Comparative root anatomy of the species under Sida rhombifolia complex (Malvaceae)

Mytheenkunju Navas a, *, Mathew Dan b, Panikamparambil Gopalakrishnan Latha a

a Division of Ethnomedicine and Ethnopharmacology, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Thiruvananthapuram 695 562, Kerala, India
b Plant Genetic Resource Division, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Thiruvananthapuram 695 562, Kerala, India

A R T I C L E   I N F O

Article history:
Received 11 April 2013
Accepted 25 September 2013
Available online 12 November 2013

Keywords:
Bala
Powder microscopy
Raw drug
Taxonomy

A B S T R A C T

Introduction: Anatomical studies of the root of four Sida species, S. rhombifolia, S. alnifolia, S. scabrida and S. rhomboidea, coming under Sida rhombifolia complex (Malvaceae) were carried out and compared. The genus Sida is the botanical source of the controversial Ayurvedic drug ‘Bala’. Nearly five species of Sida including S. alnifolia are used as Bala.

Materials and methods: After taxonomic confirmation, observed root morphology, root anatomy and powder microscopy of four Sida species as per standard procedures.

Results: Roots of all the four species are distinct in their morphology as well as anatomical characters such as pattern of bast fibres and medullary rays.

Conclusion: Based on the anatomical characters, a diagnostic key is prepared which can be used as a supporting tool for taxonomic delimitation of the species coming under the S. rhombifolia complex as well as to ascertain the identity of the drug Bala.

Copyright © 2013, Phcog.Net, Published by Reed Elsevier India Pvt. Ltd. All rights reserved.

1. Introduction

The genus Sida (Malvaceae) comprises about 200 species distributed along the tropical and subtropical parts of the world, 1 of which 20 species are found in India and 18 species in Kerala.2–4 The genus is taxonomically complicated and among its species Sida rhombifolia continues to be the hardest nut to crack. Earlier, this species was known by about 30 binomials proposed by various taxonomists due to the high rate of morphological variability within the taxa. Critical systematic studies based on the vegetative features from seedling stage to the adult plants and reproductive characters of these taxa resulted in the reduction of many species and the determination of four distinct species, viz. S. rhombifolia Linn., S. alnifolia Linn., S. scabrida Wight & Arn. and S. rhomboidea Roxb. ex Fleming from India, which are together termed as S. rhombifolia complex.5

Among these, S. alnifolia is widely used in Ayurveda as well as in Indian folklore and traditional medicine. The root of this plant is widely accepted as the source plant of the renowned Ayurvedic drug ‘Bala’ locally known as ‘Kurunthotti’, in Kerala. This drug is one of the medicaments used in the treatment of various ailments such as rheumatism, fever, neurological disorders like hemiplegia and sciatica, tuberculosis, diabetes and uterine disorders. Ksheerabala, Balarishtam, Agastyarasayanam, Aravindasavam, Dhanwantaram ointment and Rasnadi choornam are some of the important Ayurvedic formulations in the form of medicated oil, fermented drugs, medicated ghee, powder etc. in which ‘Bala’ is a major ingredient. It is also reported to have anti-tumour, anti-HIV, hepatoprotective, abortifacient, anti-microbial, and immuno-stimulant properties.6

