

Characterization and Comparison of Three Species of the Genus *Salacia*.

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ABSTRACT

Some of the plant species of the genus *Salacia* are considered as an important anti-diabetic drug in *ayurveda*. Among them *S. chinensis*, *S. fruticosa* and *S. oblonga* possess great medicinal importance, mostly because of its promising anti-diabetic activity. The increasing demand of this drug, resulted in a huge decline in its availability and it is replaced by spurious ones mainly because of lack of adequate quality standards. There is now a felt need to develop a systematic approach for the authentication of these plants and to develop well-designed methodologies for its standardization. The present study focused on the Pharmacopoeial parameters like pharmacognostical characterization and preliminary phytochemical screening of these three species. which were found to be sufficient to evaluate the raw material and can also be used as reference standards for the quality control/quality assurance purposes. The pharmacognostic study revealed that there are specific diagnostic features for distinguishing these three species among themselves and also from other related species. The external morphology of these three species are also shows variation in their colour, odour and taste. Both the species of *Salacia* ie, *S. chinensis* is having bitter taste and *S. fruticosa* is with astringent taste whereas *S. oblonga* has no characteristic taste of its own. The TLC profile showed similar pattern in *S. chinensis* and *S. fruticosa* where as *S. oblonga* showed more band in the profile than that of the other two. This reveals that the *S. chinensis* and *S. fruticosa* having similar compounds responsible for therapeutic efficacy, whereas *S. oblonga* has more chemical constituents.

Keywords: *Salacia chinensis*, *S. fruticosa*, *S. oblonga*, Pharmacognostical, Phytochemical, TLC, anti-diabetic.

INTRODUCTION

Diabetes is the commonest metabolic disorder and its frequency is on the increase all over the world. In India, diabetes is the one of the leading cause of ill health, mainly by the adoption of western life styles which leads to increase in the consumption of fast food, alcohol and a sedentary mode of life. All the systems of medicines possess various types of approaches for curing the disorders related to diabetes. Administration of various medicines internally is one among them. Some of the plant species coming under the genus *Salacia* are considered as an important anti diabetic drug in *Ayurveda* and other systems of Indian medicine. The genus *Salacia* comes under the angiosperm family Celastraceae. Among them *Salacia chinensis* L., *S. fruticosa* Wall. and *S. oblonga* Wall. possess great medicinal importance, mainly because of the anti-diabetic activity. *S. chinensis* is a straggler and flowers are greenish-yellow in colour. It is distributed mainly in Indo-Malaysia and found in sacred groves and moist deciduous forests in Kerala. Roots of *S. chinensis* is used in diabetes, haemorrhoids, leucorrhoea, leprosy, skin diseases, wounds, ulcers, liver disorders and used for alleviating *kapha* and *pitta*, amenorrhoea, dysmenorrhoea, spermatothoea and hepatothoea^{1,2}. *S. fruticosa* is a climber with greenish-yellow flowers. This endemic species is distributed in evergreen, semi-evergreen forests, sacred

groves of Western Ghats. Roots are useful in diabetes, liver disorders, ulcers and skin diseases. *S. oblonga* is a straggler with greenish-yellow flowers. It is distributed in India and Sri Lanka, in evergreen and semi-evergreen forests. Root bark is used in rheumatism, gonorrhoea, itches, asthma, and ear troubles, diabetes, hemorrhoids, leucorrhoea, leprosy, skin diseases, ulcers and flatulence³. Roots of *Salacia* spp. become one of the main ingredient in many of the ayurvedic antidiabetic formulations such as *Amrithameharichoornam*, *Katakakhadiradikashayam*, *Narayanagulam*, *Nirgundyadigulika*, *Manasamithravatakam* etc.

Though these *Salacia* species are well known for their antidiabetic activity, some another species are available in the market under the common name of the drug *pithika* (*Ekanayakam* in Malayalam). Pharmacognosy, an applied science, is concerned with the study of crude drugs of plant and animal origin. It aims at a complete and systematic knowledge of crude drugs of vegetables, animals or mineral origin. It implies not only drugs but also includes knowledge of sources, their history, properties and uses, distribution, cultivation, collection and selection.

In the pharmacognostical standardization of a plant-based raw drug, identification by macro and microscopic characters, physiochemical and photochemical (quantitative and qualitative) methods are the major steps.

