Sabaragamuwa University Journal
Volume 19 Number 1 August 2021 pp 28-41
ISSN 1391-3166 eISSN 2386-2041
http://dx.doi.org/10.4038/suslj.v19i1.7771

Morphological and Qualitative Phytochemical Comparison of Selected *Salacia* Species in Lowland Wet Zone of Sri Lanka

Semini Mudara Amarathunge* and D.K.N.G. Pushpakumara

Postgraduate Institute of Agriculture, University of Peradeniya, Sri Lanka

Department of Crop Science, Faculty of Agriculture, University of Peradeniya, Sri Lanka

*seminiamarathunga@gmail.com

Abstract

Many medicinal plants in the world are in trouble from over harvesting and destruction of habitat. Population growth, urbanization and the unlimited collection from the wild are the causes for the over-exploitation of these natural resources. Therefore, the management of the traditional medicinal plant in Sri Lanka has become a matter of necessity too. The genus *Salacia* has great importance as it contains a high medicinal value that is mostly used in traditional medicine to cure diabetes. Scientific information and knowledge about them are scant and their current usage is limited relative to their economic potential. It is necessary to examine their morphology and chemical composition as they have received a less attention for conservation and management. In Sri Lanka, the genus *Salacia* is represented by five species and only *Salacia chinensis*, *Salacia oblonga*, and *Salacia reticulata* were considered in this study. A morphological study using the specimens collected from natural habitats was done. The selected *Salacia* species could be three different species in the same genus. Anatomical studies were carried out by obtaining stem cross-sections. It also revealed that there are differences in hypodermis of selected *Salacia* species too. Qualitative phytochemical screening by using the air-dried and powdered stems was carried out to identify the different chemical compounds in water. The study confirmed the presence of tannins, terpenoids, and saponin. TLC profiling of these species was carried out using methanol extracts and it showed different R_f values. Further, the generated results could be effectively utilized for the consistency of *Salacia* species for the improvement of the Sri Lankan pharmacopeia.

Keywords: Anatomical Studies, Medicinal Value, Morphological Study, Phytochemical Screening, *Salacia* Species

Introduction

Dependency on Ayurvedic medicine is particularly high among the rural and poor populations in Sri Lanka. Production of Ayurvedic herbal products has been increased claiming a speedy raw material supply. A high percentage of herbal raw materials is supplied to the local market for Ayurvedic medicinal productions from forests. Reasons for overexploitation of these natural resources are population growth, urbanization, and the limitless collection of medicinal plants from the wild (Sharmal *et al.*, 2010). Hence, many of the medicinal plant species are becoming extinct and commercial exploitation of the important species is yet to be developed in Sri Lanka. These human activities have significantly transformed the ecosystems of lowland in Sri Lanka too. A unique feature of wet zone flora in Sri Lanka is a high degree of endemism. Wet lowlands have the highest number of endemic plant species among all the climatic zones than the other regions in Sri Lanka (Peeris, 1975; Gunatilleke and Gunatilleke, 1990). Lowland wet zone represents the area below 1,000 m in elevation and also it spreads in the southwestern quarter of Sri Lanka. Plant species of the lowland wet zone of Sri Lanka are largely distributed in the tropical lowland rainforests where elevation below 1,000 m altitude. These forests are confined to 2.14% (141,506 ha) of the total land area of the country (Ministry of Forestry and Environment, 1999).

According to National Red List 2012, *S. reticulata, S. acuminatissima, S. diandra*, and *S. oblonga* is endangered while *S. chinensis* is near threatened (MOE, 2012). It is a large genus with creeping or climbing shrubs or often small shrubs. According to National Red List 2012, the genus *Salacia* which belongs to the family Celastraceae is represented by five species, *Salacia chinensis* L. [*Salacia prinoides* (Wild.) DC.] (Heen Himbutu wel), *Salacia oblonga* Wall. Ex Wight & Arn. (Gal Himbutu), *Salacia reticulata* Wight. (Himbutu wel/Kothala Himbutu), *Salacia acuminatissima* Kosterm., spec. Nov. (celastr) and *Salacia diandra* Thw. in Sri Lanka.

