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BOTANICAL STUDIES ON RAW HERBAL SAMPLES OF *WOODFORDIA FRUTICOSA* (L.) KURZ- AN IMPORTANT AYURVEDIC PLANT

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ABSTRACT: Raw herbal samples used in the herbal medicine industry need to be properly identified for use in an herbal preparation. Plant parts of *Woodfordia fruticosa* (L.) Kurz belonging to the Lythraceae family, apart from some commercial uses, is known for its medicinal value in ethno medicines and various Indian traditional medicine systems, including Ayurveda. Flowers are reported high trade value (2000-5000 MT), are used in some Ayurvedic formulations such as Atisara, Raktapitta, Trsna, Vrana, Visarpa, Arjunarishta (Parthadyarishta), and Partharishtam. The present study aimed at botanical characterization and identification of raw leaf, flower, and stem bark herbal samples of *Woodfordia fruticosa*. Macroscopic and microscopic characters were studied using stereomicroscope and compound microscope. The morpho-anatomical description was provided for flower, leaf, and stem bark samples. Anatomical study of a leaf with a crescent-shaped vascular bundle, three different types of trichomes, and bark with linearly arranged rosette crystals crossing uniseriate medullary rays longitudinally were observed as characteristic features. Powder organoleptic and microscopic characters were described for each studied herbal sample. Characters compiled in the present study can be used as reference standards for future identification of raw leaf, flower, and stem bark samples of *Woodfordia fruticosa*.

INTRODUCTION: *Woodfordia fruticosa* (L.) Kurz belonging to the family Lythraceae, occurs in tropical and subtropical parts throughout India, especially in the Himalayas and Gangetic plains up to an altitude of 1500 m asl; and also cultivated in gardens [1, 2]. It is commonly known as Fire flame bush, Dhavi, Dhaatkikephool, Shiranjitea, Thawi, and several other names [3].

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Trade names of flower samples are Dhaiphool, Dhavadiphool, Dhataki; and are reported with a high annual trade value of 2000-5000 metric tonnes in Indian herbal market [4]. Commercially, flowers known to yield a red dye used to color silks [3].

Leaves are reported to yield pink and red dye (due to the presence of Lawsone, 2-hydroxy naphthoquinone)5, milk enhancement in livestock [6] and also in perfume, leather, and textile industries [7]. Different plant parts of *W. fruticosa* especially flowers, stem bark, and leaves are reported with medicinal importance. Flowers known used as astringent, antipyretic, appetizer, blood purifier, used in dysentery, diarrhea, leucorrhoea, skin problems, fever, asthma, liver disorder,

rheumatism, menorrhagia, and inflammatory conditions [8-13]. Flowers are known to have antibacterial [14, 15], antiviral (antiEV71) [16,] hepato-protective [17, 18], immunomodulatory [19,] antihyper-glycemic [20], antifertility [21], antitumor activity [22], cytotoxicity, anti-inflammatory, and analgesic properties [23]. Flowers of *W. fruticosa* are known used in Ayurvedic formulations including Arjunarishta (Parthadyarishta) [24], in Ayurvedic drug 'Partharishtam', [25] and other Ayurvedic formulations including Atisara, Raktapitta, Trsna, Vrana, Visarpa and known with therapeutic uses of Atisara, Raktapitta, Trsna, Vrana, Visarpa [2]. Flowers are the key ingredient used in the alcoholic preparation of "Asavas-Arishtas"[26]. Several species of yeast (such as *Pichia anomola*, *Aspergillus niger*, and *Saccharomyces cerevisiae*, *etc.* have been reported from *W. fruticosa* [27, 28]. Stem bark was reported used in jaundice [29], diarrhoea [30]. Leaves are used as disinfectant [31, 32], used in fever [15, 33], rheumatism [34], hemoptysis [35], ulcers [36,] and in gall bladder problems [12]. Leaves are known to have anti-microbial compounds [37], stem bark with analgesic activity [38]; leaves and stem bark with antibacterial [39] and antidiabetic activity [40].