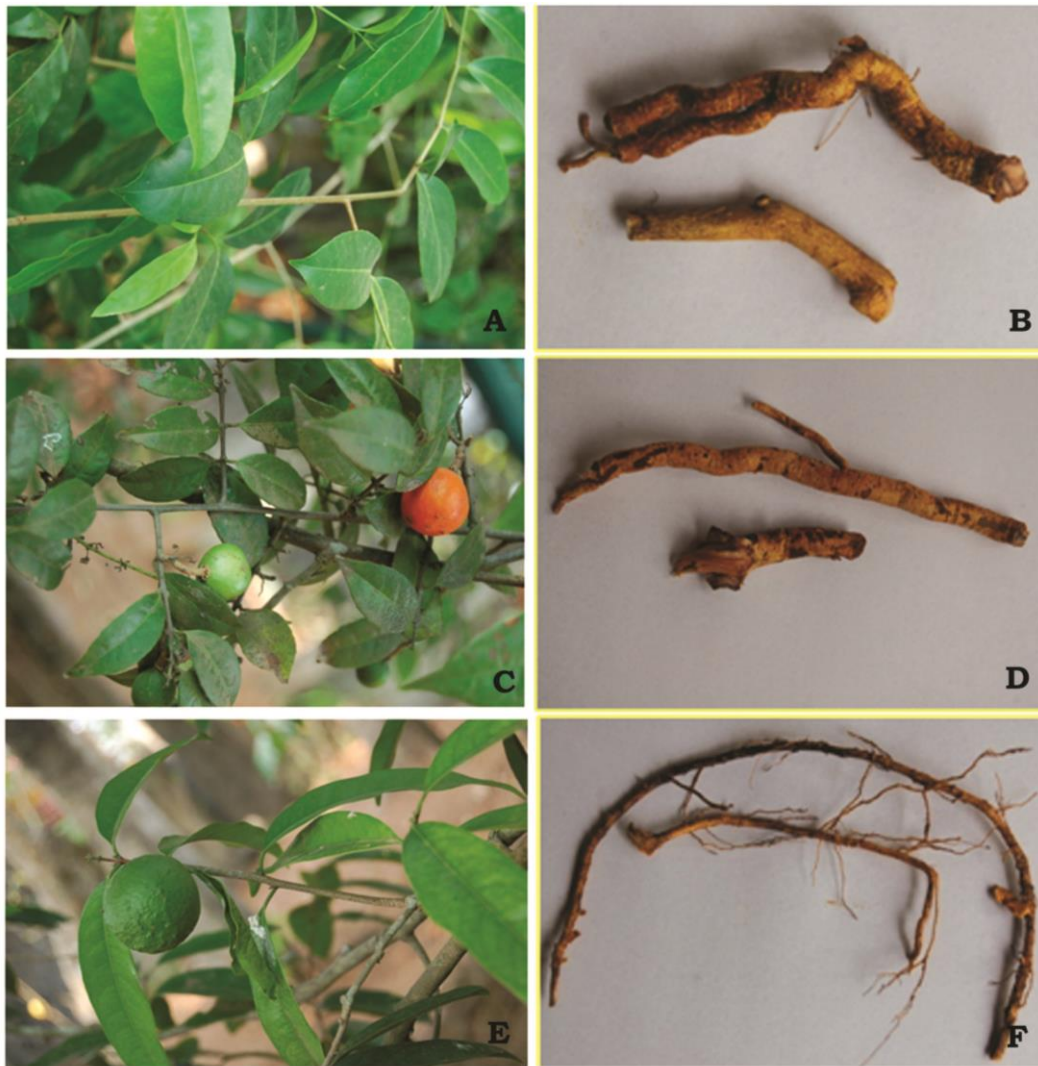


Plate 1 A & B: *Salacia chinensis* L. A. Habit - Fruiting branch; B. Root; C&D. *Salacia oblonga* Wall. C. Habit – Fruiting branch; D. Root, E&F. *Salacia fruticosa* Wall. E. Habit-Fruiting branch; F. Root.