There has been an ever increased demand from developed countries for many drugs from the plant sources during the past decade and multi-national companies try to produce medicines and nutritious products using resources from countries like Sri Lanka. Numerous Japanese patents privilege anti-diabetic properties. Consequently, degrading these patents would restrict Sri Lanka from exporting any synthetic or natural value added anti-diabetic inventions with *S. diandra* (Karunaratne, 2013). It is not a secret that problems have been arising in taking independent patents. Countries like Japan and the United States have investigated Kothala Himbutu and several publications also have been made with no reference to Sri Lankan participation (Pushpakumara *et al.*, 2002).

According to Osaki *et al* (2007), constituents of *Salacia* species are abundant and may vary depending on the type of species and place of origin. Katalanol is the most active component in the aqueous extracts of *S. reticulata*, which are traditionally used in India, Sri Lanka, and Thailand for the treatment of diabetes (Yoshikawa *et al.*, 1997; Yoshikawa *et al.*, 2002). Yoshikawa *et al* (2003) reported that the roots of *S. chinensis* contains active compounds such as triterpenes, phenolic compounds, glycosides and coloring agents. According to him, *S. chinensis* show α-glucosidase inhibitory activity too. The roots and stems of *S. reticulata* and the roots of *S. oblonga* are a specific remedy for the initial stages of diabetes in traditional Ayurvedic medicine (Flammang *et al.*, 2006; Jennifer *et al.*, 2007; Ratnasooriya *et al.*, 2003). Sharmal *et al* (2010) stated that *S. oblonga* contains Salacinol and Katalanol which are a-Glucosidase inhibitors. It is also an effective anti-diabetic agent that is superior in terms of safety to chemically synthesized products.

In Sri Lanka, experienced, scientific and technical expertise in multiple-use forest management systems are currently lacking (De Zoysa and Inoue, 2008). This study aims to reveal the interspecific relationships of selected *Salacia* species based on the variation in the qualitative phytochemical screening and phenotypic characters. *S. reticulata, S. oblonga* and *S. chinensis* are very similar in appearance. Therefore, they are used in traditional medicinal practices misleadingly. *S. reticulata* is overused, and also *S. oblonga, S. chinensis* are misleadingly used to treat diabetes. Accurate identification of *S. reticulata, S. oblonga* and *S. chinensis* using morphological data and anatomical data will help avoid the use of *S. oblonga* and *S. chinensis* instead of *S. reticulata*. Also, the evaluation of phytochemical approaches will be used to accurately distinguish those *Salacia* species. *S. reticulata* is being depleted in Sri Lanka due to over-usage.

Therefore, the conservation of this valuable plant is very important. Also for conservation purposes, correct identification of *S. reticulata, S. oblonga* and *S. chinensis* is very crucial. Since Sri Lankan Ayurvedic practitioners use *S. reticulata* to treat diabetes, these findings will be an innovation in Sri Lankan Ayurvedic medicine. Due to the increasing popularity of using *Salacia* species as an ingredient in traditional therapeutic systems, phenotypic characterization and phytochemical screening of different species is a timely requirement. The current study compares the morphological and phytochemical activity of *S. chinensis* with *S. reticulata* and *S. oblonga*.

Methodology

Site Selection

Tree samples of *S. chinensis, S. oblonga* and *S. reticulata* were collected from Kamburupitiya, Hakmana, and Pinnaduwa respectively after obtaining permission from the Department of Wildlife Conservation, Sri Lanka.

Preparation of Herbarium Sheets of Selected *Salacia* Species

Herbarium specimens were prepared by leaf samples of *S. chinensis* (Tag number 1), *S. oblonga* (Tag number 2) and *S. reticulata* (Tag number 3) and were authenticated by comparing with available classical texts and certified herbarium specimens available at the Department of National Botanic Gardens, Royal Botanic Gardens. Prepared herbarium sheets of *Salacia* species were deposited in the Department of National Botanic Gardens, Royal Botanic Gardens, Peradeniya, Sri Lanka.

Morphological Characters of Selected *Salacia* Species

Habitat of three selected *Salacia* species was recorded. Twenty plants of each species from each location were used to record morphological data. Morphological characteristics of leaves such as leaf color, leaf lamina shape, the shape of the leaf base, leaf margin, the shape of the leaf apex, leaf arrangement, venation and nature of leaf surface of three selected *Salacia* species were observed and recorded by comparing with available classical texts. Inter-nodal length, petiole length, maximum lamina width and maximum lamina length were measured using a measuring tape. The maximum length and width of lamina were measured from nine leaves for each tree. Leaf area was measured using graph papers. Morphological characters of the stem such as color, marks on the stem of three selected *Salacia* species were observed and recorded. Morphological characters of the root such as the color of three selected *Salacia* species were observed and recorded. Morphological characters of the fruit such as fruit shape, the number of seeds per fruit of three selected *Salacia* species were observed and recorded by comparing with available classical texts. The maximum fruit diameter was measured for each ripened fruit in three fields. The mean fruit diameter was calculated for three *Salacia* species separately. Floral characters such as calyx, corolla, androecium and gynoecium of three selected *Salacia* species were observed and recorded the nature and number of floral parts by comparing with available classical texts. The floral formula and floral diagram of three selected *Salacia* species were derived.