Various parts of *W. fruticosa* are reported to have tannins, such as in bark (20-27% tannins), flowers (24.1% tannins), and leaves (12-20% tannins) [41]. Chemically, leaves are known to have flavonoids [42], essential oil [43,] and phenolic compounds [44, 45]. Leaves and flowers are known to have polyphenols [46], flowers have tannins, phytophenols, anthocyains [47] and Woodfordina ABC (tannins) [48], bark with C-glucoside and bergenin [9]. In the herbal drug industry, proper identification and authentication of raw herbal samples are essential to ensure the quality, safety, and efficacy of herbal medicines [49-51]. Several pharmacopeia monographs are known to use macro-morphological and organoleptic characters of herbal drugs in the correct identification of species [52]. Botanical identification methods are considered as simple, easy, time, and cost-effective methods in the correct identification of raw herbal drugs [53-54].

The present study involved detailed qualitative and quantitative characterization of macroscopic and microscopic features of the leaf, stem bark, and flower samples. Botanical characters compiled in the present study can be used as reference standards for future identification of raw herbal samples of *Woodfordia fruticosa* used in herbal medicines preparations.

MATERIAL AND METHODS: Plant material was collected from two different locations of the U.T. of J&K's **Table 1**. Plant material was collected for herbarium sheet preparation, for raw crude herbal samples, and for botanical studies. Herbarium sheets were prepared following standard herbarium procedures [55]. Duly identified herbarium sheets were submitted to internationally recognized Janaki Ammal Herbarium (RRLH) at the Indian Institute of Integrative Medicine (CSIR-IIIM), Jammu. Oven-dried raw herbal samples (of flowers, stem bark, and leaves) were submitted to the Crude Drug Repository at CSIR-IIIM Jammu. Herbarium and crude drug accession numbers have been provided in **Table 1**.

In botanical studies, macroscopic and microscopic characters of flower, stem bark, and leaves were studied using stereo-microscope (LEICA S9i) and compound microscope. For anatomical studies, transverse sections (T.S.) of leaf and stem bark samples were obtained by freehand sectioning using a razor blade. Obtained fine sections were stained according to Kumar *et al.,* [56], with some modifications. Thin T.S. were dehydrated in different series of alcohol gradients (30%, 50%, and 70% alcohol, each for 10-15 min), stained in safranin (5-7 min), decolorized in 70% alcohol(5-10 min), staining in fast green (2-3 min) and then were again decolorized in 70% alcohol for 5-10 min. The sections were dehydrated in 90% alcohol followed by absolute alcohol (each for 5-7 min), mounted in Canada balsam, and observed under a compound microscope (Leica DM 750) with an associated camera (LEICA ICC50E). All micro-metric measurements were performed by LEICA LAS V 4.9.0 software. For powder study, samples were crushed to a powder, passed through a fine sieve, and studied in water-mounted slides under a compound microscope. An iodine test was performed to detect the presence of starch grains in powder samples. Organoleptic characters of leaf flower and stem bark samples were also noted.

RESULTS:

Botanical Description: The plant is bushy, spreading, semi-deciduous, perennial, under shrub

or shrub, 1-3 m high, growing on rocky, dry areas in hilly areas **Fig. 1A**. Plants bear several bright red flowers in axillary clusters along the branches and twigs.

Morphological Characters:

Leaf and Flower: Leaves are ovate-lanceolate to ovate, opposite or sub opposite, 8-12 × 2.4-3.5 cm, whitish and tomentose abaxially, texture leathery, margin smooth, base subcordate, apex pointed and slightly curved, petiole nearly absent **Fig. 1L**. Flowers bright red colored, axillary, present in paniculate-cymose clusters (of 3-15 flowers) **Fig. 1B, C**, dried flowers dull red **Fig. 1J**, with short pedicels (0.4-0.6 cm) **Fig. 1D**. Flower buds with 6 angular protrusions, mature flower actinomorphic (appear slightly zygomorphic) with tubular corolla (16-20 mm long, 1.6-2.7 mm thick); sepals and petals six in number, sepals triangular-shaped, small tooth-like protrusions (0.9-1.2 mm), petals are narrowly linear slightly longer than the calyx-teeth (2.5-3.5 mm) **Fig. 1F**, **G**. Stamens are 12 in number, 0.8-1.4 cm, epipetalous, inserted little above ovary base **Fig. 1E**. Pistil size ranged from 1.6-1.9 cm, stigma bifid **Fig. 1H**, ovary bilocular, anthers versatile **Fig. 1I**. Anthers fall off easily from the filament in dried flower samples **Fig. 1D**, **E,** and **2C**.

