J. Indian bot. Soc. (2024)

e-ISSN:2455-7218, ISSN:0019 - 4468

https://doi: 10.61289/jibs2024.01.30.1200

www.indianbotsoc.org

RESEARCH ARTICLE

In depth analysis of vegetative wormbush: Morphology and anatomy

Saloni P. Kothari and Devangi P. Chachad

Abstract

Spigelia anthelmia L. being a medicinal credence is suggested to be an important herbal drug. Leaves are commonly prescribed for many homeopathic treatments like helminthiasis, asthma, cardiac disorders, neuralgia, iritis and many more, in spite of being known for its poisonous nature. The whole plant and its parts are used for various ailments. Knowing morphology and anatomy are the simplest methods used for the correct identification of any plant. This study reveals the macroscopical and anatomical features of the whole plant of *S. anthelmia*, which will be helpful to detect the adulteration if any.

Keywords: Macromorphology, Micromorphology, *Spigelia anthelmia* L.

Introduction

Plants have been used by humans excessively, for the maintenance of our health and well-being since time immemorial. These are considered to be a biosynthetic laboratory due to the presence of many active principle constituents (Bhogaonkar and Lande 2015). Plant based medicine is beneficial in many ways because of its safe and cost-effective nature, easy availability with minimum or no side effects. (Prakash and Gupta 2005). Herbal medicine has tremendous advantages against synthetic drugs and is extensively in demand. However, being the victim of adulteration or misidentification or having ambiguity due to vernacular names for two or more entirely different species is a reason for adulteration many times in herbal medicine or in natural products. (Chanda 2014). To avoid all these errors, the solution would be to have proper identification techniques, physical standardization and anatomical characteristics for these plants. *Spigelia anthelmia* L. is a medicinal plant widely used in homeopathic medicines. It is used in treatments of various diseases and contains plant based natural bio-active constituents such as spiganthine and ryanodine, 20-deoxyspiganthine, 8 α-hydroxyspiganthine, 20-hydroxyryanodine, 10-*epi*-ryanodine, 20-norspiganthine-5-carboxylic acid and 8,9-dehydro-10-*epi*-ryanodine (*Awotedu et al.* 2020, Iwu 2014). The preliminary phytochemicals majorly present in this plant are alkaloids, saponins, flavonoids, phenolics and tannins (Awotedu and Ogunbamowo 2019). It also shows the presence of two volatile alkaloids such as isoquinoline and an actinidine isomer; along with three quaternary alkaloids i.e., choline, benzoylcholine and 2, 3-dimethylacrolyl choline (Wagner *et al.* 1993). Plant has been reported to be a poisonous as it contains an alkaloid Spigeline and Spiganthine which affects the nervous system, can be used to repel intestinal parasite and also acts on heart especially the endocardium (Meena and Yadav 2010, Liang *et al.* 2019, Hegde *et al.* 2013). The decoction of its roots is used as an effective vermifuge. It is also considered to be a criminal poison (Hegde *et al.* 2013). The plant shows potency against simple problems like toothache as well as the most complicated diseases like cancer and HIV. It is also used as a laxative and an antibacterial. (Awotedu and Ogunbamowo 2019, Danlami *et al.* 2017). The tincture prepared from leaves is popularly used in the management of asthma and helminthiasis in Homeopathy.

S. anthelmia (Loganiaceae) is commonly known as Wormbush, Worm-grass and West Indian Pinkroot. This genus mainly consists of 95 species and is known to be a

Department of Botany, Jai Hind College, Basantsing Institute of Science, J.T. Lalvani College of Commerce, Mumbai University, Mumbai, Maharashtra, India.

***Corresponding Author:** Devangi P. Chachad, Department of Botany, Jai Hind College, Basantsing Institute of Science, J.T. Lalvani College of Commerce, Mumbai University, Mumbai, Maharashtra, India., E-Mail: devangi.chachad@jaihindcollege.edu.in

How to cite this article: Kothari, S.P. and Chachad, D.P. 2024. In depth analysis of vegetative wormbush: Morphology and anatomy. J. Indian bot. Soc., Doi: 10.61289/jibs2024.01.30.1200

Source of support: Nil

Conflict of interest: None.

