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Morphological and microscopic studies on fruits of *Drooping Fig* [*Ficus semicordata* Buch.-Ham. ex Sm.]

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ABSTRACT

Ficus semicordata Buch.-Ham. ex Sm. is a small tropical tree used as food, fodder, and medicine in India's different parts. Its fruits are rich in flavonoids, phenols, tannins, saponins, anthraquinone, and glycosides. They are used to treat several

health issues, including abdominal problems, urogenital problems, colic pain, visceral obstructions, diabetes, jaundice, leprosy, hepatitis, etc. The present study aimed at the detailed macroscopic and microscopic characterization of fruits of *F. semicordata*. Morphological, anatomical, and powder characters of fruits were studied using a compound microscope and stereomicroscope. Morphological study of fruit revealed characteristic protruded dark brown spot like structures on the fruit surface, orifice covered with triangular-shaped bracts, and cut surface showed several pink to silver-colored feebly stalked seeds attached to thin creamy white to pink colored fruit wall. Powder microscopy revealed numerous unicellular trichomes, some colored cork cell fragments, seed testa fragments, etc. Botanical characters compiled in the present study can be used as a reference standard for identifying raw fruit samples of *F. semicordata* in both fresh and dried forms.

Keywords: Drooping fig, Macroscopic study, Microscopic characterization, Bhui goolar, Khaina, Figs.

INTRODUCTION

Ficus genus belonging to the Moraceae family (in Ficeae tribe), is one of the largest genera (about 1100 species in 37 genera worldwide) known for woody trees, shrubs, vines, epiphytes, and hemiepiphytes with its various species occurring in tropical and temperate regions in Indian and adjoining continents up to an altitude of 1200 m asl (Dhyani and Khali, 1993; Clement and Weiblen, 2009; Berg, 2001; Somashekhar et al., 2013; Arab et al., 2019). It is considered a

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very important genus with many species known for their food, fodder, economic (lac/silk, jelly/jam, fiber, fuelwood, small timber, etc.), medicinal (ethnomedicinal value and in Ayurveda), and religious values (in Buddhism, Hinduism, and Jainism) (Dhyani and Khali, 1993; Lansky and Paavilainen, 2012; Somashekhar et al., 2013). Different plant parts (leaf, stem, stem bark, fruit, latex, root, etc.) of several *Ficus* species are reported to be used to treat several health ailments in different parts of India (Pande et al., 2007; Hazarika et al., 2012; Murugan et al., 2013; Hazarika and Pongener, 2018; Athokpam et al., 2014). Figs are globally well known for their edible and medicinal uses. Fruits of *Ficus semicordata* Buch.-Ham. ex Sm. are known to have medicinal and edible values. Fruits of *F. semicordata,* being wild and easily accessible, are considered a cheaper nutrition source for poor people. Its fruits are reported to have high protein content, fat, vitamin A, iron, zinc, and phosphorous (Gupta and Acharya, 2019). Fruits of *F. semicordata* are also rich in flavonoids, phenols, tannin, saponin, carbohydrates, alkaloids, lignins, anthraquinone, and tannin, glycosides, and carbohydrates (Gandhi et al., 2019).