Stem Anatomy

Five plants from each species were used to record anatomical data. Transverse sections were taken from matured stems of selected *Salacia* species by using sharp blades. Those were mounted on the grease-free microscopic slides with glycerin and covered with a cover-slip and were examined through a light microscope (Brand: SVBONY, Model: SV601, Magnification: 40X-1600X, Country of origin: China).

Characteristics of Stem Powder of Selected *Salacia* Species

Five plants from each species were used to identify the characteristics of stem powder. Stem powder of three selected *Salacia* species was made by grinding small stem pieces separately. Pinch matured stem powder of selected *Salacia* species was placed on the grease-free microscopic slides along with a drop of glycerin and water (1:1) by staining with safranin and then it was covered with a clean coverslip, observed under the light microscope (Brand: SVBONY, Model: SV601, Magnification: 40X-1600X, Country of origin: China).

Thin Layer Chromatography (TLC) of Selected *Salacia* Species

A more detailed phytochemical analysis by TLC was conducted to evaluate any significant difference between the three *Salacia* species. Twenty plants from each species were used to record qualitative data. Five grams of *S. chinensis, S. oblonga* and *S. reticulata* matured stem powder sample was weighed and extracted with dichloromethane overnight following cold extraction protocols separately. TLC was observed under UV at 366 nm, 254 nm (Biradar and Rachetti, 2013) after spraying vanillin sulfuric.

Mobile phase: Methanol 0.1: Dichloromethane 9.9 (double developed)
The movement of the active compound was expressed by the retention factor (R_f).
R_f value = Distance traveled by compound from the origin/Distance travelled by solvent from the origin

Qualitative Phytochemical Screening of Selected *Salacia* Species

Twenty plants from each species were used to record qualitative data. All quantitative results are means of 20 replicates per plant and ten individuals per each *Salacia* species were used. The matured stem of selected *Salacia* species was thoroughly washed and air dried, powdered using a grinder and the powder (20 g) was extracted. Water extracts were obtained by boiling (20 g) of powdered stem materials of each specimen in 50 ml of distilled water and were filtered. All the extracts were subjected to preliminary phytochemical screening in standard procedures for the stem of *Salacia* species (Khandelwal, 2008).

Determination of Tannins of Selected *Salacia* Species - A few drops of 0.1% $FeCl_3$ were added to 2-3 ml of *S. chinensis*, *S. oblonga* and *S. reticulata* stem extract. Brownish green or a blueblack coloration indicated the presence of tannins.

Determination of Saponins of Selected *Salacia* Species - *S. chinensis, S. oblonga* and *S. reticulata* stem extracts were vigorously shaken to obtain a stable persistent froth. Then mixed the frothing with 3 drops of olive oil. Formation of emulsion indicated the presence of saponins.

Determination of Flavonoids of Selected *Salacia* species- A few drops of 1% NH_3 solution were added to the *S. chinensis, S. oblonga* and *S. reticulata* stem extract in a test tube. A yellow coloration indicated the presence of flavonoids.

Determination of Terpenoids of Selected *Salacia* Species- Extract of *S. chinensis, S. oblonga* and *S. reticulata* stem sample (5 ml) was mixed with 2 ml of $CHCl_3$ in a test tube carefully and was added 3 ml of concentrated H_2SO_4 to the mixture to form a layer. An interface with a reddish-brown coloration indicated the presence of terpenoids.

Determination of Alkaloids of selected *Salacia* Species - Mayer's test – 1 ml of Mayer's reagent (Potassium bismuth iodide solution) was added to the 1 ml of *S. chinensis, S. oblonga* and *S. reticulata* stem extract. Whitish yellow or cream-colored precipitate indicated the presence of alkaloids.