Received: 10/09/2023 **Accepted:** 23/11/2023 **Published online**: 30/01/2024

neotropical genus, distributed from Southern America to northward to southern United States and naturalized in many tropical countries. It is usually found in a habitat with occasional wastelands and deciduous forests (Swamy and Jalander 2021).

The aim of the study is to carry out the critical analysis on anatomical features and morphological studies of root, stem and leaf to serve as a possible tool for identification of *S. anthelmia* L.

Material and methods

The whole plants of *S. anthelmia* for the present work was collected from open sunny sites growing nearby railway tracks (between wooden sleepers) at Dadar – Parel in Mumbai (India). The plant was authenticated by Blatter's Herbarium, St. Xavier's College, Mumbai, India.

Macroscopy: The morphological characters visible to naked eyes or magnifying glass of the whole plant parts were observed and described (The Ayurvedic Pharmacopoeia of India, 2001).

Microscopy: Hand cut cross-sections were taken from freshly collected material. Sections were then stained with safranin and observed under a Olympus compound microscope and microphotographs were taken with the help of a digital camera at 10X magnification. (Fahn 1997, Jackson and Snowdon 1990)

Leaf Constants: A detailed study of stomatal number and stomatal index was carried out using procedures outlined by Khandelwal 2008. Stomatal types were determined according to Chachad and Vaidya 2016. The leaf clearing technique was performed according to Hickey 1973 with minor modifications for studying leaf architecture. Palisade ratio was determined as per Khandelwal 2008. Trichome type and its density was assessed by a standard method suggested by Dilcher 1974.

Observation

Systematic Description

Annual, erect herb, about 10-60 cm in height. Stem erect, terete and short stalked with glabrous simple or sparsely branched; Feather like lobed leaves in a whorl of four, opposite decussate, ovate- oblong or ovate- lanceolate. One pair larger than the other; lower leaves linear-lanceolate, 1-1.5 x 0.5 cm, lateral veins 2-3 pairs; the upper pairs of leaves in whorls ovate lanceolate or ovate-oblong 3.5-8 x.0.7-3 cm, lateral veins 4-5 pairs; entire margin with acuminate or acute apex, decurrent base, slightly scabrous above, glabrous, or minutely puberulent along the mid nerve beneath; stipules deltoid. Inflorescence 3-8 cm long, terminal usually in slender secund and cincinnus cyme of 10-15 flowers having very short peduncle. Flowers whitish with purple stripes, bisexual, sessile or sub-sessile, 1.5 - 2cm, bracteole linear – lanceolate, 1.2 – 1.8 mm long. Calyx 5 lobed, free, linear – lanceolate, 2.5 – 3.5 mm long. Corolla tubular – campanulate; tube 5 – 10 mm long, white with pink nerves in the middle; 5 lobbed, lobes ovate, acute at the apex, whitish purple strips, 2 – 3 mm long as long as wide with two purple strips. Stamens 5 inserted slightly below the middle of the corolla tube, 4 – 5 mm long, anthers yellow. Ovary globose, superior, placentation axile,2 – locular, 6 – 8 ovules per locules; style 6 – 7 mm long. Capsules cordiform 3 – 5 x 4 -6 cm, 2 lobbed, 4 valved; finely muricate with persistent portion of style and rhombic calyx. Seeds ovoid, black, several per locule, obliquely ellipsoid – reniform, 1.8 – 2.0 x 1.2 – 1.5 mm (Swamy and Jalander 2021, Meena *et al.* 2014; Prajapati *et al.* 2021; Awotedu and Ogunbamowo 2019) (Figure 1) (Table 1 and Table 2).

Micromorphology

Transverse Section of Root

Single layered tangentially elongated phellem filled with tannins with a few root hair is seen on the outer side. The Cortex is composed of parenchymatous cells and is

Table 1: Macroscopic Characters of Root and Stem in S. *anthelmia*

Macromorphology	*Stem Characteristics*	*Root Characteristics*
Size	*Length: 15.2 cm*	*Length: 1.3cm*
	Breath: 2.0-3.5mm	*Breath: 1.0 - 1.6mm*
Shape	Round	Round
Type	Slightly woody	Tap root
Node	Very Few	N.A.
Internode	Very Few	N.A.
Odour	None	--
Taste	Leafy	--
Colour	Light Green	--
Texture	Smooth	--