Ficus semicordata is known to occur along sub-Himalayan tracts (Bakshi et al., 2001) to an altitude of up to 1200 m asl (Kunwar and Bussmann, 2006). It is commonly known as Drooping fig, Bhui goolar, Khanayo, Khaina, Dumur, Lata dumur, and Aminsep (Kaur et al., 2016; Khatun et al., 2016; Hazarika et al., 2016). A total of eight synonyms are available at www.theplantlist.org, including *Ficus conglomerata* Roxb., *Ficus cunia* Buch.-Ham. ex Roxb., *Ficus hapalophylla* Kurz, and *Ficus semicordata* var. *conglomerata* (Roxb.) Corner. It is used as a fodder for goats and cattle (Amatya, 1996). Leaves are commonly reported as fodder, fruits for edible and medical purposes in India's different regions (Khatun et al., 2016; Murugan et al., 2013; Sajida and Barua, 2011; Shin et al., 2018). Among medicinal uses, different parts are reported with antibacterial and antioxidant properties (Gupta and Acharya, 2019). Fruits, leaves, and stem bark are reported to have antifungal activity (Gandhi et al., 2019), and fruits are reported with antioxidant properties (Tamuly et al., 2015). Gupta and Acharya (2018) compiled ethnomedicinal uses of different parts of *F. semicordata*. In different regions of India, fruits are used for the treatment of a variety of health-related problems, including abdominal problems, ulcers, visceral obstructions, diabetes, colic pain, jaundice, hepatitis, etc. (Gupta and Acharya, 2018). Fruits are used in diarrhoea and relieve headaches (Manandhar, 1992; Bhattarai, 2002; Shashi and Acharya, 2018) and constipation and indigestion (Dhami, 2008). Fruit and bark are used in urogenital problems (Hazarika et al., 2016), leprosy (Kaur et al., 2016), and aphthous complaints (Murugan et al., 2013).

Ficus genus, characterized by hypanthodium inflorescence, is known as one of the largest genera with several species. Fruit samples of *Ficus* (in Hypanthodium inflorescence) are characterized by the presence of fleshy receptacle enclosing several males and female flowers topped by ostiole with an opening surrounded by bracts (Kumari and Rani, 2020). The identification of herbal samples of different species belonging to the same genus is comparatively difficult. The species differentiation is generally complex

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based on fruit morphology. However, 'Figs' reported displaying many variations in fruit shape and size, fruit wall thickness, fruit wall (the composition of ground tissue), flowers in an inflorescence, etc. (Berg and Corner, 2005; Fan et al., 2019; Clement and Weiblen, 2009; Oyama and Souza, 2011). In the herbal drug industry, herbal samples used for herbal preparations are generally procured in dried and unorganized form. Identifying crude herbal samples may require a botanical reference standard for correct identification of raw herbal samples. Because of the similarity in Figs or Hypanthodium inflorescence of different *Ficus* species, the present study aimed at the detailed macroscopic and microscopic characterization of raw fruit samples of *F. semicordata* using stereomicroscope and compound microscope. Botanical characters summarised in the present study can be used as a future reference standard for identifying and authenticating fruit samples of *F. semicordata* in both fresh and dried forms.

MATERIAL AND METHODS

Plant material was collected from two different locations of the Union Territory of Jammu & Kashmir (Table 1). Aerial plant specimens were collected to prepare herbarium sheets, and fruits were collected for crude drug specimens and botanical studies. Herbarium sheets were prepared following standard herbarium procedures (Rao and Sharma, 1990). Duly identified herbarium sheets were submitted to the internationally recognized Janaki Ammal Herbarium (RRLH) at the Indian Institute of Integrative Medicine (CSIR-IIIM), Jammu. Oven-dried fruit samples were submitted to the Crude Drug Repository at CSIR-IIIM Jammu. Botanical studies involved the study of macroscopic and microscopic characters (2-5 samples of each accession) using a stereomicroscope (LEICA S9i) and compound microscope (Leica DM 750).

Table 1. Details of the studied plant specimens.

Location	Herbarium accession number	CDR accession number	Geographical coordinates	Altitude (masl)
Nandini Wildlife Sanctuary, Udhampur district, UT of J&K, India	RRLH-23724	4154	32°50.674 N 074°56.660 E	529
Bhinipul, Billawar, Kathua, UT of J&K, India	RRLH-24344	4221	32°33.350 N 075°32.190 E	585

For a macroscopic study, the shape, size, surface appearance, color, and texture of fruit samples were studied. To study cut fruit features, freshly collected fruits were cut longitudinally (through the ostiole and peduncle) and transversally (across mid-region). The microscopic study involved studying anatomical characters of transverse sections (T.S.) of the fruit wall (from the middle part of the fruit) and pedicel. Thin T.S. of the fruit wall and pedicel were obtained by freehand sectioning using a razor blade. Transverse sections of fleshy fruit wall were observed in water-mounted slides, while T.S. of pedicel was stained in safranin and fast green, according to Kumar

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et al. (2020b). The final sections were observed under a compound microscope with an associated camera (LEICA ICC50E). For microscopic powder analysis of fruit samples, oven-dried fruit samples were crushed to a fine powder and were observed in water mounted slides under a compound microscope. An iodine test was performed to detect the presence of starch grains in fruit powder.

