Micro-Morphological Study of ‘BALA’ Plant (Sida cordifolia L., Malvaceae) With Special Reference to Its Propagation Technique

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Abstract
‘Bala’ (= Sida cordifolia L.) of the family Malvaceae, is being considered as an important medicinal herb from the time immemorial. The whole plant is an important ingredient of a number of Ayurvedic and Siddha formulations. Root is used extensively in the treatment of neurological and urinary problems, asthma, chronic dysentery, rheumatism, phthisis etc. The present paper deals with micro-morphological studies, chemical overviews and propagation technique of this medicinal herb. The micro-morphological studies were done to identify correct raw drug from that of the common adulterants and substitutes.

Keywords: ‘Bala’; Sida cordifolia L.; Medicinal uses; Micro-morphology; Propagation technique

1. Introduction
‘Bala’, botanically known as Sida cordifolia L., belongs to the family Malvaceae. The plant attracts the attention of Ayurvedic, Siddha, Homeopathic and modern Allopathic physicians for its immense medicinal properties. Even some pharmaceutical companies have marketed slimming capsules, the main ingredient of which is ‘Bala’. The botanical, pharmacognostical and other microscopical information of ‘Bala’ are previously reported [1, 2, 3, 4, 5, 6, 7, 8, 9]. The plant is variously known, as in Sanskrit ‘Bala’; in Bengali ‘Swet berela’, ‘Berele’ and ‘Brela’; in Hindi ‘Kungui’ etc. According to some authentic literature as ‘Charaka Samhita’, ‘Sushruta Samhita’ and ‘Astanga Hridayam’, ‘Bala’ is named as ‘Sahadeva’, ‘Vatyalika’ and ‘Vatapushpi’ respectively. Besides, ‘Bala’ is mentioned variously in different classical literature and different parts of India as ‘Audanika’, ‘Badiyalaka’, ‘Bindi’, ‘Bhadra’, ‘Bhadrabala’, ‘Jayanti’, ‘Kanaka’, ‘Krura’, ‘Nilaya’, ‘Vaghnini’, ‘Vati’ etc. [9].

The plant is a pan tropical weed distributed throughout the tropical and subtropical regions of India up to an elevation of 1800m. The plant is very common in India but comparative scarcity occurs in Arunachal Pradesh, Himachal Pradesh, Jammu & Kashmir, Sikkim and Uttarakhand. ‘Bala’ is a very popular medicinal plant and the whole plant is being used by Ayurvedic physicians for treatment of different diseases. The raw drug also forms a chief ingredient of several important formulations and preparations, as for eg. ‘Ksirabala’, ‘Dhanvantaram’, ‘Balaristam’, ‘Asvagandhadileham’, ‘Balataila’ etc. The drug is well reputed in Ayurvedic and Siddha system of medicine for ailment of different diseases. Though seed contain the maximum amount of active constituent, ‘ephedrine’ but root is used extensively. Root is used as astringent, diuretic and tonic. The infusion of root is given for the treatment of neurological and urinary problems as well as blood and bile disorder. It is also used for the remedy of bleeding piles, cystitis, leucorrhoea, gonorrhoea, chronic dysentery and asthma. Root powder is given with cow milk to treat leucorrhoea and frequent micturition [10]. It is also useful in throat diseases, phthisis and insanity [11]. The root bark is used mixed with sesame oil (Sesamum indicum, Pedaliaceae) and cow milk in curing facial paralysis and sciatica pain [12]. Root decoction mixed with ginger is effective in curing intermittent fever and healing of wounds [11]. Seeds are aphrodisiac, given in gonorrhoea, cystitis, colic pain, piles, tenesmus etc. Leaves are demulcent and febrifuge, used in dysentery. The cooked leaves are eaten in case of bleeding piles. The extract of whole plant, mixed with water is prescribed to cure spermatorrhoea, rheumatism and gonorrhoea [12, 11].

Materials and Methods
Plant specimens were collected from different localities of West Bengal and from the campus of Acharya Jagadish Chandra Bose Indian Botanic Garden, Shibpur, Howrah and National...
Research Institute of Ayurveda for Drug Development (NRIADD), Bidhannagar for macro- and microscopic study. Suitable portion of specimens were also preserved in F.A.A. soln. for future anatomical study. The specimens were identified with the authentic taxonomic literature [2, 13]. Mature seeds were collected for propagation purpose. From freshly collected specimens, macro-morphological studies were done with the help of simple dissecting microscope. Microscopic studies were performed based on hand sections of the roots, stems, leaf-blades and petioles as observed under compound microscope (model no. Olympus KIC 29567). Permanent slides were prepared after gradual dehydration as per the standard protocol [14]. Palisade ratio, stomatal index and vein-islet number, determined using chloral hydrate treated leaf specimens, are represented graphically. All these numerical values are considered as a diagnostic constant and will help to identify the plant species.