Table 2: Macroscopic characters of *S. anthelmia* Leaves

Evaluating features	*Leaf Characteristics*
Size	*Length: 6.2cm*
	Breath: 2.9 cm
Shape	Ovate
Apex	Acute
Margin	Entire
Veins	Unicostate Reticulate
Veinlets	Quinternery
Lamina	Upper surface slightly rough
Base	Rounded
Petiole	None
Odour	Leafy
Taste	Bitter
Colour	Green
Texture	Slightly Rough

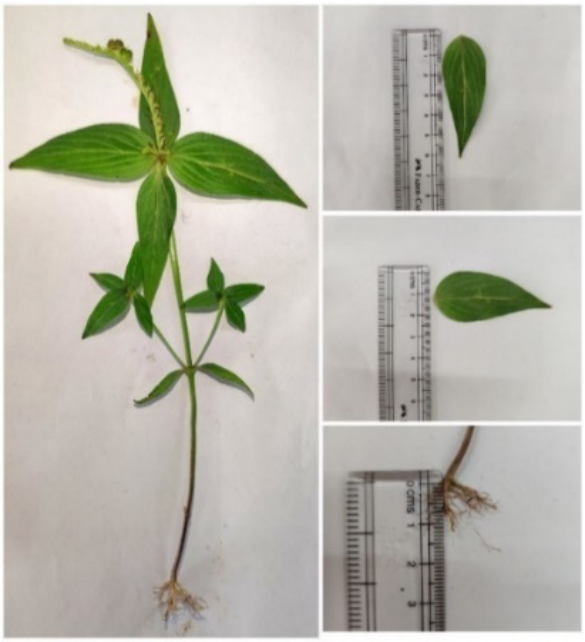


Figure 1: Macroscopy

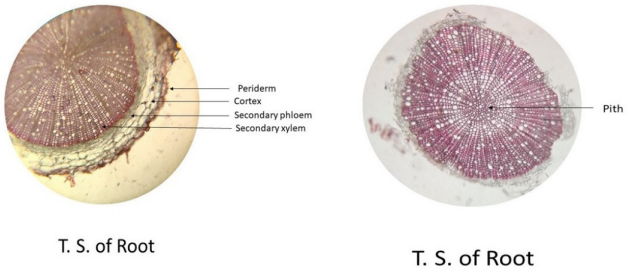


Figure 2: Transverse Section of Root and Root showing pith

3-4 layered. Endodermis is single layered, strictly devoid of starch. Vascular bundle consisting of primary phloem and secondary phloem followed by primary xylem and secondary xylem. Pith is almost obliterated (Figure 2).

Transverse Section of Stem

Double layered epidermis is seen with cuticle. It also shows presence of hypodermis which is differentiated in two zones i.e., chlorenchymatous and sclerenchymatous, in the young stem whereas in older stem the sclerenchyma layer becomes discontinuous. Cortex is crushed. Primary vascular bundles are conjoint, collateral and arranged in a ring. Origin of Xylem is endarch. Secondary growth is seen where a ring of xylem is formed with Uniseriate medullary rays. The pith shows presence of large parenchymatous cells along with which starch grains are present. In the older stem the pith disintegrates, making the stem hollow (Figure 3).

Transverse Section of Leaf

Single layered epidermis with small evenly distributed conical, glandular trichomes. Thin layer of cuticle is present. Mesophyll

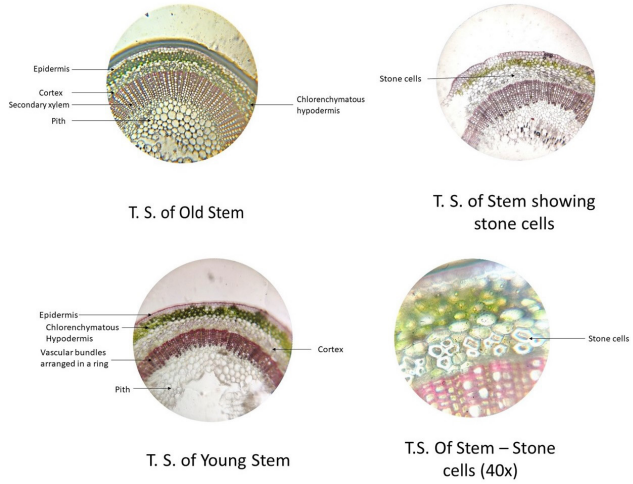


Figure 3: (a)Transverse Section of old stem and Stem showing stone cells(10X) (b) Transverse section of young stem and Stem showing stone cells (40X)