Since the root of Sida is the source of the important raw drug ‘Bala’, its identity is controversial.7 The Ayurvedic physicians and local healers of Kerala use S. alnifolia as Bala where as in northern India S. cordifolia is used.5 Various species of Sida such as S. rhombifolia, S. rhomboidea, S. scabrida (3 species of the Sida rhombifolia complex), S. acuta, S. cordifolia, S. fryxellii, etc. are used as ‘Bala’.9 A few species of allied genera such as Abutilon, Pavonia, Urena etc. are also used as ‘Bala’ in the raw drug market either as substitute or as adulterant.10,11 The demand of this drug in the market is very high. It is estimated that the annual demand of Sida spp. in Indian Systems of Medicine is 9339 tons/year.12 Its annual demand in four Southern districts of Kerala during the mid 1990’s was 713.99 tons per year,13 and that by six major pharmacies in Kerala is 589 tons per year.14 A recent study15 showed that the demand of Sida root is increased to 1194 tons per year by the Ayurvedic drug manufacturing industry in Kerala. All these reports showed bulk requirement of this drug in health care industry of the country. But these species are collected only from the wild and so far there is no organised cultivation.
Due to the close morphological resemblance, the identification of most of the species of *Sida* especially the species belong to the *Sida rhombifolia* complex is very difficult. Since the useful part is the root, the complication of identification of the raw drug is too high. Normally, the taxonomical delimitation in this group is possible only in the reproductive stage, especially based on the mericarp morphology. In these circumstances, in order to identify the plant species in the vegetative stage and for the authentication of the genuine drug, the root anatomy and powder microscopy of the species in the *Sida rhombifolia* complex were studied and its taxonomic significance is discussed. There were a few earlier attempts, to ascertain the identity of the genuine drug Bala, through root anatomy of selected species of *Sida*. The comparative root anatomy of the species under *Sida rhombifolia* complex and its taxonomic significance is discussed for the first time.

2. Materials and methods

2.1. Collection and authentication of the plant

Four species of *Sida* coming under the *S. rhombifolia* complex were collected from different localities of Kerala for the present study. Specimens of all the species were collected in the flowering stage, studied their morphology, compared with authenticated specimens and determined their taxonomic identity. Voucher specimens were deposited in the Herbarium of Jawaharlal Nehru Tropical Botanic Garden and Research Institute (TBGT).

2.2. Macroscopic studies

Morphology of the fresh roots was examined by naked eye and detected organoleptic characters by standard procedures.

2.3. Microscopic studies

The fresh root samples were fixed in FAA and free hand sections were taken for anatomical studies. Sections were taken from 2 cm below the stem-root transitional point of each sample to ensure a uniform growth stage of the root. Good sections were stained with safranin and observed under Leica 1000 binocular microscope and photographs were taken with Leica D-Lux 3 camera.

2.4. Powder studies

Fresh roots were collected, washed thoroughly with tap water and shade dried. Dried roots were powdered coarsely and observed under Leica 1000 binocular microscope. Powder taken randomly and placed over slides, mounted over glycerine, stained with safranin and covered with 1 mm cover slips, observed under microscope and recorded the details.

3. Observations & results

3.1. Root morphology of *Sida rhombifolia* complex

All species of *Sida* possess a taproot system. The shape of the roots varies very much even within a species.

3.2. *Sida rhombifolia* Linn.

Erect branched under shrubs to 1.5 m tall. Stem terete, green or purplish-grey, stellate-tomentose to glabrescent. Leaf blades 0.5–5 × 0.5–4 cm, leaves towards stem base always obovate with retuse or emarginate apex, rarely truncate; upper leaves obovate elliptic-lanceolate with rounded, subobtuse or acute apex, obtuse or rounded at base, margins irregularly serrate-dentate or crenate to the distal half, entire towards the proximal half. Flowers axillary, orange-yellow, solitary, sometimes in apparent racemes due to reduction of upper leaves. Schizocarp 4–5 mm long; mericarps 8–10, 4 × 3 mm, included in the calyx, trigonous with acute angles, pale when mature, distinctly and transversely rugose on the sides and back: apex with a pair of short glabrous, divergent awns; awns 1–1.5 mm long, as long or slightly shorter than calyx. Seeds 2 mm long, black, minutely hairy at hilum.

3.2.1. Root morphology

The taproot is light brownish, straight and with many fibrous lateral roots. The lateral roots are longer, thinner and possess numerous rootlets. The lateral roots are straight, more often wavy and brownish yellow in colour. The outer surface is rough (Fig. 1A).