Results

A. Macro-morphological study

Perennial subshrub; stem upto 1m high, erect, grayish-green, densely pubescent with minute stellate hairs mixed with simple hairs. Leaves 2.5-6.5 x 1.5-3.5 cm, orbicular, ovate to oblong, cordate at base, obtuse or acute, occasionally rounded or truncate at apex, crenate-serrate along margin, 5-7 nerved at base, densely velutinous with stellate hairs on both the surfaces; petioles 2-3cm long; stipules free-lateral, filiform, densely stellate-hairy mixed with few simple hairs. Flowers 10-15 mm diam., axillary, solitary, or 2-5 in cymes, clustered particularly towards apical portion of twigs; pedicels 4-6 mm long. Calyx campanulate, accrescent; lobes triangular, 4-6 mm long, acute to acuminate, densely stellate-hairy mixed with few simple hairs. Flowers 10-15 mm diam., axillary, solitary, or 2-5 in cymes, clustered particularly towards apical portion of twigs; pedicels 4-6 mm long. Calyx campanulate, accrescent; lobes triangular, 4-6 mm long, acute to acuminate, densely stellate pubescent mixed with some simple hairs outside. Corolla light yellow or creamy white, 10-15 mm across; petals obovate, truncate at apex, 6-8 mm long. Staminal column 6-8 mm long, either with simple hairs or glabrous. Ovary conical, stellate hairy; style 5-7 mm long; stigma penta-fid. Fruits depressed-globose, schizocarp, with a pair of horny structure at the lateral sides of the apex; seeds c. 1.5 mm across, flattened, reniform, glabrous, dark brown or black (Plate 1).

B. Microscopic study

(i) Root

Transverse section of root is circular in outline with a very wide central woody part. Bark is thin with cork consisting of 4-7 rows of thin-walled, tangentially elongated cells of which the outer 1-2 rows of cells are light brown in colour. Phellogen layer is consisting of single layer of rectangular shaped cells. Cortex narrow and is comprising of 3-4 rows of tangentially elongated cells. Calcium oxalate crystals and minute starch grains are frequent within cortex. Secondary phloem found in conical strands composed of 5-6 tangential bands of thick-walled groups of bast fibres, alternating with thin walled phloem elements. Some of the phloem parenchyma cells contain clusters of crystals. Almost all the phloem ray cells contain cluster of calcium oxalate crystals. Secondary xylem consists of vessels, xylem parenchyma, xylem fibers and medullary rays. Vessels occur in groups of 3-4, or solitary, variable in size and shape. Xylem parenchyma, surrounds the vessels, contain starch grains and very thick-walled abundant fibers. Medullary rays many, uni- or bi-serriate, cells radially elongated, most of them contain calcium oxalate crystals. Four distinct primary xylem arch present at the center of the wood.

(ii) Stem:

Transverse section of stem is rounded in outline with stellate trichomes on epidermal layer. Epidermis is composed of oval to oblong, radially elongated, thin-walled cells covered by thin cuticle. Epidermis is followed by the frequent occurrence of 1-2 layers of chlorenchyma. Chollenchyma is 4-6 cell-layered thick, composed of rounded to oval shaped cells. Clusters of crystals of calcium oxalate are frequent in large polygonal parenchyma cells found in this layer. Band of fibres covering the phloem consist of extra phloem fiber (bast fiber) bundle cap as sclereid of 6-8 or more. Calcium oxalate crystals are often found in many phloem cells. Xylem is consisting of xylem parenchyma, tracheid, vessels and medullary rays which also contain starch grains. Medullary rays are uni- or multiseriate. Pith large and is composed of parenchyma cells having more amounts of starch grains and calcium oxalate crystals (Fig. 1. & 2).


(iii) Leaf

Leaf-blade-

Transverse section of leaf-blade shows thin cuticle on both upper and lower epidermis with stellate trichomes. Upper epidermis single cell-layered, thick, and is composed of oval to oblong cells. Mesophyll tissue is consisting of compactly arranged, rectangular, elongated palisade cells followed by rounded to oval shaped, loosely arranged spongy cells. In the midrib region, the upper epidermis is very thinly cuticularised with different types of trichomes. The cells are tangentially elongated on upper and radially elongated on lower epidermis. Next to the upper epidermis 4-5 rows of rounded to oval cells followed by parenchymatous cells surrounding the vascular bundles. The vascular bundles are collateral, with higher amount of xylem and scanty phloem tissues. Vascular bundle sheath is distinct and composed of sclerenchymatous cells. Xylem elements are radially elongated and followed by phloem elements. A few calcium oxalate crystals are present in this layer.

Stomata is anisocytic type. The average stomatal index is 21.58 on upper surface and 26.57 on lower surface. Palisade ratio is 1.32 and vein-islet number is 38.59 (Table I & II; Fig. 5 & Fig. 6).