Macroscopic identification involves the morphological and organoleptic characterization of the crude drug. Microscopic characterization involves anatomical and powder characteristics. Morphological characters involve size, shape, texture, surface characters, and markings of the plant material. Appearance, hardness, odour, taste and nature of fracture together constitute the organoleptic characters. Morphological and anatomical characters play a vital role in crude drug standardization⁴. As stated by the Metcalfe and Chalk⁵, microscopical methods are often necessary to establish the botanical identity of commercial samples of medicinal plants, timbers, fibers *etc.*, and may play an important part in checking adulteration and substitution.

MATERIALS AND METHODS

Materials used

Plant materials were collected from various sacred groves of Thrissur and Malappuram Districts of Kerala State. The useful part *ie*, roots were collected along with the plant and kept it for drying in shade. For anatomical studies roots

were fixed in FAA (Formalin-Acetic acid-Alcohol mixture) immediately after collection.

Method of study

Botanical study

Material processing and slide preparation were carried out as per the Indian Pharmacopoeial standard methods⁶. All the materials for anatomical study were fixed in FAA. Hand sections and microtome sections were taken and histological and histochemical staining was carried out as per standard methods⁷. The sections were stained with safranin and fast green for general studies and Sudan black for oil, iodine for starch, Ferric chloride for tannin and KOH for antraquinone⁸. Maceration was carried out by Jaffery's method. Quantitative values such as measurements of the xylem vessel elements and fibres were taken using micrometer scale. The data were subjected to statistical analysis. Photomicrographs were taken using 'Canon Photospot G3 camera, attached to the Ziess microscope.

Phytochemical studies

Roots were shade dried and coarsely powdered. Physicochemical parameters such as moisture content,

Table 1: Comparative macroscopic and microscopical studies of three species of *Salacia*.

Parameters observed	<i>S. chinensis</i>	<i>S. fruticosa</i>	<i>S. oblonga</i>
Macroscopy	The root is dark yellow externally and internally light yellow colour. It has a characteristic odour and bitter in taste	The root is Yellowish brown externally with circular striations and smooth. Characteristic odour with astringent taste	The root is pale yellow externally, with easily peelable bark, and the cut end with yellow colour. No characteristic taste and odour
Cork	Cells are suberised and rectangular in shape which is highly thickened.	Cork cells are 5-6 layers, cells are tangentially elongated rectangular cells with dark brown deposits in the outer most layers. Inner layers contain yellowish depositions	Cork is thick walled, oval; cork cambium is radially elongated, square shaped.
Cortex	8-12 rows of oval or rounded thick walled cells with brown colour content. All the cells are fully filled with starch grains	Cortical cells are thick walled, oval, round and tangentially elongated, with brown content and starch grains	Stone cells are present in the cortex, cortical cells slightly thick, tangentially elongated and oval shaped. Presence of ceratenchyma cells or obligatory cells traverses through the cortex.
Phloem	Wide, is transversed with uniseriate phloem ray	Comparatively more wide, traversed with phloem groups of phloem fibres and uniseriate and triseriate phloem rays	Phloem ray is present with rosette crystals.
Xylem	Xylem tissue is traversed with 5-7 concentric rings of non lignified parenchymatous tissues. Vessels solitary and in groups of 2 to 3	Xylem composed of usual xylem elements. Vessels solitary rarely in groups of 2	Xylem composed of usual xylem elements. Xylem cells are mostly solitary rarely in groups of 2 to 3. Vessels are comparatively large and some of them contain reddish brown depositions
Xylem rays	Xylem rays uniseriate, rarely biseriate	Xylem rays uniseriate, rarely biseriate and triseriate	Xylem rays uniseriate, oval in shape. Many of the medullary ray cells contain red contents and starch grains
Cell inclusions	Brown coloured contents in medullary rays, cortex region and in pericycle region Rosette crystals of calcium oxalate in cortex and phloem cortical cells, ray cells	Rosette crystals and acicular (needle like) crystals are found in wood parenchyma. A powdery substance is present in it. Cortex is with brown content and starch grain. No crystals are present in phloem.	Starch grains compound or simple. Rosette crystals are present in the cortex and phloem. Prismatic crystals are also present in cortex.

Table 2: Comparative quantitative studies of roots of three *Salacia* species.