3.2.2. Root anatomy

The comparative root anatomy of most of the species of *Sida* coming under the *S. rhombifolia* complex and its taxonomic significance is discussed for the first time.

3.3. *Sida alnifolia* Linn.

Woody herbs or subshrubs to 50 cm, usually low and strongly branched. Branches prostrate or ascending, terete, green or purplish-grey, stellate-tomentose to glabrescent. Leaf blades 0.5–5 × 0.5–4 cm, leaves towards stem base always obovate with retuse or emarginate apex, rarely truncate; upper leaves obovate elliptic-lanceolate with rounded, subobtuse or acute apex, obtuse or rounded at base, margins irregularly serrate-dentate or crenate to the distal half, entire towards the proximal half. Flowers axillary, orange-yellow, solitary, sometimes in terminal clusters due to reduction of distal leaves. Schizocarp 4 mm long; mericarps 7–10, 2.5–3.5 mm, included in the calyx, trigonous with acute angles, pale when mature, distinctly and transversely rugose on the sides and back, with a pair of short stellately hairy mucro at apex, mucro obtuse, retuse or emarginate at apex. Seeds 2 mm long, black, glabrous except for a puberulent hilum.
3.3.1. Root morphology

The taproot is tortuous; numerous branched lateral roots are produced from the taproot, mainly concentrated in the upper region. The lateral roots are long, wavy and thin having few wiry rootlets. The outer surface of the root is rough and cream coloured (Fig. 1B).

3.3.2. Root anatomy

T.S of the root is more or less circular in outline with wavy margin (Fig. 1B1). The outermost layer cork consists of 4−9 rows of cells. The cells are thin walled, elongated and rectangular. In some region the outermost layer of cells are peeled off and peels are recurved. Below this layer, a single layer of phellogen present with

thin walled and narrow cells. Next to the phellogen two rows of thin walled elongated cells are present which contains stellate crystals.

Cortex consists of conical strands of bast fibres, arranged in 3–8 rows. Smaller groups of cells are seen towards periphery and larger groups are seen towards inner side, consists of 2–20 cells present in between the fibre groups towards the periphery. The conical strands of bast fibres are separated by phloem rays contain stellate crystals and starch grains. Crystals are abundant in cortical region. Starch grains are simple and small, sometimes in group of three. Below this layer a complete ring of phloem present which contains 4–7 layers of cells and the cells are thin walled and polygonal. Some phloem ray cells contain stellate crystals. Inner to the phloem, wood present which forms the major part of the root and it composed of xylem parenchyma, vessels, fibres and medullary rays (Fig. 1B2). Vessels are polygonal and small in size, most of them are seen singly, group of 2–3 and rarely group of 5–7 are also seen. Some vessels are filled with resinous substances, tyloses are present. Xylem parenchyma is associated with vessels and filled with large starch grains. Xylem fibres are thick walled. Medullary rays are uniseriate and biseriate. Ray cells are filled with starch grains.

3.3.3. Powder characters
Powder is cream coloured, no characteristic odour. The powder microscopy showed elongated fibres, starch grains, stellate crystals, and bordered pitted vessels.

3.4. Sida scabrida Wight & Arn.

Erect branched subshrubs to 2 m tall. Stem terete, green or slightly tinged with purple, pubescent with minute stellate hairs intermingled with scattered, long simple hairs. Leaf blade 6–8 × 3–4 cm, concordous, rhomboid or oblong-lanceolate, truncate at base, acuminate at apex, basally 3-nerved, lateral nerves 4–5 pairs, margins serrate-crenate distally and entire towards base. Flowers axillary, yellow, solitary, sometimes in clusters of 3–5 due to reduction of distal leaves. Mericarps 7–10, 3 × 3 mm, enclosed in the calyx, trigonous with acute angles, black when mature, minutely stellate-pubescent on the back towards the apex, prominently reticulate or transversely rugose on the sides and back, apex with a pair of linear divergent simple and stellate-pubescent awns.