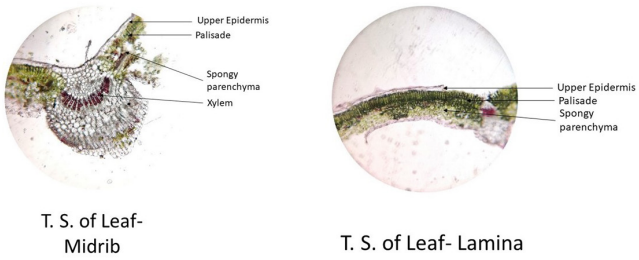


Figure 4: Transverse Section of Leaf midrib and Leaf lamina

Figure 5: Unicellular trichome (40X)

is differentiated in palisade and spongy parenchyma. Palisade is single layered whereas spongy parenchyma is 4-5 layered with small intercellular spaces included within the spongy parenchyma. The lower epidermis is also single layered but shows presence of small cavities/pits indicating sunken stomata. Vascular tissue observed in the midrib region, in the form of xylem showing metaxylem towards the ventral surface and phloem with non-lignified cells at dorsal surface. The vascular tissue is surrounded by parenchyma cells. U epidermis shows anisocytic stomata whereas the lower epidermis shows anomocytic stomata (Figures 4-6).

Leaf Constants

- Stomatal Number: The stomatal number was calculated from three different fields on upper and lower epidermis separately. The results are given in Table 3 (Figure 7).
- Stomatal Index (I): The ratio of stomata and total epidermal cells was calculated as Stomatal Index = Number of Stomata/ (Number of Epidermal cells + Number of Stomata) X 100, and the observations are given in Table 4.
- Palisade Ratio: The palisade ratio was calculated and the results were recorded in Table 5 (Figure 8).
- Leaf Architecture (Figure 9 and Figure 10): Leaf organization is simple. With respect to leaf shape and size, the length of the whole leaf is 36 mm and the width is 12 mm. The lamina is symmetrical; base is symmetrical; form is elliptic lanceolate; apex is attenuate and base is acute normal. The margin is serrate. The leaf texture is coriaceous. There are no glands and the petiole is normal. The type of venation is pinnate reticulodromous. Primary vein (1^0) is massive; its course is straight and unbranched. Secondary veins (2^0) are present; angle of divergence is acute narrow. The variation in the angle of divergence is nearly uniform. The relative thickness of secondary veins is moderate; its course is curved and unbranched. Inter-secondary veins are composite. Intramarginal vein is absent. Tertiary veins (3^0) are present; angle of origin exmedial to admedial side is RO/OO/RR/OA*. The pattern is orthogonal reticulate and the course is simple and forked. The higher order venation forming a reticulum in which vein orders are distinct. Quaternary veins (4^0) are thin; its course is reticulate. Quintenary veins (5^0) are thin; its course is reticulate. The highest vein order of the leaf is 5^0. The marginal ultimate venation is fimbrial. Areoles are well developed; arrangement is random, and shapes are triangular, quadrangular, pentagonal. Veinlets are simple, linear or once branched.

  * RO(Right Obtuse)/OO (Obtuse Obtuse)/RR(Right Right)/ OA(Obtuse Acute)
- Trichome Density: The trichome density was calculated by Trichome Density = Number of Trichome/ (Number of Epidermal cells + Number of Trichome) X 100 and the results ere recorded in Table 3.