Table I: Sida cordifolia L.-Palisade ratio

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<th>II.</th>
<th>III.</th>
<th>IV.</th>
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<tr>
<td>Average</td>
<td>1.3 1.35 1.2 1.6</td>
<td>1.3 1.35 1.2 1.6</td>
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Table II: Sida cordifolia L.-Vein-islet number

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<td>Range: 34-42.7 Mean-38.59</td>
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Fig. 5: Sida cordifolia L.: Palisade ratio
Petiole
Transverse section of petiole shows obcordate outline. Stellate hairs present throughout the epidermal layer. Epidermal cells are tabular or rectangular in shape, 1-cell layered and followed by 2-7 layers of collenchymatous cells which is often interrupted. Next the parenchymatous cortical zone where there are three vascular bundles as petiolar traces. Vascular bundles of petioles are conjoint, collateral open. Vascular bundle is ensheathed with sclerenchymatous sheath cells. The ground tissue is of parenchymatous, cells of which are devoid of any contents. The cells are penta-, hexa-gonal or somewhat rounded, thin-walled and compactly arranged without any intercellular spaces (Fig. 3 & 4).

Chemical overview
A systematic examination of the drug plant by extraction with different solvent systems showed the presence of the alkaloids in whole plant body including leaves, stems, seeds and roots though previously root of ‘Bala’ plant was thought to be the major official part. Seeds contain comparatively larger quantity of alkaloids than other plant parts. The major portion of alkaloid has been identified as ‘ephedrine’ which is also found in Ephedra. Seeds of ‘Bala’ contain fatty oil, phytosterol, mucins, resin, resin acids, proteins, steroids, potassium nitrate, pseudoephedrine, linoleic acid, malvalic acid and sterulic acid while tannin and glucosides are totally absent [15, 11]. Aerial parts contain hydrocarbon and fatty acids (palmitic acid, stearic acid, hexacosanoic acid and β-sitosterol) whereas root contains acylsterylglucoside, sitoindoside, C-28 phytocedysones viz. sidasterone A, sidasterone B, carboxylated tryptamines, quinazoline alkaloids, sympathomimetic amides, β-phenethylamine, ψ-ephedrine, choline, phytosterol, resin acid, hypaphorine, vasicinone and vasicinol [16, 17, 18].
Propagation technique

‘Bala’ (= Sida cordifolia L.) is a popular medicinal plant, the root of which was once thought as major source of active constituents. Thus uprootation of whole plant was obvious for extraction purpose. Comparing to the other species of Sida L., distribution of ‘Bala’ is becoming narrower gradually due to over exploitation from its natural habitat in different states of India. Seeds of ‘Bala’ contain maximum amount of alkaloids necessitating cultivation of the plant as presently there is no Government registered variety of this species. A well-drained sandy-loam to clay-loam soil rich in humus is suitable for cultivation of this plant. The plant grows well in tropical and subtropical climate but the growth in tropical region is better. Warm as well as moist condition throughout the year is also preferable for good growth of the plant. ‘Bala’ can be grown both from the seeds and by stem cuttings [19]. The plants propagated from stem cuttings flower earlier than that of seed propagation. In case of seed propagation, one year old dormant seeds are either directly sown in the field in-situ or in nursery bed. After emerging out, seeding of 7-14 days old are transplanted in a space of 75 x 85cm in the field. In vegetative propagation, the stem cuttings obtained from lateral stems are used. Beside these the propagation is also reported from tissue culture practices. Multiple shoot formation from mature nodal explants of ‘Bala’ on MS medium supplemented with 2.0 mg 1^-1^-6^- benzyl amino-purine, 0.5 mg 1^-1^-naphthalene acidic acid, 1.0 mg 1^-1^- adenine sulfate and 10% (v/v) coconut milk are reported [20]. In this method, multiple shoots are initiated within 21st day and each explant is capable of inducing formation of more than twenty shoots. The regenerated plants are induced for rooting and plantlets are introduced in soil. This material may be useful for germplasm conservation and genetic improvement of ‘Bala’. The land is repeatedly ploughed to a fine tilth and weeds, pebbles etc. are removed. For a hectare of land, 20-25 tons FYM for low fertile and 15-18 tons for moderately fertile soil are essential. Green manuring is effective where irrigation facilities are available. Groundnut cake, bone-meal, rapeseed cake and vermin-composting are beneficial for better growth of the plant. Neem oil is recommended to remove mites and nematodes from the fields. The seedlings should be transplanted in a space of 75 x 85 cm in the field. Plenty of water is not required for its cultivation. The crop is given 2-3 periodic weeding and hoeing at interval of 20-30 day. After one year of plantation, the crop is ready for harvest. Harvesting is done manually [21]. Retail market price of ‘Bala’ root is 30/kg [22]. Leading exporters engaged in the cultivation, harvesting and processing of this plant are Mother Herbs (P) Ltd., India, Tan-Hoard Exports, India etc. The drug plant ‘Bala’ (= Sida cordifolia L.) is often adulterated with Sida retusa L. (Kerala), Abutilon indicum (L.) Sweet and Urena lobata L. [23].

Discussion and conclusion

The morphological and anatomical features both are the important characters for the identity of ‘Bala’ plant. The cultivation with proper method can provide good as well as suitable drug which help in identification of adulterants and substitutes. Natural cultivation is more fruitful than tissue culture method. The unscientific and skill-less uprooting during harvesting of the whole plant seems to trend deterioration of the natural habitat in the near future. The only solution is to use the required portion of the plant scientifically and its ex-situ or in-situ cultivation.

References

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