3.4.1. Root morphology
The taproot is slightly tortuous; numerous branched lateral roots are produced from the taproot, concentrated mainly in the lower region. The lateral roots are long, wavy and thin. Root surface is smooth and brownish with cream tinge (Fig. 1C).

3.4.2. Root anatomy
T S of mature root is more or less circular in outline (Fig. 1C1). The bark is thin and the wood constitutes the major portion. Outer most layer of the cork consists of 13–18 layers of rectangular elongated cells. Cork is followed by 2–3 layers of cortical cells with rosettes of calcium oxalate crystals.

Secondary phloem consists of bast fibres which are arranged as conical strands, but not prominent. Most of the strands are scattered in the secondary cortex, which contains 2–28 cells in each group. Phloem consists of more or less rounded or oval cells towards the secondary xylem. Small starch grains and calcium oxalate crystals are present in the phloem cells. Crystals seen in the phloem are larger when compared to crystals of cortical cells. In the secondary cortex, the inner most fibre layer is seen as a broken ring, just outer to secondary xylem. Starch grains are smaller in outer phloem and larger towards the inner side, 2–3 cambial cells are present. Wood consists of xylem vessels which are rounded, oval and elliptic in shape, xylem parenchyma and medullary rays (Fig. 1C2). Traeheary elements are seen associated with the xylem. Xylem vessels are single, rarely group of three in radial arrangement. Medullary rays are extending to the phloem region contains rosette crystals. Crystals are abundant in the secondary phloem. Medullary rays are uniseriate, rarely biseriate and very rarely triseriate. Ray cells are elongated and barrel shaped, contains small starch grains. Cells become broader towards the phloem region.

3.4.3. Powder characters
Powder is light brown with yellowish tinge. No characteristic odour or taste. On microscopic observation powder showed: thin walled parenchyma cells with crystals and starch grains, thick walled xylem parenchyma; simple pitted, bordered pitted, spiral annular, scalariform and reticulate vessels; long fibres with tapering ends, twisted fibres and bundles of fibres; thin walled stone cells, parts of bark; rounded or oval starch grains, compound grains of diads or triads; stellate calcium oxalate crystals, prismatic crystals, white crystal like masses and reddish brown resinous blocks.

3.5. Sida rhomboidea Roxb. ex Fleming

Erect much branched subshrubs to 3 m tall. Stem terete, usually purplish, minutely pubescent with small stellate hairs. Leaf blades on younger shoots are much larger (7–8 × 5–6 cm), obovate or suborbicular, truncate or rounded at base, subobtuse or acute at apex; those on flowering shoots are smaller, 1–5 × 0.5–3 cm, rhomboid to lanceolate, 3-nerved from base, lateral nerves 3–5 pairs, nerves raised on the lower surface, margins coarsely serrate to crenate, entire towards base. Flowers axillar, pale yellow, solitary; pedicels 6 mm in flowers, to 30–40 mm in fruits, filiform, glabrous, articulated at about the middle. Schizocarp 3–4 mm long; mericarps 8–10; completely included in the calyx, closely coherent, 3 × 2 mm, indehiscent, trigonous with acute angles, prominently reticulate on the sides, reticulate or rugose on the back, apex beaked with a single, glabrous muticous process.

3.5.1. Root morphology
The taproot is tortuous; numerous branched lateral roots are produced from the taproot, concentrated mainly in the upper region. The lateral roots are long, wavy and thin. Root surface is smooth and cream coloured (Fig. 1D).

3.5.2. Root anatomy
T S of the root is more or less circular in outline (Fig. 1D1). In certain region outermost part of the cork cells slightly detached due to exfoliations. Cork cells are narrow elongated, 5–7 layers, followed by one or two layers of phellogen and one or two layers of phelloderm. Crystals are rarely seen in the thicker portion (at the upper part of the root i.e. just below the stem) and absent in the lower portion. Bast fibres form the major portion of the cortex. They are conical strands with mostly single row or two or three rows, loosely arranged. Each strand consists of 12–20 groups of fibres with 2–30 cells. Cortical cells are transversely long, somewhat angular with starch grains.