RESULTS

Botanical description

Ficus semicordata is a small to medium-sized tree, up to 15 m tall and up to 2 m in circumference with a spreading irregular crown (Figure 1A). Leaf-blade 5-15 cm long, 2-5 cm broad, rough on both sides, mostly elliptic to oblong, lance-shaped, coarsely toothed leaf margin with tapering tip and characteristic unequal base (Figures 1B). Figs are present on underground branches embedded in soil (Figure 1C) and also on aboveground branches.

Macroscopic characters of fruit

Fruit occur in inflorescence, also called as hypanthodium or fig. They are spherical, globular to pear-shaped. Freshly collected ripe fruits are slightly yellowish with brown spots on the surface with a size range of 17.93×17.46 mm to 22.72×19.98 mm (Figures 1D, E). Dried fruits brown, surface rough, wrinkled, or corky with a spot like protrusion over the surface (Figures 1G, H); with small stout pedicel at the basal region (4-6 mm) (Figure 1D, G); white hairs on young figs (Figures 1G). The apical opening called 'orifice' is guarded by triangular-shaped hairy bracts present in alternate patterns at the fruit (compactly packed or separated from each other) (Figures 1F, I).

Cut surface of the fruit

The longitudinally cut surface of fresh ripe fruit (across the orifice to peduncle) appeared slightly oval-shaped (Figure 1J), while the transversally cut surface appeared nearly circular (Figure 1K). Cut ripe fruit was observed with several small fruit-lets enclosed in creamish colored, fleshy, thick fruit wall (2.5-4.0 mm thick). Fruit wall comparatively thicker near the peduncle and orifice region. In the fruit's cut surface, several small, oval-shaped, compactly packed reddish coloured flowers or florets or seeds were observed with thin, fleshy, long, or short stalks attached to the fruit wall (Figure 1L, 2B, C). The dried mature fruit wall's inner side appeared silver coloured with several seeds present in papery hair-like structures (Figure 1M). Mature dried seeds oval, brown, covered by papery covering with beak-like pointed protrusion (Figure 1N, O).

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Figure 1: Morphological studies on fruit samples of *F. semicordata*; A). Plant habit, B) Leaf morphology, C) Fruits on the underground stem; fruit samples: D) Fresh, G) Dried; Fruit surface: E) Fresh, H) Dried; Bracts covering orifice, F) Fresh, I) Dried; J) Longitudinally cut fruit, K) Transversally

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cut fruit, L) Fruit-lets morphology, M) Dried fruit inner surface, N, O) Seeds morphology.

Microscopic characters of fruit

In the microscopic study, T.S. of the fruit wall and of pedicel were studied for an anatomical arrangement of cells and tissues.