Name of the Species	Vessels		Fibres	
	Length (µm)	Width (µm)	Length (µm)	Width (µm)
<i>S. chinensis</i>	293.14 ± 137.5	93.29 ± 31.04	290.43 ± 114.3	18.28 ± 5.47
<i>S. oblonga</i>	294.71 ± 159.34	39 ± 22.91	366.71 ± 112.65	17.71 ± 3.35
<i>S. fruticosa</i>	394.28 ± 187.89	120.43 ± 58.68	487 ± 161.19	17 ± 3.65

water soluble extractive, alcohol soluble extractive, ash value and acid insoluble ash were studied as per the standard procedure¹. Powdered roots (5gm) were extracted with methanol using the Soxhlet. The extracts were filtered, concentrated and made up to 10 ml. This extract was used for TLC profiling.

Thin layer Chromatography

TLC analysis of the methanolic extract of the roots of the three species was done on pre-coated silica gel 60F₂₅₄ plates (E. Merck) of uniform thickness of 0.2 mm using the solvent system Toluene: Chloroform: Methanol (8: 1: 1). The solvents used were Gr quality (E. Merck). The developed plate was derivatized using Anisaldehyde Sulphuric acid reagent. *R_f* value of major bands were determined.

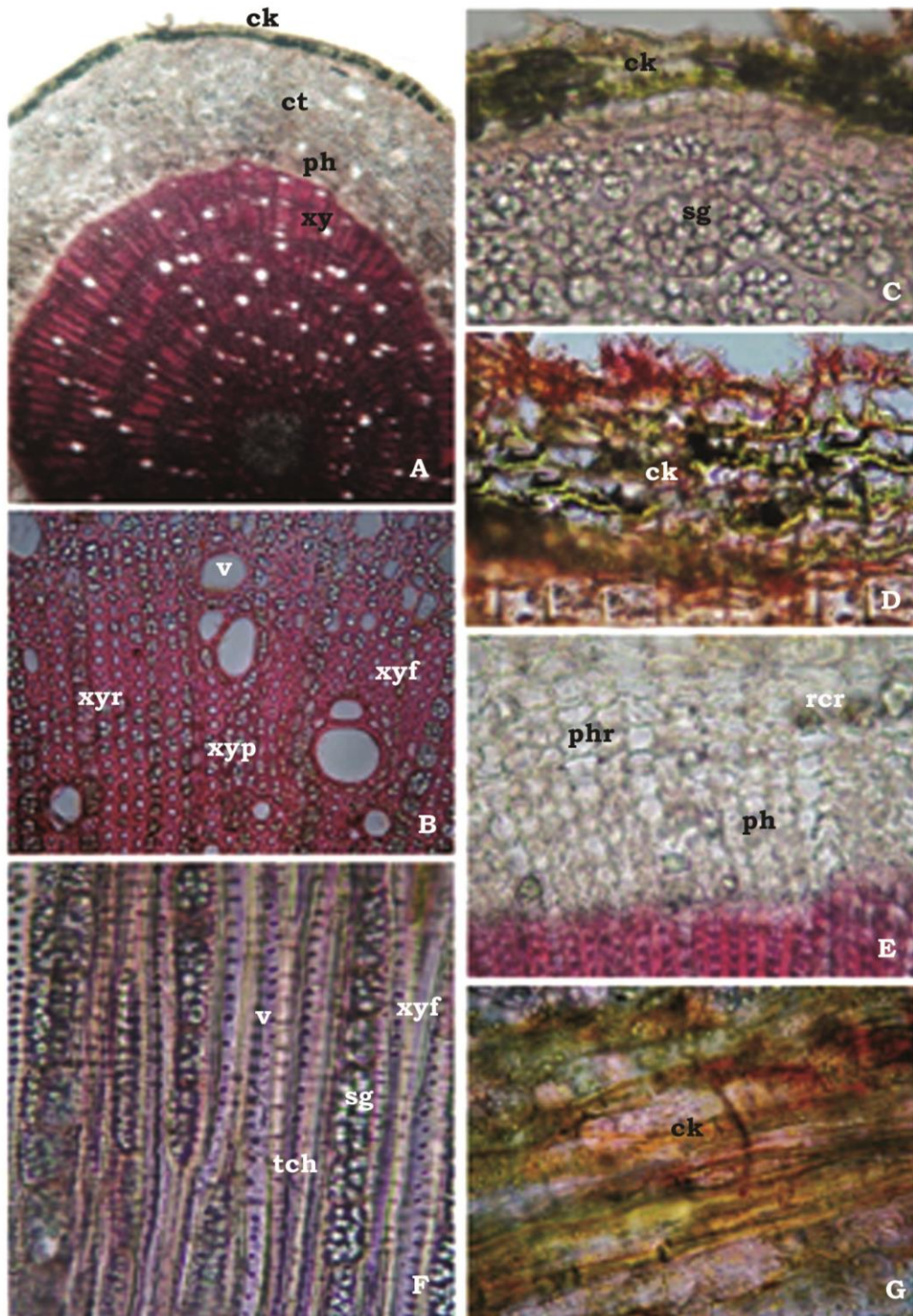