Results and Discussion

Morphological Characters of *S. chinensis, S. oblonga* and *S. reticulata*

According to the results, *S. chinensis, S. oblonga* and *S. reticulata* are terrestrial woody shrubs with a similar appearance. According to the results, *S. chinensis* has opposite leaf arrangement, leaves with ovate shape, acute base, acuminate apex with entire margin, unicostate-reticulate venation and without leaf curlings. *S. oblonga* have opposite leaf arrangement, leaves with thinly coriaceous leaf surface with a slightly glossy upper surface, lanceolate shape, acute base, unicostate-reticulate venation, shortly acuminate apex and entire margin. *S. reticulata* has the opposite leaf arrangement, leaves with coriaceous, glabrous leaf surface with a shining upper surface, light color under the surface of the leaf, greenish-yellow color soft petiole, ovate shape, acute base, paler with unicostate-reticulate venation, abruptly acuminate apex, margin with minute rounded teeth and leaf curlings (Plate 1 and Table 1).

These *Salacia* are used to treat illnesses in ayurvedic medicine in Sri Lanka (Arunakumara and Subasinghe, 2010). All the *Salacia* species are more or less morphologically similar except for a few characters as reported by Dhanasiri *et al* (2013).

The length of a leaf of *S. chinensis* is about 4.2-10.5 x 2.2-4.0 cm. Leaves of *S. oblonga* are elliptic-oblong or oblong-lanceolate, the length is about 4.8-11.2 x 2.2-5.2 cm. *S. reticulata* is a woody climber or scandent shrub. It is dichotomously branched and the stem is relatively tough (Dissanayake and Clayton, 1996). The height of two years old *S. reticulata* and *S. oblonga* plants are between 170 cm and 191 cm (Dhanasiri *et al.*, 2013).

There is a vast problem in the accurate identification of plants due to the use of common names for adulterants and their medicinal plants. Due to similar appearances, these species are incorrectly identified and used. Hence, we cannot obtain the correct and expected medicinal output. *S. chinensis* has a brownish colored bark. *S. oblonga* stem contains brownish color bark and a more swollen large stem than the other two *Salacia* species. *S. reticulata*, stem contains grey color bark (Plate 2 and Table 2). The bark of *S. reticulata* is greenish-grey color and it is smooth (Arunakumara and Subasinghe, 2010).

S. chinensis roots are orange in color. *S. oblonga* roots are large and brownish-orange in color. *S. reticulata* roots are light yellowish-brown in color (Plate 3 and Table 2).

According to the results, *S. chinensis* has fleshy indehiscent triangular-shaped fruit, smaller than the fruit of *S. oblonga* mostly with one seed. *S. oblonga* has fleshy indehiscent triangular-shaped fruit which is more similar to the fruit of *S. chinensis* and mostly contains 1 seed. *S. reticulata* has fleshy indehiscent un-ripened fruit with "kothale" (pot) shape and ripened fruit with clearly distinguishable "kothale" shape and mostly one seed per fruit. Seeds are almond shape (Plate 4 and Table 2).

The fruit of *S. chinensis* is a drupe, globose, 1.0-0.5 cm in diameter, 1-celled, 1-seeded, supported on the disk, and somewhat enlarged, persistent calyx smooth and maturity red or orange-red.

Table 1: Leaf characters of three *Salacia* species

Leaf characters	***S. reticulata***	***S. oblonga***	***S. chinensis***
Leaf surface	Coriaceous, glabrous	Thinly coriaceous	Cretaceous
Shape	Ovate	Lanceolate	Ovate
Apex	Abruptly acuminate	Shortly acuminate	Acuminate
Margin	Minute rounded teeth and leaf curlings	Entire	Entire without leaf curlings
Mean maximum lamina length (cm)	5.52 ± 0.45	5.01 ± 0.40	5.43 ± 0.45
Mean maximum lamina width (cm)	4.54 ± 0.69	4.25 ± 0.60	4.44 ± 0.76
Mean inter-nodal length (cm)	2.21 ± 0.71	4.25 ± 0.70	3.24 ± 0.69
Mean petiole length (cm)	1.21 ± 0.45	1.25 ± 0.46	1.24 ± 0.40
Mean leaf area (cm^2)	5.01 ± 0.49	2.55 ± 0.45	4.84 ± 0.46