Medullary rays become broad with 15–20 cells when reaching the periphery. Inner cortical cells contain more starch grains than outer. Starch grains are generally compound. Outer cortex contains few crystals. Secondary phloem and fibre strands are followed by 2–3 layers of angular cambial tubes, followed by wood. Wood consists of vessels, xylem parenchyma and medullary rays (Fig. 1D2). Vessels are angular, mostly single and rarely in group of 2–4 cells and very exceptionally group of 6 cells also seen. Medullary rays are mostly uniseriate and rarely biseriate, contains large...
Sida rhombifolia and lateral branches which ends in numerous rootlets. The taproot of

Comparative root anatomy of the four species are given in Table 1. Comparative root anatomy of the four species in the S. rhombifolia complex and its taxonomic significance is discussed.

Roots of all the four species are with a main cylindrical taproot and lateral branches which ends in numerous rootlets. The taproot of Sida rhombifolia and S. scabrida are fibrous while other two species are less fibrous. The taproot of Sida alnifolia and S. rhomboidea are tortuous and with few lateral roots. In the case of S. rhombifolia and S. scabrida, taproot is straight and the lateral roots are numerous. The colour, shape, branches, taste and odour are slightly varying between the species. In the case of S. rhombifolia, the colour is brownish and all other species are in various shades of cream colour. The general morphology and organoleptic characters of the root of four species in the S. rhombifolia complex are given in Table 1.

The anatomical features of the four species are varying. They are almost circular in outline with slight wavy margin. Bark region is thin and wood occupies a major portion. The bark is with a tendency to break, detach and recurve. It is followed by few layered cortex and then secondary phloem which consists of bast fibres and phloem rays, with starch grains and crystals. The wood consists of vessels, parenchyma and medullary rays. Medullary rays are uniseriate to triseriate with starch grains. The colour, size, shape, branches, taste and odour are slightly varying between the species. In the case of S. rhombifolia, the colour is brownish and all other species are in various shades of cream colour. The general morphology and organoleptic characters of the root of four species in the S. rhombifolia complex are given in Table 1.

The anatomical features of the four species are varying. They are almost circular in outline with slight wavy margin. Bark region is thin and wood occupies a major portion. The bark is with a tendency to break, detach and recurve. It is followed by few layered cortex and then secondary phloem which consists of bast fibres and phloem rays, with starch grains and crystals. The wood consists of vessels, parenchyma and medullary rays. Medullary rays are uniseriate to triseriate with starch grains. The colour, size, shape, branches, taste and odour are slightly varying between the species. In the case of S. rhombifolia, the colour is brownish and all other species are in various shades of cream colour. The general morphology and organoleptic characters of the root of four species in the S. rhombifolia complex are given in Table 1.

Due to the complexity in the identity and usage of the Sida species in terms of drug, there were a few attempts to ascertain the identity of the root of selected Sida species, through comparative root anatomy. Comparative root anatomy of the four species of Sida rhombifolia complex and its taxonomic significance is discussed.

Roots of all the four species are with a main cylindrical taproot and lateral branches which ends in numerous rootlets. The taproot of Sida rhombifolia and S. scabrida are fibrous while other two species are less fibrous. The taproot of Sida alnifolia and S. rhomboidea are tortuous and with few lateral roots. In the case of S. rhombifolia and S. scabrida, taproot is straight and the lateral roots are numerous. The colour, size, shape, branches, taste and odour are slightly varying between the species. In the case of S. rhombifolia, the colour is brownish and all other species are in various shades of cream colour. The general morphology and organoleptic characters of the root of four species in the S. rhombifolia complex are given in Table 1.