Plate 2: Microscopic characters of *S. chinensis* root . A. TS of root x 200 B. Wood portion enlarged x 400. C. Cortex region enlarged x 400; D. Cork region enlarged x 400; E. Phloem region enlarged x400; F. Longitudinal view of xylem, G. LS of cork. ck, cork; ct, cortex, ph, phloem; phf, phloem fibres; rcr, rosette crystals of calcium oxalate; sg, starch grains; tch, tracheids; v, vessels; xyf; xylem fibre; xyp, xylem parenchyma; xyr, xylem ray.

Table 3: Comparative physicochemical parameters of *Salacia* spp.

Name of plant	Water soluble extractive %	Alcohol soluble extractive %	Total ash %	Acid insoluble ash %	Moisture content %
<i>S.chinensis</i>	10.86	13.54	3.33	2.18	9.96
<i>S.oblonga</i>	12.7	13.24	4.03	2.47	11.35
<i>S.fruticosa</i>	19.28	24.66	2.29	2.65	9.16

RESULT AND DISCUSSION

Taxonomy and morphology

Salacia chinensis Linn.

A small erect or straggling tree large, woody, climbing shrub found Leaves ovate to lanceolate; flowers 2-6, clustered together on axillary tubercles, yellowish; fruit small globose, 1-2 cm in diameter, red when ripe one-

seeded; seeds so rounded by an edible pulp. Ripe fruits are eaten⁹ (Plate1 A & B).

Salacia fruticosa

S. fruticosa is a climber with greenish yellow coloured bark. It is endemic to Western Ghats and found in the evergreen, semi-evergreen forests, sacred groves and in plains¹⁰ (Plate.1 C&D).