Table 2: Stem, root, and fruit characters of three *Salacia* species

Stem, root & fruit characters	***S. chinensis***	***S. oblonga***	***S. reticulata***
Stem	Grey color bark with lichens	Brownish color bark with lichens and more swollen large stem than other 2 *Salacia* species	Brownish colored bark with more lenticels and lichens
Marks on stem	Longitudinally shallow marks	No marks	No marks
Stem diameter (cm)	12 ± 0.10	18 ± 0.12	11 ± 0.10
Root	Light yellowish-brown	Large and brownish orange	Orange
Fruit shape	Distinguishable “kothale” shape	Triangular shaped fruit which is more similar to the fruit of *S.reticulata*	Triangular shaped fruit smaller than the fruit of *S. oblonga*
Fruit diameter (cm)	2.02 ± 0.12	3.05 ± 0.12	1.84 ± 0.10

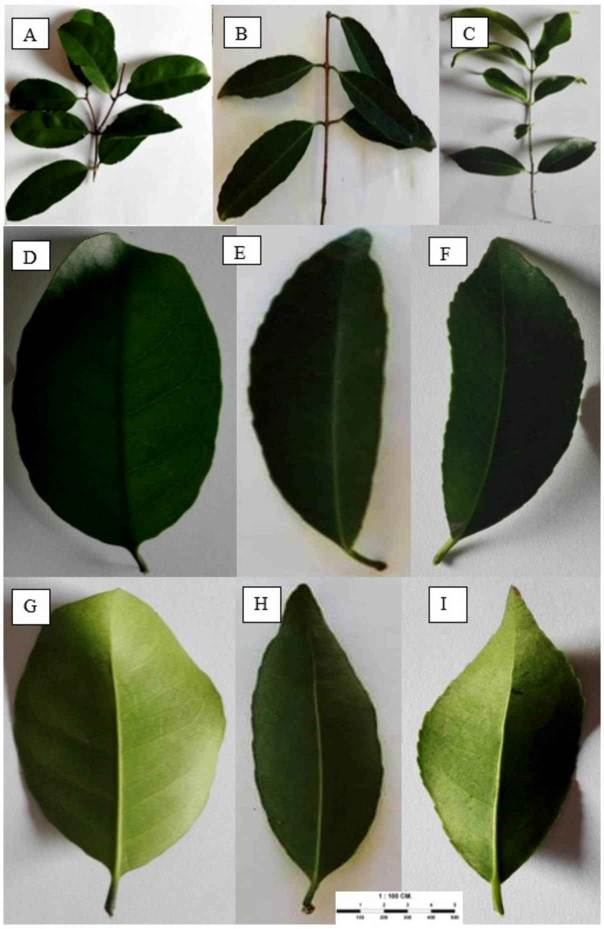

Plate 1: Leaf arrangement of (A). S. *chinensis*, (B). S. *oblonga* and (C). S. *reticulata*; Upper surface of a leaf of (D). S. *chinensis*, (E). S. *oblonga* and (F). S. *reticulata*; Lower surface of a leaf of (G). S. *chinensis*, (H). S. *oblonga* and (I). S. *reticulata*

Plate 2: Stem characters of (A). S. *chinensis*, (B). S. *oblonga* and (C). S. *reticulata*

Seeds are globose, up to 8 mm in diameter. Fruits of *S. oblonga* are baccate, globose, or sub globose, 2.0-4.8 cm in diameter, supported on persistent calyx tuberculate, bright orange-red when ripe, contained 1-8 seeds, angular, 2.7 x 1.5 cm. Fruits of S. reticulata are drupaceous, globose, 6-3.8 cm in diameter. They are tuberculate, bright pink-orange when ripe. It contains one seed and it is almond-like (Dissanayake and Clayton, 1996).

According to the obtained results, the flower of *S. chinensis* is a pedicellate yellowish light green color racemose inflorescence with actinomorphic symmetry. It has no epicalyx. Calyx contains fused five sepals with valvate aestivation. Corolla