Stem Bark: The bark is thin (0.5 cm or more in thickness), smooth, reddish-brown colored, freshly peeled bark dark brown on the outer side and light creamy colored on the inner side. Bark surface observed with transverse and longitudinal cracks, peeling off in flakes (near the base of the stem) and in thin and fibrous strips (middle stem region) become curved on drying **Fig. 1K**. Dried bark samples are irregularly curved **Fig. 1K** with the outer surface dark brown colored and inner surface reddish-grey colored.

Microscopic Characters:

Flower: Cut view of flower showed superior and bilocular ovary (0.5-0.65 cm), with several rounded to oval ovules **Fig. 2C**, **F**, style elongated (0.9-1.2 cm), bifid stigma **Fig. 1H**, and with versatile anthers **Fig. 1I**.

Stem Bark: T.S. of bark showed outer flaky, thin cork zone (81.08 ± 6.45 µm) with compactly packed cells. Cork zone followed by a broad parenchymatous secondary cortex zone (166.66 ± 12.43 µm) with oval to slightly transversally elongated thick-walled cells. Cortex followed by a continuous thick phloem zone with phloem cells interspersed in cortex cells. Phloem formed a major part (thickness of 630.59 ± 17.06 µm) in studied cross-section of bark (of a total thickness of 788.64 ± 5.83 µm). Phloem comprised of oval-shaped cells interspersed with vertical medullary rays.

Phloem cells observed with several rosette crystals arranged in a transverse parallel row-like pattern. Medullary rays were nearly uniseriate on the inner phloem and showed dilatation growth (up to 4-10 cells wide) in the outer phloem near the cortical region. The inner zone of the phloem was comprised of the phloem fiber zone, followed by the xylem region consisting of well distinct xylem fibers and vessels **Fig. 2A**, **D**. Quantitative microscopic characters are shown in **Table 2** and **3**.

Transverse section of leaf blade (from midrib region) showed typical dicot leaf anatomy with central midrib region (with a notch in the center) and wing-like extended lamina region **Fig. 2B**. The Lamina region consisted of a single-layered, cuticularised upper epidermis with rectangular-shaped cells. Lamina epidermis consisted of few oval-shaped glandular trichomes and few curved, pointed trichomes with a broad base and abruptly tapering tip **Fig. 2E**. Epidermis followed by palisade layer with compactly packed elongated cells, then by spongy parenchyma zone. The lower epidermis region consisted of several uniformly thickened curved trichomes. The Midrib region consisted of a single-layered cuticularised epidermis followed by an inner collenchymatous patch and then by a broad parenchymatous tissue zone. The vascular zone was crescent-shaped with well distinguishable xylem facing the upper epidermis, followed by a less distinct phloem zone sheathed by a well-differentiated continuous sclerenchymatous zone. Xylem vessels were present in linear rows with comparatively broader vessel lumen diameter towards the abaxial side than the adaxial side. The vascular zone was followed by a broad parenchymatous zone (8-10 cell wide) with rosette crystals in some cells, aninner 2-3 cell wide collenchymatous tissue zone, and a single-layered lower epidermis. Quantitative microscopic characters are shown in **Table 4**.

FIG. 1: MORPHOLOGICAL STUDIES ON RAW HERBAL SAMPLES OF *W. FRUTICOSA*, A). PLANT HABIT, B). FLOWERS CLUSTER ON THE PLANT, C). FRESH RAW FLOWER SAMPLES, D). SINGLE FLOWER MORPHOLOGY, E). THE FLOWER OPENED (SHOWING STAMEN ATTACHMENT TO FLOWER TUBE), F). FLOWER PART SHOWING SEPALS, PETALS, AND STAMEN FILAMENTS, G). SEPAL, PETAL MORPHOLOGY AND FLOWER TUBE, H). STIGMA MORPHOLOGY, I). ANTHER MORPHOLOGY, J). DRIED FLOWER SAMPLES, K). DRIED STEM BARK SAMPLES, L). DRIED LEAF SAMPLES, M). FLOWER POWDER SAMPLE, N). STEM BARK POWDER SAMPLE, O). LEAF POWDER SAMPLE