Salacia oblonga

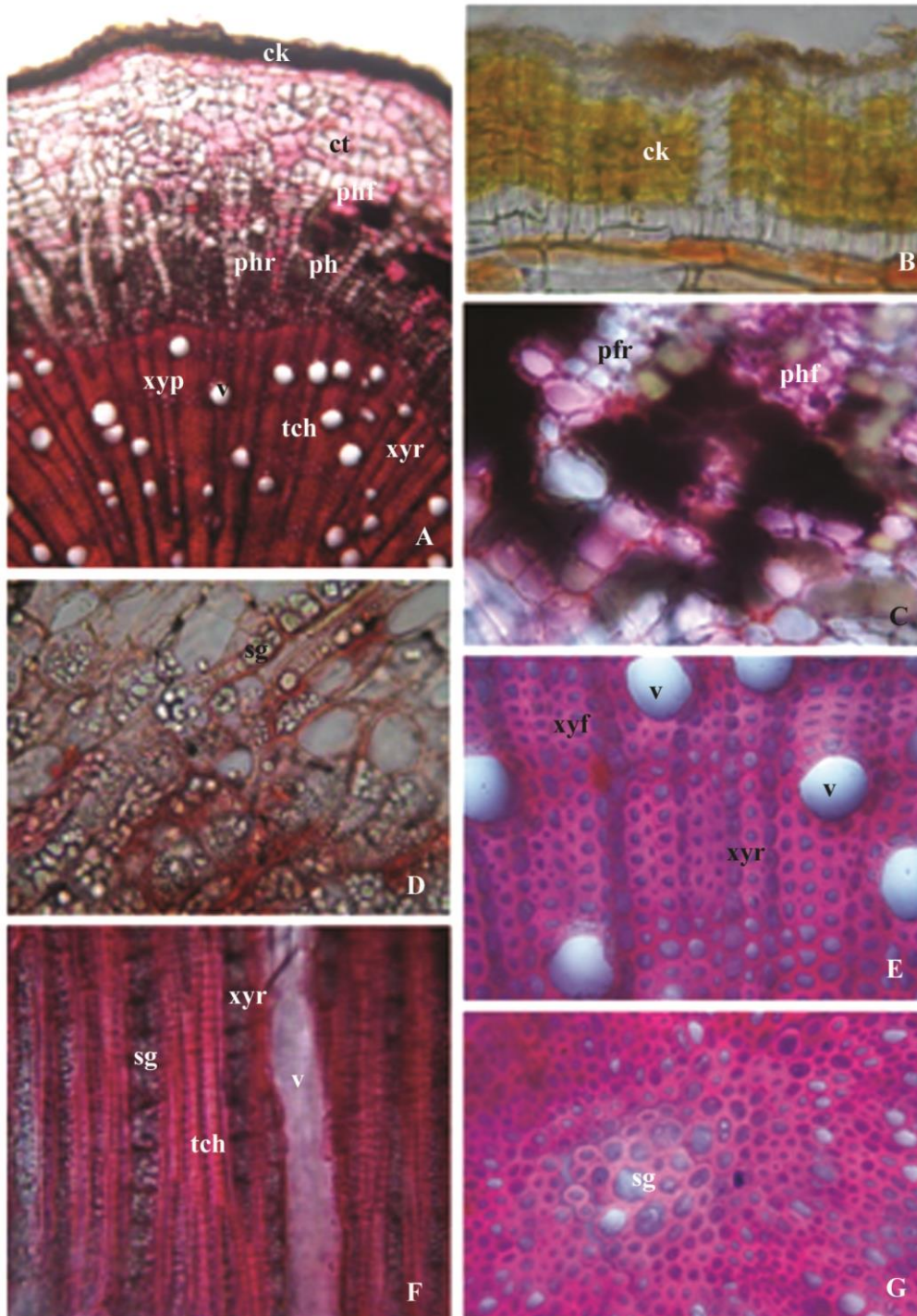


Plate 3: Microscopic characters of *S. fruticosa* root. A. TS of root x 200 B. Cork region enlarged x 400. C. Phloem region enlarged x 400; D. Cortex region enlarged x 400; E. Wood region enlarged x400; F. Longitudinal view of xylem elements, G. Central portion of the root x 400. ck, cork; ct, cortex, ph, phloem; phf, phloem fibres; rcr, rosette crystals of calcium oxalate; sg, starch grains; tch, tracheids; v, vessels; xyf; xylem fibre; xyp, xylem parenchyma; xyr, xylem ray.



Plate 4: Microscopic characters of *S. oblonga* root . A. TS of root x 200 B. Cork region enlarged x 400. C. Cortex region enlarged x 400; D. Phloem region enlarged x 400; E. Cent region enlarged x 400; F. Wood region x 400. ck, cork; ct, cortex, ph, phloem; rcr, rosette crystals of calcium oxalate; sg, starch grains; st, stone cells; tch, tracheids; v, vessels; xyf, xylem fibre; xyp, xylem parenchyma; xyr, xylem ray.

Salacia oblonga Wall ex wight is a climbing shrub. Leaves ovate or ovate-lanceolate; flowers greenish yellow, in short congested cymes, fruits globose, 3 cm in diameter, tuberculate, light brown or orange when ripe, seeds 1-8, angular, imbedded in pulp⁹ (Plate 1 E&F).

Distribution

S. chinensis is distributed almost throughout India including Andaman and Nicobar Islands thriving along seashore and river banks as well as in forests at altitudes up to 750m^{9,11}.

