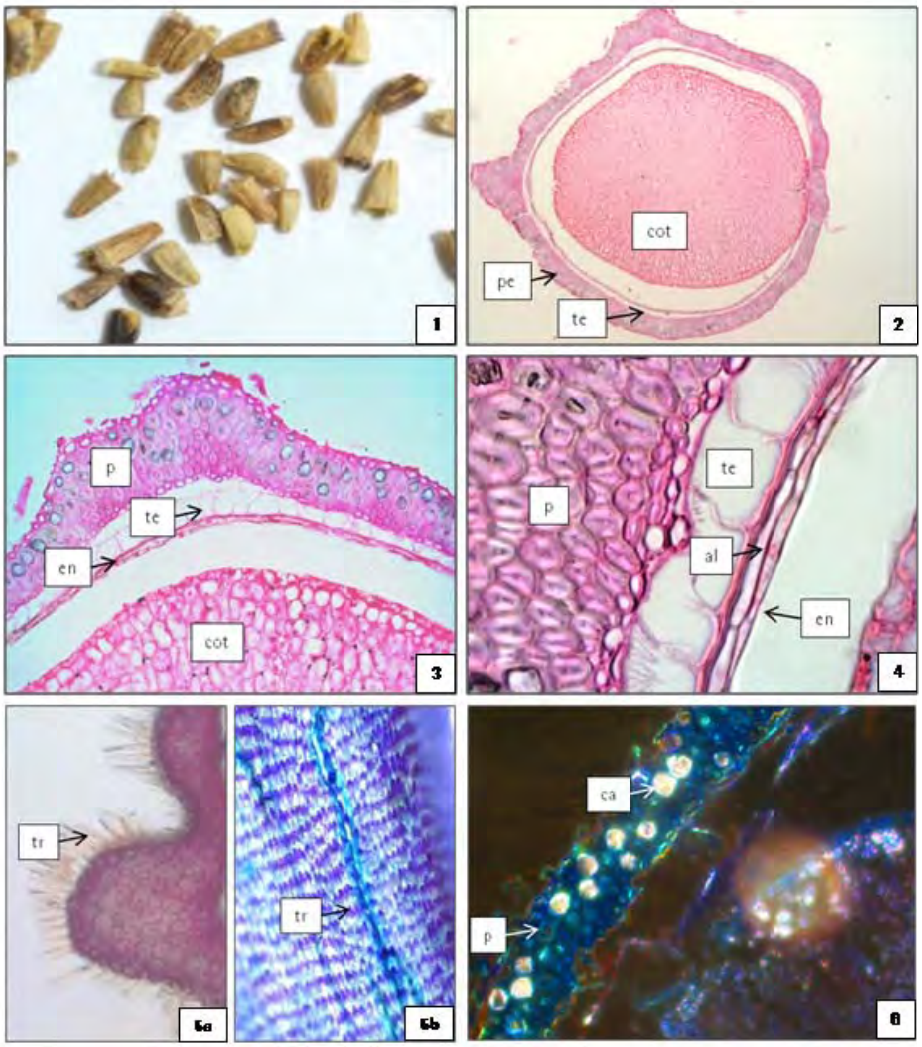

Fig. 1 The samples of *C. intybus* seeds; **Fig. 2** The cross-section of *C. intybus* seed shows pericarp (pe) testa (te) and cotyledons (cot); **Fig. 3** The cross-section of the seed stained with safranin showing the outer pericarp (p) with thick-walled, lignified compactly packed, circular or elongated or pentagonal cells, followed by testa (te), endosperm (en), and cotyledons (cot); **Fig. 4** An enlarged portion of the cross-section of the seed stained with safranin showing the pericarp (p), testa (te), and endosperm (en) cells with aleurone layer (al); **Fig. 5a** The cross-section the seed stained with safranin showing the trichomes (tr) on the epidermis. **Fig. 5b** Achenial trichomes arranged in rows, appressed to the seed coat in the direction of the pappus; **Fig. 6** The cross-section of the seed showing calcium oxalate (ca) crystals in pericarp cells (p), observed under polarizer.

Fig. 7 The cross-section of a whole cotyledon and a portion enlarged, stained with toluidine blue showing the outer epidermis enclosing multilayered palisade cells (pc) on adaxial surface and compactly packed spongy cells (sc) on the abaxial surface.