Powder Study: Organoleptic features including color, odor, texture, and taste of each drug sample (flower, stem bark, and leaf samples) were observed characteristic. Organoleptic characters of flower leaf and stem bark samples are provided in **Table 1**. A microscopic study of powder samples was observed with characteristic features for each drug type. Microscopic powder study of leaf samples was observed with cork cell fragments, non-glandular trichomes, few golden yellow fragments, and few rosette crystals; flower samples with few cork cells, several rounded pollen grains (mean size of 17.64 ± 0.21 × 16.88 ± 0.27 µm), a few unicellular trichomes, few cork cells, and few

prismatic crystals. Microscopic powder study of stem bark sample was observed with cork cell fragments, starch grains (mean size of 12.18 ± 0.74 × 9.71 ± 0.47 µm), and rosette crystals (mean size of 14.58 ± 0.96 × 12.46 ± 0.84 µm). Iodine test revealed abundant starch grains in stem bark powder sample while starch was not detected in leaf and flower powder sample. The mean size and range of starch grains, rosette crystals, and pollen grains in studied powder samples are shown in **Table 3** and **4**.

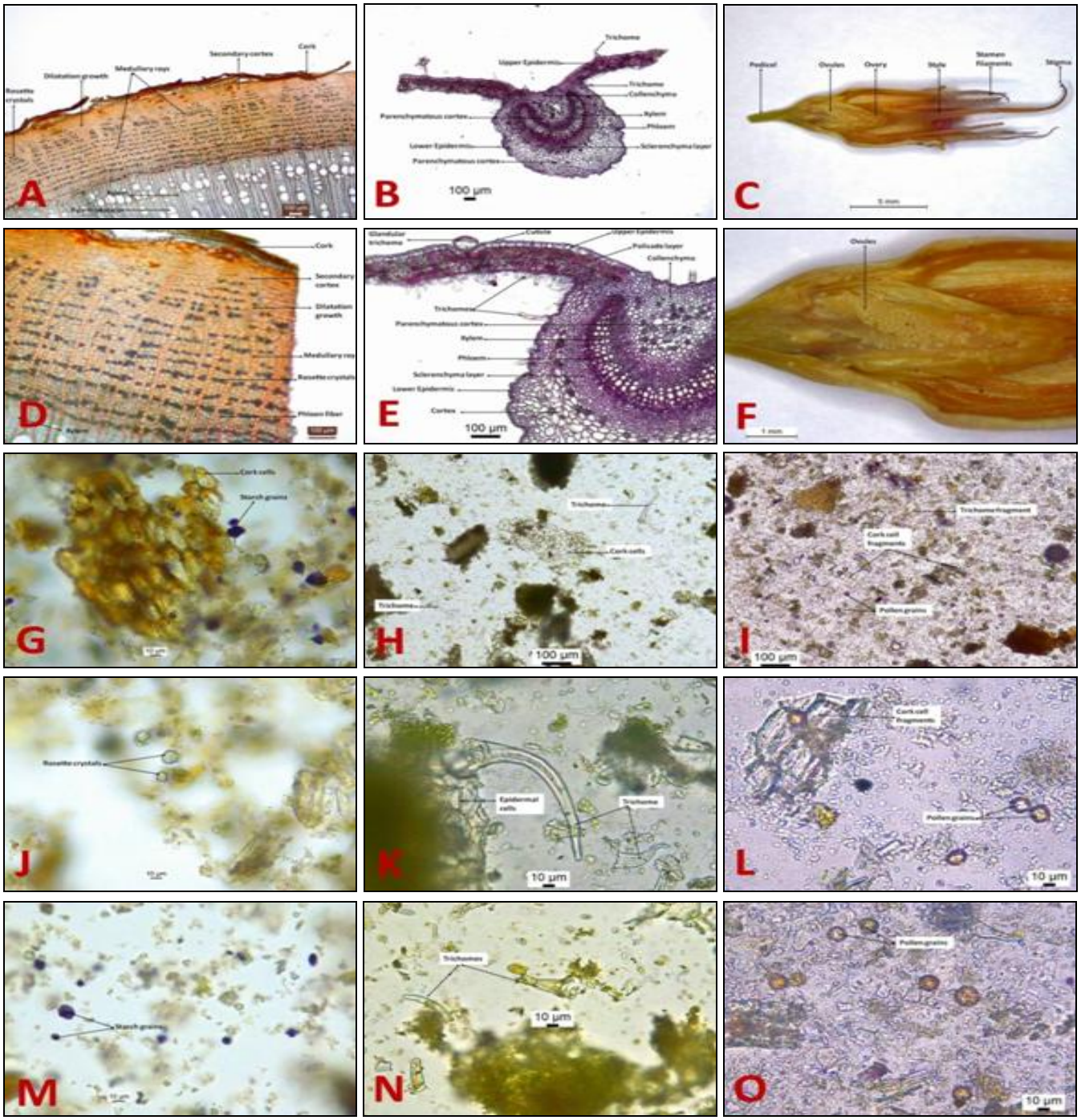

FIG. 2: MICROSCOPIC STUDIES ON RAW HERBAL SAMPLES OF *W. FRUTICOSA*, A). PLANT HABIT, B). FLOWERS CLUSTER ON THE PLANT, C). FRESH RAW FLOWER SAMPLES, D). SINGLE FLOWER MORPHOLOGY, E). THE FLOWER OPENED (SHOWING STAMEN ATTACHMENT TO FLOWER TUBE), F). FLOWER PART SHOWING SEPALS, PETALS, AND STAMEN FILAMENTS, G). SEPAL, PETAL MORPHOLOGY AND FLOWER TUBE, H). STIGMA MORPHOLOGY, I). ANTHER MORPHOLOGY, J). DRIED FLOWER SAMPLES, K). DRIED STEM BARK SAMPLES, L). DRIED LEAF SAMPLES, M). FLOWER POWDER SAMPLE, N). STEM BARK POWDER SAMPLE, O). LEAF POWDER SAMPLE