Table 3: Stomatal number and Trichome density

Field	*Number of Stomata per sq. mm*		*Trichome density*	
	Upper Surface	*Lower surface*	*Number Epidermal cells*	*Number of Trichome*
1	4	83	67	1
2	6	77	73	2
3	8	73	58	1
Stomatal Number/ Trichome density	6	77.66	1.98	

Table 4: Stomatal Index

Field	*Upper Epidermis*		*Lower Epidermis*	
	Stomatal Number	*Epidermal cell Number*	*Stomatal Number*	*Epidermal cell Number*
1	4	39	83	57
2	6	39	77	73
3	8	48	73	68
Stomatal Index	54.05		12.5	

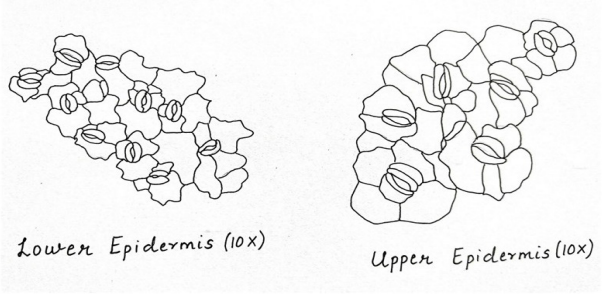


Figure 6: Stomata (Camera lucida drawing)

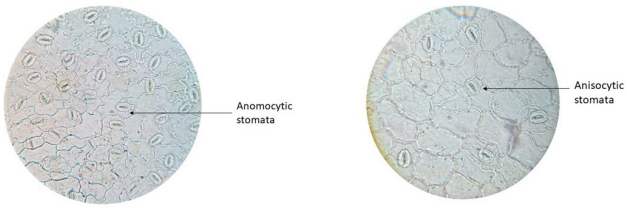


Figure 7: Stomata (40X)

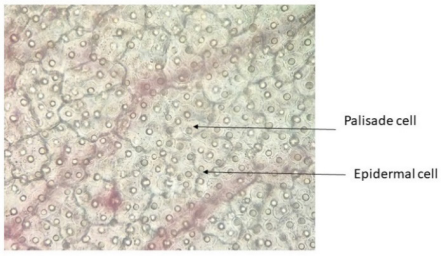


Figure 8: Palisade Ratio

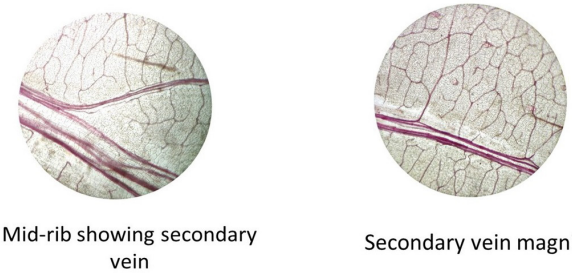
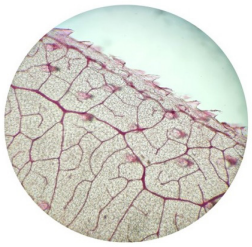


Figure 9: Leaf midrib showing Secondary vein and Secondary vein magnified

Table 5: Palisade Ratio

Field	*Number of Palisade cells beneath on epidermal cell per sq. mm*		
	Number Epidermal cells	*Number of Palisade cells*	*Palisade Ratio*
1	6	59	9.83
2	6	61	10.17
3	6	60	10
Palisade Ratio (Average)	9.83 – 10.17		



Leaf margin (10x)

Figure 10: Leaf margin showing trichome

Discussion and Conclusion

Despite the history of *S. anthelmia* in homeopathic medicine and being poisonous, till now less scientific research is done on the anatomical and pharmacognostical potential of this species. The literature review done for this study revealed that this was the first attempt made to study detailed anatomy of *S. anthelmia* although work has been carried out by Elufioye1 and Olaifa, 2015 and Awotedu, 2019 where microscopic studies were performed superficially and more emphasis was given on proximate analysis of the drug. Though the findings like presence of anomocytic stomata, and uniseriate trichome were found to be coinciding during this study. There is a controversy in their work regarding the presence of calcium oxalate and calcium carbonate crystals in the plant. During the current study, the presence of calcium oxalate crystals was confirmed. The detailed descriptions for plant parts will be of a great help in identification and standardization of this plant which is unavailable in the Homeopathic pharmacopoeia of India and in any of the literatures. Therefore, this study will be giving solutions against the problems such as adulteration and misuse of this drug using anatomy as a tool.

Acknowledgement

We deeply appreciate the kind help of Prof. (Dr.) Meenakshi Vaidya (Department of Botany, Mithibai College, Mumbai University) for guiding us in performing Palisade ratio, Leaf architecture and types of stomata.