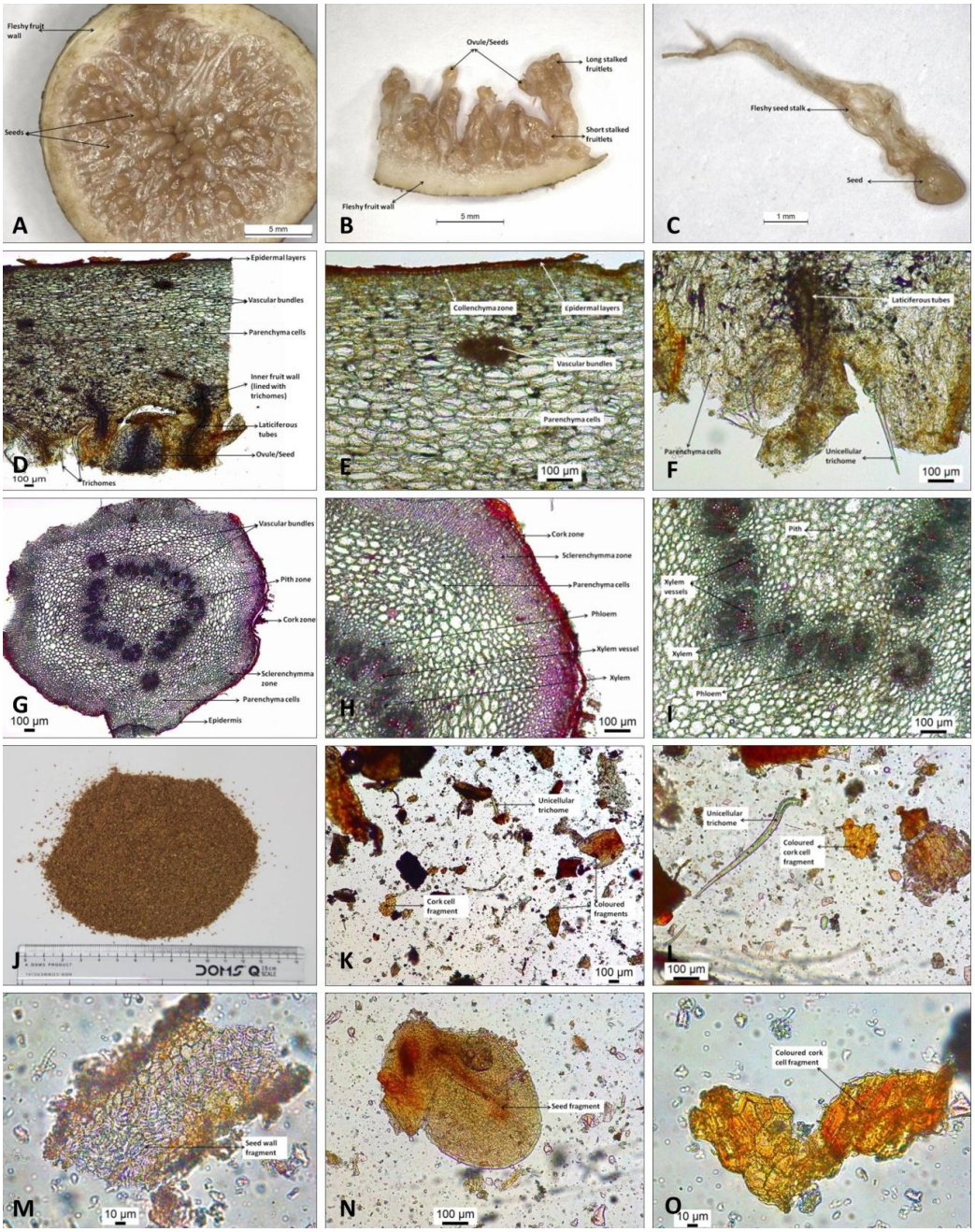

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Figure 2: Microscopic studies on fruit samples of *F. semicordata*; A). Ripe fruit cut view, B) Cut fruit wall with attached seeds, C) Seed morphology; T.S. of fruit wall: D) Whole view of the section, E) Outer region, F) Inner region; T.S. of peduncle: G) Whole view of the section, H) Outer region, I) Pith region; J) Powder sample, K-O) Microscopic studies.

Anatomy of the fruit wall

The transverse fruit wall section was observed with outermost few broken cork patches and continuous, compactly packed, dark-colored epidermal cell layers. Epidermal layers followed by parenchymatous multilayered (45-50 layered) cell layers (Figure 2D). In the parenchymatous zone, few compact, randomly distributed, small patches of vascular bundles (116.432±17.01×194.24±28.29 µm) were observed (Figure 2D, E). The inner side of T.S. is irregular in outline, observed with oval-shaped seeds enclosed in cavity-like structure, few unicellular trichomes, and few dark-colored elongated laticiferous tubes (Figure 2F).