TABLE 1: COLLECTION DETAILS AND POWDER STUDIES OF DIFFERENT SAMPLES OF *WOODFORDIA FRUTICOSA*

	Collection details of plant samples		
	GPS location	**Herbarium accession number**	**CDR accession number**
Nandini WLS (J&K)	32°50.674N, 074°56.660E (529m asl)	23811	Flower (4174), Stem
Pallan (Billawar, J&K)	32°33.320N, 75°33.751E (633m asl)	23395	(4175 bark), Leaves (4220)
	Powder organoleptic characters		
	Flower	**Stem bark**	**Leaf**
Colour	Soil like brown colored (Figure 1M)	Soil colored (Figure 1N)	Light green to creamish green (Figure 1O)
Odor	Slightly characteristic odor	No characteristic odor	Characteristic odor
texture	Slight granular	Sand like granular	Smooth to slightly rough
Taste	No characteristic taste	Slightly bitter with a rough mouthfeel	Characteristic, slightly bitter

TABLE 2: QUANTITATIVE MICROSCOPIC CHARACTERS OF THE T.S. OF STEM BARK OF *WOODFORDIA FRUTICOSA*

Character	Min	Max	Mean (±S.D.)
	Stem bark (µm)		
T.S. thickness	746.49	818.49	788.64±5.83
Cork thickness	55.36	115.62	81.08±6.45
Cortex thickness	123.66	250.96	166.66±12.43
Phloem thickness	514.12	683.96	630.59±17.06
Intermedullary ray width	19.93	80.08	48.81±6.34

TABLE 3: QUANTITATIVE MICROSCOPIC CHARACTERS OF STEM BARK OF *WOODFORDIA FRUTICOSA*

	Min	Max	Mean (±S.D.)	Min	Max	Mean (±S.D.)
Stem bark cell size (µm)	**Length**			**Breadth**		
Cork	11.69	21.45	17.50±1.06	6.76	16.80	12.16±0.89
Cortex	21.07	43.13	30.08±2.07	9.03	16.36	13.20±0.69
Phloem parenchyma	12.33	22.47	16.07±1.00	10.14	13.65	11.99±0.43
Stem bark medullary ray	474.01	709.03	626.25±30.25	13.30	24.19	17.48±1.08
Starch grains	9.69	16.65	12.18±0.74	7.91	11.91	9.71±0.47
Rosette crystals	9.97	19.13	14.58±0.96	7.29	15.24	12.46±0.84