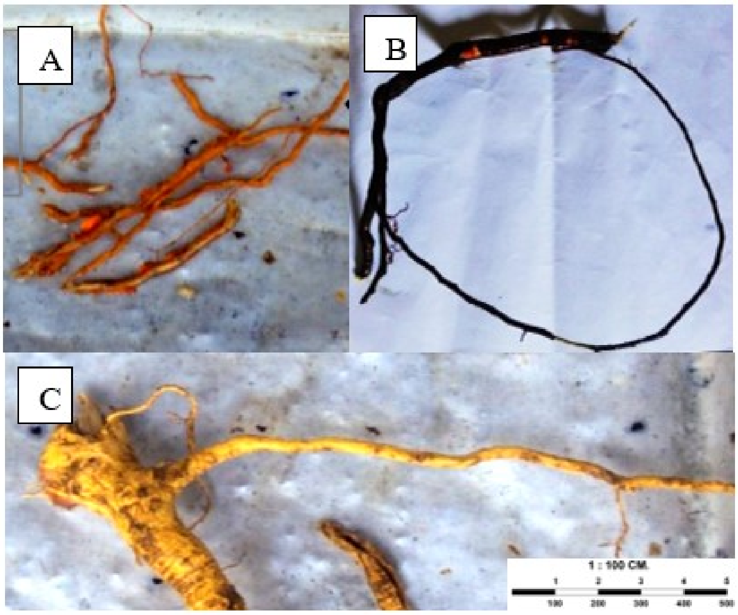

Plate 3: Root characters of (A). S. *chinensis*, (B). S. *oblonga* and (C). S. *reticulata*

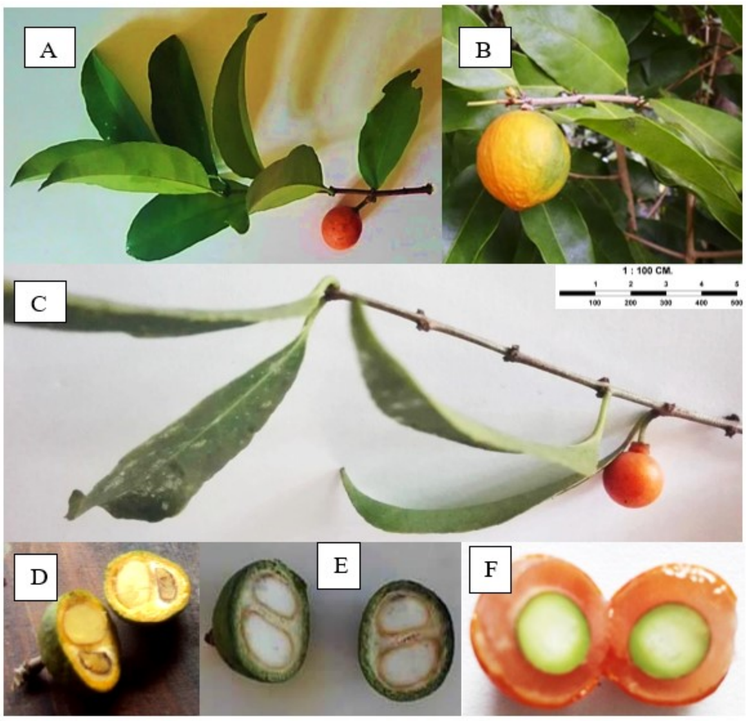

Plate 4: Fruit characters of (A). S. *chinensis*, (B). S. *oblonga* and (C). S. *reticulata*; Transverse section of the fruit of (D). S. *chinensis*, (E). S. *oblonga* and (F). S. *reticulata*

contains fused five petals with twisted aestivation. Androecium of *S. chinensis* contains three yellow color stamens which are attached to the corolla. Gynoecium of *S. chinensis* contains a superior ovary with two fused carpels and one free capitate-shaped stigma which forms a small narrow corona than the corona of *S. reticulata* (Plate 5 and Table 3).

The flower of *S. oblonga* is a half-opened brownish light green color racemose inflorescence with actinomorphic symmetry and a small pedicel than the other two *Salacia* species. It has no epicalyx. Calyx contains free five sepals with (2+2+1) imbricate aestivation. Corolla contains