References

Anonymous (2001). *The Ayurvedic Pharmacopoeia of India* Govt. of India Ministry of health and Family Welfare. The Controller of Publication New Delhi Reprint Vol 1.

Achenbach H, Hübner H, Vierling W, Brandt W and Reiter M (1995). Spiganthine, the cardioactive principle of *Spigelia anthelmia*. *Journal of natural products* ***58*****(7):** 1092-1096.

Awotedu OL and Ogunbamowo PO (2019). Evaluation of pharmacognostic and phytochemical profile of *Spigelia anthelmia* Linn. leaves. *Mod Phytomorphol* **13:** 41-46.

Awotedu OL, Ogunbamowo PO, Ariwoola OS and Chukwudebe EP (2020). Phytochemical and Phytomineral Status of *Spigelia anthelmia* Linn Leaves. *Int. J. Biochem. Res. Rev.* **29(2):** 33-40.

Bhogaonkar PY and Lande SK (2015). Anatomical characterization of Lepidagathiscristata Willd. A ethnomedicinal herb. *J. Glob. Biosci* **4:** 2282-2288.

Chachad DP and Vaidya M (2016). Stomatal studies on some selected plants of Malvaceae. *W. J. Pharma. Sci.* ***5***: 1060-1068.

Chanda S (2014). Importance of pharmacognostic study of medicinal plants: An overview. *J. Pharma. Phytochem.* ***2*****(5):** 69-73.

Danlami U, Cecilia OE and Ifeanyi OM (2017). Evaluation of the phytochemicals and antimicrobial activities of the ethanolic, hexane and ethyl acetate extracts of *Spigelia anthelmia* leaves. *Int. J. Pharma and Chem.* **3:** 29-32.

Dilcher DL (1974). Approaches to the identification of angiosperm leaf remains. *The botanical review* **40:** 109.

Elufioye OT and Olaifa AO (2015). Pharmacognostic evaluation of *Spigelia anthelmia* Linn (Loganiaceae). *European j. med. plants* ***8*****(2):** 87-96.

Fahn A (1997). Plant anatomy. Aditya Books Pvt. Ltd.

Foster A S (1952). Foliar venation in angiosperms from an ontogenetic standpoint. *Am. J. Bot.* 752-766.

Hegde G, Hebbar SS and Hegde G R (2013). *Spigelia* L. (Spigeliaceae/ Strychnaceae): Addition of a genus to the flora of Karnataka. *Res. Rev. J. Bot* ***2*****(2):** 9-11.

Hickey LJ (1973). Classification of architecture of dicotyledons leaves. *Amer. J. Bot* ***60*****(1):** 17-33.

Iwu MM (2014). *Handbook of African medicinal plants*. CRS Press Taylor and Francis Group 311-312.

Jackson BP and Snowdon DW (1990). *Atlas of Microscopy of Medicinal Plants culinary herbs and spices*. Stanely Thornes Publishers Ltd.

Khandelwal K (2008). *Practical pharmacognosy*. Pragati Books Pvt. Ltd.

Liang D, Jiang GB, Wang GT, Guo YN, Liu ZY and Wang RJ (2019). *Spigelia* L. (Loganiaceae), a newly recorded genus in China. *Phytotaxa* ***402*****(1):** 38-44.

Meena KL and Yadav BL (2010). *Spigelia anthelmia* L. (Spigeliaceae): A New Generic Record to the Flora of Rajasthan. *J. Indian Bot. Soc.* ***89*****(3&4):** 258-261.

Prakash PAGN and Gupta N (2005). Therapeutic uses of *Ocimum sanctum* Linn (Tulsi) with a note on eugenol and its pharmacological actions: a short review. *Indian J. of physiol. Pharmacol.* ***49*****(2):** 125.

Swamy J and Jalander V (2021). *Spigelia anthelmia* L. (Loganiaceae): A New Generic Record to the Flora of Telangana, India. *Indian Forester* ***147*****(6):** 596-597.

Wagner H, Seegert K, Odenthal KP, Espositoavella M, Villarreal E, Solis P and Gupta MP (1993). Preliminary pharmacologic evaluation of *Spigelia anthelmia* aerial parts. *Int. J. Pharmacogn.* ***31*****(1):** 7-14.