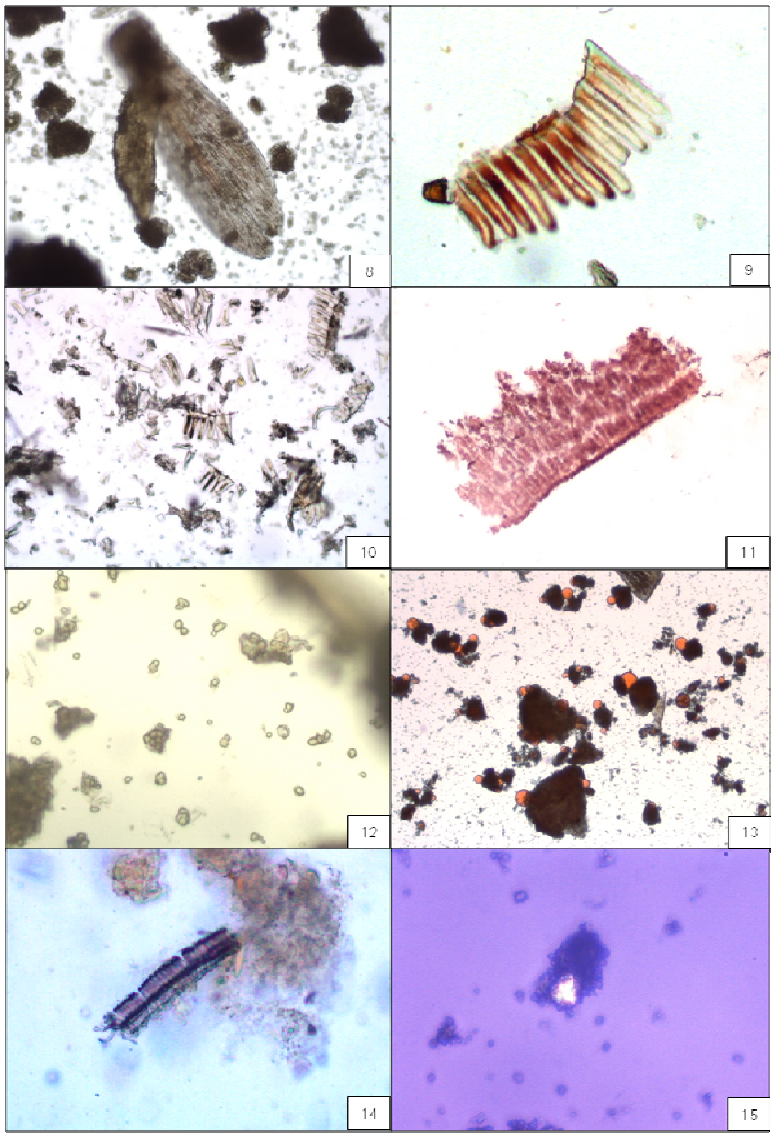

Fig. 8 The powdered material showing papilla from the anterior portion of the seed; **Fig. 9** The powdered material showing nonglandular trichomes; **Fig. 10** The powdered material showing the achenial trichomes from its seed surface with dark contents; **Fig. 11** The powdered material showing cotyledon cells; **Fig. 12** The powdered material showing aleurone grains; **Fig. 13** The powdered material stained with sudan-III showing oil globules; **Fig. 14** The powdered material showing spiral vessel elements; **Fig. 15** The powdered material observed under polarizer showing glittering calcium oxalate crystals.

3.3 Powder microscopy

The powdered material was creamish to brown, soft, oily, and sticky, without any characteristic odor. The microscopical observations of the powdered material showed papilla hair from the anterior portion of the seed (Fig. 8) and non-glandular achenial trichomes from seed surface are frequent (Fig. 9-10). Cotyledon cells (Fig. 11), aleurone grains (Fig. 12), oil globules (Fig. 13) and spiral vessel elements (Fig. 14) are frequent. Calcium oxalate crystals are also present here and there (Fig. 15).

4 Discussion

Cichorium intybus is a commercial herb, the green leaves, roots, and seeds of which are used for various purposes. Although it was reported to be obtained from the wild source (Ved, 2007), most of the commercial material was obtained from commercial cultivation. Mandal et al. (2018) described that the seeds are creamish to brown in color, whereas they are mixture of various shades of creamish to brown to darker throughout the samples we have collected. Jana and Mukherjee (2014) had described the structure of cypselar trichomes of 7 species of Asteraceae (Jana and Mukherjee, 2014), where they described that *C. intybus* seeds possess non-glandular, papillate trichomes that were adpressed with the surface, these results were concored with our observations. Cihan and Palser (1982) reported that there were large prismatic crystals in the pericarp regions as conspicuous elements at the maturity and the *C. intybus* has the thickest pericarp among the species studied and our observations were comparable (Cihan and Palser, 1982; Pandey et al., 1978). In 2018, Mandal et al. reported that the endocarp and the testa are fused and cannot be differentiated in achenes but, these 2 layers can be distinguished as endocarp with single-layer, thick-walled, lignified cells and the testa with thin-walled, nearly square-shaped cells. The current study reveals the basic botanical pharmacognostical details of *C. intybus* seeds with the organoleptic, macroscopic, and microscopic details, which help identify this important species from closely related species of Asteraceae.

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