TABLE 4: QUANTITATIVE MICROSCOPIC CHARACTERS OF FLOWER AND LEAF OF *WOODFORDIA FRUTICOSA*

	Min	Max	Mean (±S.D.)	Min	Max	Mean (±S.D.)
	Flower characters					
Pollen grains	**Equatorial axis**			**Polar axis**		
	16.56	18.57	17.64±0.21	15.63	18.07	16.88±0.27
	Leaf characters					
	Length			**Breadth**		
Upper epidermis (midrib region)	5.85	9.09	7.17±0.32	3.24	6.42	5.08±0.39
Upper epidermis (lamina region)	14.22	23.85	18.66±1.05	12.47	24.5	17.37±1.36
Lower epidermis (midrib region)	5.29	9.87	7.12±0.45	4.86	7.93	6.77±0.30
Adaxial cortical cell size	11.69	24.1	18.48±1.16	8.36	17.31	13.10±0.91
Abaxial cortical cell size	16.26	45.36	26.97±3.21	12.34	34.20	19.99±2.81
Trichome (Curved)	25.10	94.24	50.32±6.88	8.86	18.65	11.66±1.13
Trichome (Straight)	42.42	181.72	79.94±16.22	17.93	43.88	29.65±2.22
Palisade thickness	59.10	73.45	65.28±1.60			
Xylem length	65.75	114.09	93.11±5.13			
Xylem vessel diameter	10.24	29.56	19.20±1.90			

DISCUSSION: Identification of entirely unknown raw herbal samples without a reference standard is considered problematic [57]. Detailed macroscopic and microscopic characterization, including qualitative and quantitative features, can be more useful in the identification of raw herbal samples [54, 58]. Macroscopic and microscopic characterization has been performed in different types of herbal samples such as whole plant [59], heartwood [60]; leaves [61], root [62], rhizome [63], stem bark [64], flowers [26], *etc.* Botanical-based identification methods vary for different plant samples [65]. Anatomical characters have been used for the identification of raw leaf and bark drug samples in several species

[66-68]. Kotina *et. al.,* [68], observed characters such as trichomes, sclereids, secretory canals, druse crystals, brown contents in parenchyma cells as diagnostic microscopic features in the identification and differentiation of raw leaf and bark herbal material from adulterant samples.

For identification of stem bark, macroscopic characters (such as shape, size, surface color, texture, *etc.*), microscopic features (of rhytidome, cork, cortex, ray dilation, sclereids in phelloderm, secondary phloem, phloem fibers, starch grains, the shape of crystals, stone cells, tannins, *etc.*) and powder features were known helpful in species characterization [69-72].

In the present study, botanical identification studies with macroscopic, microscopic, and powder characterization were performed on the leaf, stem bark, and flower samples. Studies performed included descriptions of qualitative and quantitative macroscopic and microscopic botanical characters. Botanical studies with anatomical characterization have been done in some previous studies on leaf samples [73] and flower samples [26, 74, 75]. Leaf anatomical characters observed in the present study also corresponded with anatomical features studied by Birajdar *et al.,* [73] In the microscopic study of flower powder of *W. fruticosa*, Baravalia *et al.,* [75], observed unicellular trichomes, rosette, and calcium oxalate crystals. However, in the present study, rosette crystals were not observed in flower powder microscopic study. Microscopic studies for stem bark samples were described for the first time in the present study. In the present study, the anatomical study of leaf samples revealed some characteristic features, including a notch in the central region of the midrib, crescent-shaped vascular bundle, varied types of trichomes (oval-shaped glandular trichomes; curved and straight non-glandular trichomes). Transverse section of stem bark was observed with uniseriate longitudinal medullary rays (with dilation growth near cortical region) and phloem parenchyma cells with rosette crystals in a transverse arrangement.

Powder study of stem bark was observed with few cork cell fragments, abundant oval to elongated starch grains, and rosette crystals. Starch grains were not detected in leaf and flower powder samples.

CONCLUSION: The present study involved detailed morphological, anatomical, and powder studies with qualitative and quantitative characterization for the raw leaf, flower, and stem bark samples of *W. fruticosa*. Some characteristic features of the leaf (crescent-shaped vascular bundles with rosette crystals in cortex cells), flower (macroscopic, microscopic features), and stem bark samples (characteristic arrangement of rosette crystals in phloem cells to uniseriate medullary rays) have been summarised in the present study. Botanical characters described in the present study can be used as a rapid reference identification standard for future identification of raw samples of *W. fruticosa* in fresh as well as dried form.

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CONFLICTS OF INTEREST: No

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