Anatomy of pedicel

The transverse section of the pedicel appeared nearly irregular circular in outline with clearly differentiated cork, sclerenchyma, parenchyma, vascular, and pith zones (Figure 2G). The outer zone being cork layer thin (50.26±4.32 µm), with broken, dark-colored with compactly packed cells was followed by 5-7 cell thick sclerenchyma zone (162.04±16.48 µm),

then by 15-18 cell wide parenchymatous with few light-colored fragments (Figure 2J), odor slightly characteristic, texture granular, and with a slightly characteristic taste. Microscopic study of fig powder showed the presence of several elongated, pointed, unicellular trichomes (mid-region thickness of 31.37±3.10 µm) (Figure 2L), few seed testa fragments (Figure 2M), coloured oval shaped seed fragments (Figure 2N), few golden yellow fragments of cork and other tissues (Figure 2O) and few prismatic calcium oxalate crystals (Figure 2K-O). No xylem or phloem cells or sclerenchymatous cells were observed in powder. Starch was not detected in the iodine test.

zone (408.02±11.69 µm thick) with compactly packed, slightly elongated to oval-shaped cells (Figure 2H). The center region formed a nearly circular-shaped vascular ring interrupted by thin parenchymatous cell layers (and with two vascular extensions). The vascular region formed a thickness of 216.84±9.27 µm and followed by a central circular-shaped pith with a radius of 339.40±13.33 µm. Pith cells are compactly packed and nearly oval-shaped (Figure 2I).

Powder study

In the powder study, organoleptic and microscopic characters of fruit powder were studied. Fruit powder was greenish-brown

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Table 2. Quantitative macroscopic and microscopic characters of the T.S. of the fruit of *F. semicordata*.

Character	Parameter	Min	Max	Mean (±S.D.)
Fruit size				
Fresh fruit	Length (mm)	17.93	22.72	19.86±0.50
	Breadth (mm)	17.46	19.98	18.16±0.43
Dry fruit	Length (mm)	13.42	16.07	14.40±0.23
	Breadth (mm)	12.28	13.90	13.16±0.21
Fruit wall (T.S.)				
Parenchyma cell size	Length (µm)	59.54	114.96	85.231±4.94
	Breadth (µm)	31.94	64.28	47.868±3.00
Vascular bundle size	Length (µm)	390.76	76.97	194.24±28.29
	Breadth (µm)	260.69	68.25	116.432±17.01
Pedicel (T.S.)				
Parenchyma cell size	Length (µm)	28.73	53.89	44.63±2.48
	Breadth (µm)	17.58	42.27	26.55±2.17
Pith cell size	Length (µm)	21.92	43.11	31.67±2.64
	Breadth (µm)	18.18	35.29	24.59±1.74
	Vessel diameter (µm)	4.64	10.78	7.51±0.64
Tissue zone thickness	The radius of studied T.S. (µm)	1102.37	1303.68	1227.19±24.89
	Cork thickness (µm)	36.70	80.08	50.26±4.32
	Sclerenchyma thickness (µm)	93.10	272.86	162.04±16.48
	Parenchyma thickness (µm)	364.7	487.22	408.02±11.69
	Vascular region thickness (µm)	173.82	257.68	216.84±9.27
	Pith radius (µm)	285.36	405.23	339.40±13.33
Powder study	Trichome thickness (µm)	18.06	51.60	31.37±3.10