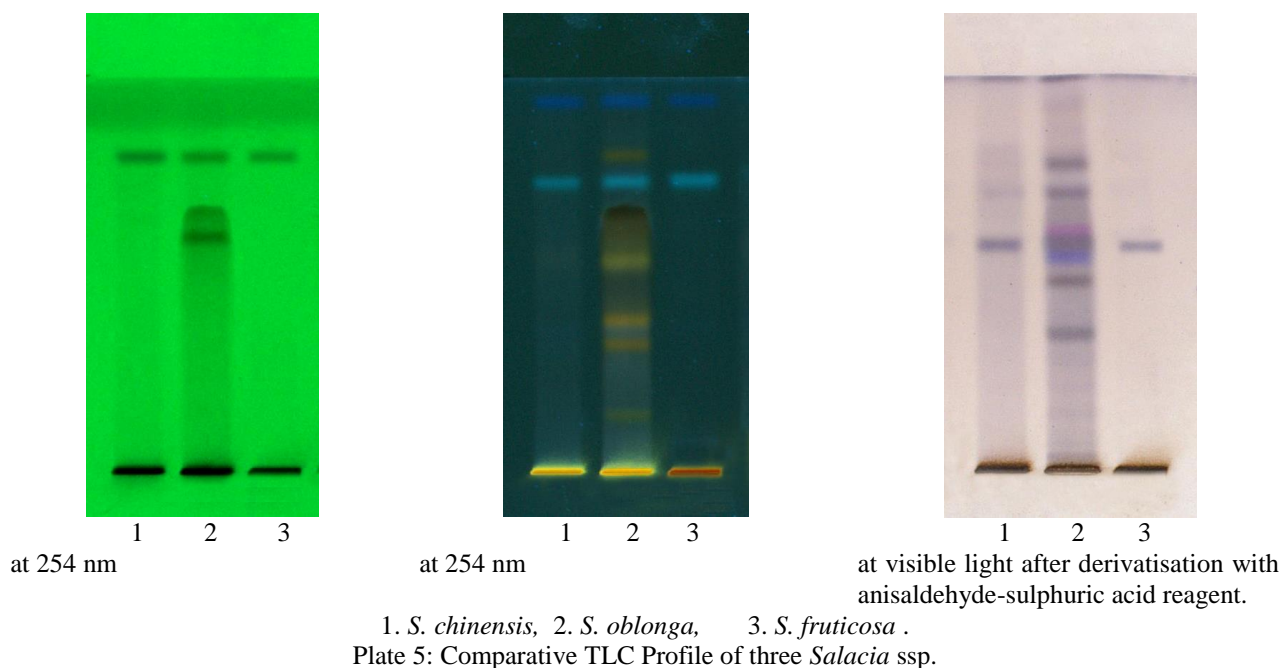


Table 4: Comparative R_f values of TLC Profile of three *Salacia* spp.

R_f values of *Salacia* spp. studied (UV at 254 nm)

<i>Salacia chinensis</i>		<i>Salacia oblonga</i>		<i>Salacia fruticosa</i>	
R _f value	Colour	R _f value	Colour	R _f value	Colour
0.80	Dark green	0.60	Dark green	0.80	Dark green
		0.67	Dark green		
		0.80	Dark green		
R _f values of <i>Salacia</i> spp. Studied (UV at 366 nm)					
0.73	Light Blue	0.15	Light green	0.73	Light blue
		0.33	Brown yellow	0.93	Dark blue
		0.39	Pale yellow		
		0.53	Yellow		
		0.66	Dark green		
		0.73	Light blue		
		0.80	Dark yellow		
		0.93	Dark blue		
		0.93	Dark blue		
R _f values of <i>Salacia</i> spp. studied (After Derivatization)					
0.59	Dark blue	0.34	Dark blue	0.59	Dark blue
		0.48	Dark blue		
		0.54	Violet		
0.70	Light Blue	0.59	Dark blue		
		0.61	Purple		
0.83	Blue	0.70	Light blue		
		0.78	Dark blue		
		0.87	Pale blue		

The plant *Salacia fruticosai* s widely distributed, throughout in India¹².

S. oblonga is a woody climber, distributed in Sri Lanka and southern regions of India^{13,14}. It is also reported in other south-east Asian countries¹⁵. It is called as *Chundan* in Tamil and *Ponkoranti* in Malayalam. *S. oblonga* is found in the rain forests of Western Ghats from south Konkan to southwards.

Macroscopic and microscopic comparison of the roots of three species of *Salacia* were carried out and the results are shown in table- 1 and plates 2-4.

In the comparative maceration study it was observed that roots of *S. fruticosa* have large vessels with a length of $394.28 \pm 187.89 \mu\text{m}$ and a width of $120.43 \pm 58.68 \mu\text{m}$ and long fibres were also observed in *S. fruticosa* ($487 \pm 161.19 \mu\text{m}$) but the width of fibres is large in *S. chinensis* which was $18.28 \pm 5.47 \mu\text{m}$. The dimensions of xylem vessels and fibres of the three *Salacia* species studied are given in table 2.