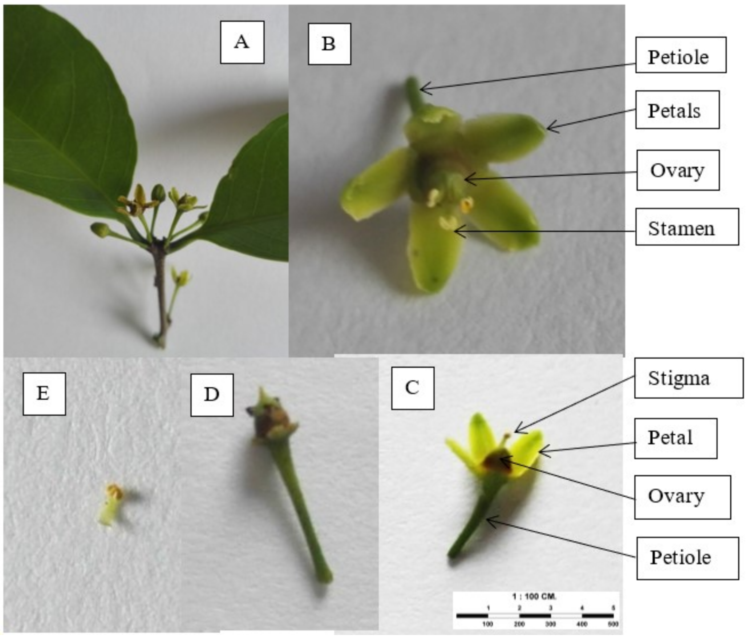

Plate 5: (A) Inflorescence; (B) Full flower; (C) The half flower; (D) Stigma and (E) Stamen of *S. chinensis*

free five petals with (2+2+1) imbricate aestivation. Androecium of *S. oblonga* contains three white color-free stamens which are attached to the corolla. Gynoecium of *S. oblonga* contains a superior ovary with two fused carpels and one free stigma (Plate 6 and Table 3).

The flower of *S. reticulata* is a pedicellate orangish light green color racemose inflorescence with actinomorphic symmetry. It has no epicalyx. Calyx contains fused five sepals with valvate aestivation. Corolla contains free five petals with (2+2+1) imbricate aestivation. Androecium of *S. reticulata* contains three orange color stamens which are attached to the corolla. Gynoecium of *S. reticulata* contains a superior ovary with one free carpel and one free capitates shaped stigma which forms a large wide corona with the flat upper part than the corona of S. chinensis (Plate 7 and Table 3).

Indian *S. reticulata* produces greenish-white to greenish-yellow color flowers as clustered (two to eight) in leaf axils and they are bisexual, entire lobes, anthers dehiscing transversely (Arunakumara and Subasinghe, 2010; Dhanasiri *et al.*, 2013). Flowers of *S. chinensis* are yellow or yellowish-green and few to many in fascicles on axillary bracteate tubercles. The diameter of the flower is about 6-7 mm. The calyx is shallowly 5 broadly ovate lobed and 1 mm long. It is obtuse, puberulous, and persistent. Anthers are transversely dehiscent. The ovary is triangular or ovoid. Flowers of *S. oblonga* are axillary, 1-3, in short, pedunculate heads, 4-5 mm across, dull yellow (Dissanayake and Clayton, 1996).

About the morphological analysis, the results indicated that the leaf characters (Table 1), and stem, root, fruit characters (Table 2) are mostly similar in three plant species. But the floral characters (Table 3) of the three plant species are different. These three *Salacia* species have three different floral formulas and according to Figure 1, these three *Salacia* species have three different floral diagrams too. According to Kalpan (2001), plant morphology is a useful tool for systematic, classification, and phylogenetic approaches.

Accurate identification of *S. reticulata* and *S. oblonga* using anatomical data will help avoid the use of *S. oblonga* instead of *S. reticulata*. Therefore, the conservation of this valuable plant is very important. For conservation purposes, correct identification of *S. reticulata* and *S. oblonga* is very important.

Anatomical Characters of *S. chinensis*, *S. oblonga* and *S. reticulata*

Cross-sections that were taken from stems of three selected *Salacia* species are mentioned below. *S. chinensis* contains red-colored pigmented cells