The anatomical features of the four species are varying. They are almost circular in outline with slight wavy margin. Bark region is thin and wood occupies a major portion. The bark is with a tendency to break, detach and recurve. It is followed by few layered cortex and then secondary phloem which consists of bast fibres and phloem rays, with starch grains and crystals. The wood consists of vessels, parenchyma and medullary rays. Medullary rays are uniseriate to triseriate with starch grains. The colour, size, shape, branches, taste and odour are slightly varying between the species. In the case of S. rhombifolia, the colour is brownish and all other species are in various shades of cream colour. The general morphology and organoleptic characters of the root of four species in the S. rhombifolia complex are given in Table 1.

The anatomical features of the four species are varying. They are almost circular in outline with slight wavy margin. Bark region is thin and wood occupies a major portion. The bark is with a tendency to break, detach and recurve. It is followed by few layered cortex and then secondary phloem which consists of bast fibres and phloem rays, with starch grains and crystals. The wood consists of vessels, parenchyma and medullary rays. Medullary rays are uniseriate to triseriate with starch grains. The colour, size, shape, branches, taste and odour are slightly varying between the species. In the case of S. rhombifolia, the colour is brownish and all other species are in various shades of cream colour. The general morphology and organoleptic characters of the root of four species in the S. rhombifolia complex are given in Table 1.
S. alnifolia is creamy and S. scabrida is light brown with a cream-tinge. Stellate crystals are seen in S. rhombifolia and S. rhomboidea where as in S. alnifolia rosette crystals and in S. scabrida rosette and prismatic crystals are seen. Thin walled stone cells are seen in S. scabrida and S. rhomboidea while stone cells are not seen other two species. Fibres are narrow and long in all the species and twisted fibres are seen in S. scabrida. Vessels are diagnostic features in case of powder drugs. In S. rhombifolia only bordered pitted vessels are seen in the powder where as in S. alnifolia bordered pitted and scalariform vessels are seen. In S. scabrida both simple and bordered pitted vessels along with spiral, annular, scalariform and reticulate vessels are seen. In S. rhomboidea simple and bordered pitted and annular vessels are seen. Variation in the vessels, starch grains and crystals are useful as supporting characters for taxonomic delimitation of species.

Anatomical features of the four species are different. Present anatomical and microscopical study on the root of four species in Sida rhombifolia complex, supports the taxonomic status of the four species coming under the complex as reported by Sivarajan and Pradeep (1994). Based on the anatomical features, an artificial key is prepared and given below.

5. Key based on anatomical characters

1 a Vessels are rounded and horizontally arranged, Cork cells in more than 10 layers.......................................................S. scabrida

1 b Vessels are mostly angular and vertically arranged, Cork cells in less than 10 layers.....................................................2

2 a Bark region narrow, medullary starch grains smaller, Medullary rays usually biseriate....................................................S. rhombifolia

2 b Bark region broad, medullary starch grains larger, Medullary rays usually uniseriate..............................................3

3 a Bast fibre narrow tapering, compact, Phloem rays dilated at distal end.......................................................S. rhomboidea

3 b Bast fibre broad, not compact, Phloem rays not dilated at distal end..................................................S. alnifolia

6. Conclusion

The species status given to the four species in the Sida rhombifolia complex,2 is well supported in the present study. The anatomical features can be used as markers for the taxonomic delimitation of the Sida rhombifolia complex. In the herbal drug industry, especially in Ayurveda, this data will help for the identification of genuine botanical source S. alnifolia which is popularly used as the drug Bala.

Conflicts of interest

All authors have none to declare.

Acknowledgements

The authors are thankful to the Director, for providing facilities and Dr S. Rajasekharan, former Head, Division of Ethnomedicine and Ethnopharmacology for encouragement and support. Thanks are also due to Mr. N. P. Rajith for setting the photo plate.

References