Phytochemical Studies

Preliminary phytochemical studies

The comparative study on physicochemical parameters revealed that *S. fruticosa* showed high values for water

soluble and alcohol soluble extractives and they were 19.28% and 24.66% respectively. Total ash value and moisture content were found to be high in *S. oblonga* which were 4.03% and 11.35% respectively, but the acid insoluble ash was high in *S. fruticosa* (2.65%) (Table 3).

Comparative TLC Profile of three *Salacia* spp.

From the comparative TLC of the three species of *Salacia* shows that the *S. chinensis* and *S. fruticosa* shows almost similar profile pattern, where as *S. oblonga* shows more spots than the other two.

DISCUSSION

Comparative anatomical characters in the case of three species of *salacia*, that is *S. chinensis*, *S. fruticosa* and *S. oblonga*, which are employed as a raw – drug in the Ayurvedic medicine manufacturing process. The present study is a preliminary investigation on the anatomical and chemical differences between these three species. The observations are presented and discussed below under appropriate heads.

The root of *S. chinensis*, is dark yellow externally and internally light yellow in colour. It has a characteristic odour and bitter in taste. The T.S of *S. chinensis* root show, many layered, suberised cork, cells rectangular, highly thickened lower tangential walls, and multi storied cork cells are present. Starch cells are present in the cork region. Brown coloured contents are present in Medullary rays, in cortex region and in pericycle region. Prismatic and rosette crystals are present in cork and phloem. Cortex is filled with dark brown content.

The root of *S. fruticosa* is yellowish brown externally with circular striations and smooth. Characteristic odour with astringent taste is noted. The T.S shows that the cork cells are tangentially elongated rectangular cells with dark brown deposits in the outer most layers. Cells are thick walled. Starch grains are also present in cortex region. The presence of a powdery substance is also observed. substitute

The root of *S. oblonga* is pale yellow externally, with easily peelable bark, and cut end with yellow colour. No characteristic taste and odour is noted. T.S shows thick walled cork cells. Stone cells are present in the cortex, obligatory cells traverse through the cortex, which was not reported so far. Many of the cells contains red contents, starch grains can be compound or simple present. Rosette crystals are present in the cortex and phloem. Presence of prismatic crystals also observed in cortex.

The comparative maceration studies of these three species shows, *S. fruticosa* have large vessels with a length of $394.28 \pm 187.89 \mu\text{m}$ and a width of $120.43 \pm 58.68 \mu\text{m}$ and long fibres were also observed in *S. fruticosa* ($487 \pm 161.19 \mu\text{m}$) but the width of fibres is large in *S. chinensis* which was $18.28 \pm 5.47 \mu\text{m}$.

In the comparative study on physicochemical parameters revealed that *S. fruticosa* showed high values for water soluble and alcohol soluble extractives and they were 19.28% and 24.66% respectively. Total ash value and moisture content were found to be high in *S. oblonga* which were 4.03% and 11.35% respectively, but the acid insoluble ash was high again in *S. fruticosa* (2.65%).

From the comparative TLC of the three species of *Salacia* shows that the *S. chinensis* and *S. fruticosa* shows almost similar profile pattern, whereas *S. oblonga* shows more spots than the other two.

CONCLUSION

The three species of *Salacia* ie, *Salacia chinensis*, *Salacia fruticosa* and *Salacia oblonga* are used as an antidiabetic drug in ayurveda. The present study is a preliminary to analysis the anatomical and histochemical differences between the three species. The external morphology of these three species is also dissimilar in their colour, odour and taste. Both the species of *Salacia* ie, *S. chinensis* is having bitter in taste and *S. fruticosa* is with astringent taste where as *S. oblonga* has no characteristic taste of its own. The present study also showed that the three species show significant differences in histochemical and anatomical level. But the TLC shows similar profile pattern in *S. chinensis* and *S. fruticosa* where as *S. oblonga* shows more spots than the other two. This reveals that the *S. chinensis* and *S. fruticosa* having similar compounds responsible for therapeutic efficacy, where as *S. oblonga* has more chemical constituents and should keep apart from the other two species and cannot be used as substitute of the other two species.

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