DISCUSSION

In the herbal drug industry, correct identification and authentication of herbal drugs are considered essential to ensure herbal medicines' quality and safety (Shinde et al., 2009; Sahoo et al., 2010). Species in the same tribe or family may appear similar in resemblance and are comparatively difficult to identify (Khan et al., 1996). Microscopic studies are known to help in the botanical identification of herbal samples (Metcalfe and Chalk, 1957). Botanical identification methods of herbal drugs involving macroscopic and microscopic studies vary for different plant parts. Botanical studies have been performed for the identification of raw herbal samples. In several botanical based herbal drug standardization studies, some macroscopic characters (such as shape, size, surface appearance, color, texture, etc.); some anatomical characters including the thickness of various tissue zone to the total size of studied cross-section; cell size of epidermal, vascular and ground tissues; the appearance of vascular bundles, lumen diameter of xylem vessels; some powder characters such as shape and size of starch grains, crystals, trichomes, etc. have been observed significant for characterization and identification of different types of raw herbal samples (Lens et al., 2008; Fritz and Saukel, 2011; Manohan et al., 2013; Sereena and Sreeja, 2014; Hassan et al., 2015; Ginkgo et al.; 2016; Ya'ni et al., 2018; Kumar et al.,

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2018; Park et al., 2019; Kumar et al., 2020a; Kumar et al., 2020b; Singh et al., 2020).

For fruit and seed samples, morpho-anatomical characters such as shape, size, color, external appearance, coat surface, some other special characters or combination of characters are known as significant in taxonomic identification of a different group of plants (Khafagi et al., 2018; Barkatullah et al., 2014; Zoric et al., 2010; Srivastava et al., 2006; Kalsoom et al., 2019). In the Moraceae family, morphological and anatomical characters of flower, fruit, and seeds are considered helpful in studying phylogenetic relationships at the generic and family level (Arab et al., 2019). Morphological and anatomical studies are available on some fruit samples belonging to the Moraceae family (Oyama and Souza, 2011). Botanical studies, including macroscopic and microscopic characterization of fruit samples, are available for *Ficus hispida* (ICMR, 2008).

In *F. semicordata*, macroscopic and microscopic studies are available on the stem, stem bark, and leaf samples (Shashi et al., 2019; Gupta et al., 2020). However, detailed botanical studies of fruits of *F. semicordata* are scarce. The present study involved macroscopic, anatomical, and powder studies on fig samples of *F. semicordata*. In previous botanical studies, various characters of figs such as shape, size, the surface appearance of fruit and seeds, orifice features, receptacle characters, fruit-lets characters, epidermal, ground tissue and vascular characters in cross-sections of pedicel and receptacle, laticifers, crystals, etc. were used in the morpho-anatomical description of fig (ICMR, 2008). Fan et al. (2019) studied comparative anatomical characters of the inflorescence of 22 *Ficus* species and observed some botanical characters such as fig diameter, longitudinal cut surface passing through ostiole and peduncle, fig wall thickness, cross-section characters such as epidermal cells, ground cells, vascular tissue characters, trichomes, lenticels, laticifers, druses, etc. as variable features in different species of *Ficus*. In the present study, various qualitative and quantitative characters, including shape, size, color, surface features of fruit and seeds; anatomical characters of fruit, transverse section of fruit wall and pedicel; and organoleptic and microscopic characters of powder sample were observed helpful in species characterization. Fan et al. (2019) observed fruit wall thickness as positively correlated with a fig's size. In the present study, the fruit wall was comparatively thicker near the orifice and pedicel region. Morpho-anatomical and powder characters compiled in the present study can be used as a reference standard in future identification of raw fruit samples of *F. semicordata*.

CONCLUSION

A combination of macroscopic and microscopic characters described in the present study can help taxonomic studies identify fresh and dried raw fig samples of *F. semicordata.* Studied morpho-anatomical characters of fruit wall, fruit surface characters such as the presence of brown spots, alternate triangular-shaped bracts over the orifice, anatomical features of fruit wall and peduncle, and powder features (coloured cork cell fragments, coloured oval shaped seed fragments, unicellular trichomes, few prismatic crystals, absence of starch grains, etc.), were described for fruits

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of *F. semicordata*. Botanical characters compiled in the present study can be used as a reference standard for identifying fruit samples of *F. semicordata* in the herbal medicine industry and in comparative taxonomic studies with various other *Ficus* species.

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