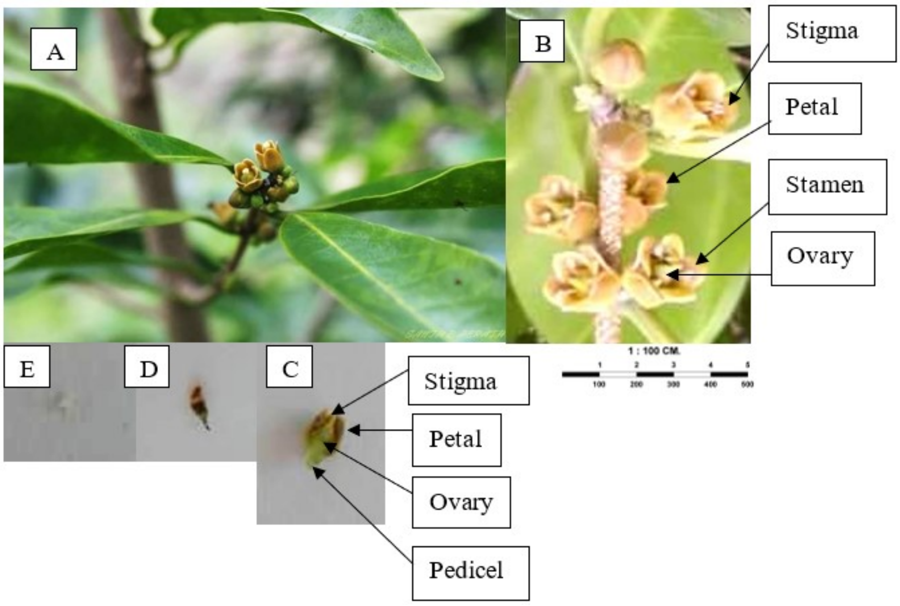

Plate 6: (A) Inflorescence; (B) Full flower; (C) The half flower; (D) Stigma and (E) Stamen of *S. oblonga*

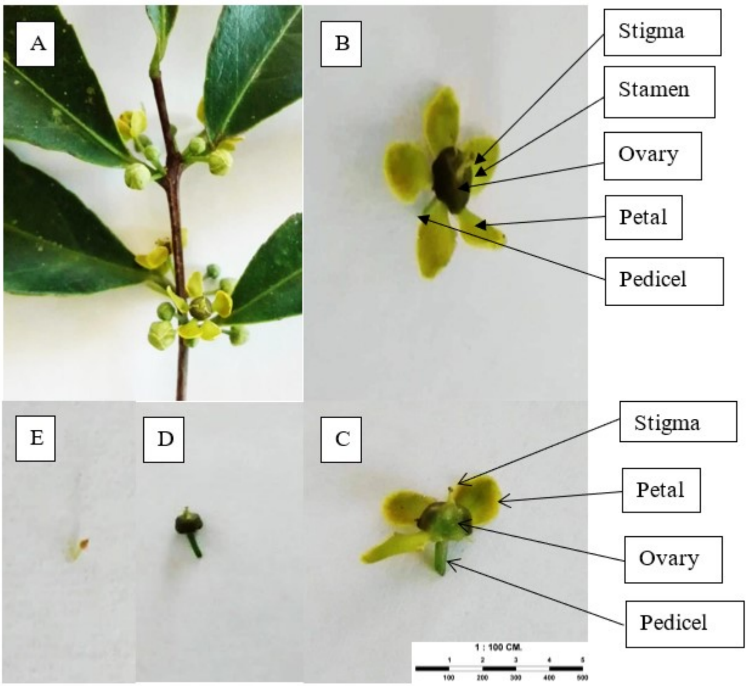

Plate 7: (A) Inflorescence; (B) Full flower; (C) The half flower; (D) Stigma and (E) Stamen of *S. reticulata*

in the hypodermis (Plate 8). It may be due to the accumulation of polyphenolic compounds. Those two layers appear in light yellowish color and dark brown color in *S. oblonga* and are also present in *S. chinensis*. *S. oblonga* does not contain pigmented cells in the hypodermis (Plate 9). It contains two specific layers after the hypodermis such as the outermost layer which is light yellowish and the next layer which is a dark brown color and is marginalized by vascular rays. *S. reticulata* does not contain pigmented cells in the hypodermis and two specific layers in the endodermis (Plate 10). The stem of *S. chinensis* has more similar characters with the stem of *S. oblonga* than the stem of *S. reticulata*. The present study has discovered an easy practice to identify three *Salacia* plant materials microscopically.

Table 3: Floral characters of three *Salacia* species

Floral character	***S. reticulata***	***S. oblonga***	***S. chinensis***
Inflorescence	Pedicellate orangish light green color racemose	Half opened brownish light green color racemose	Pedicellate yellowish light green color racemose
Calyx	Fused 5 sepals	Free 5 sepals	Fused 5 sepals
Corolla	Free 5 petals	Free 5 petals	Fused 5 petals
Androecium	3 orange color stamens	3 white color stamens	3 yellow color stamens
Flower formula	$^{*}♂K_{(5)}\overline{C_{2+2+1}}\,A_3\underline{G}_1$	$^{*}♂K_{(5)}\overline{C_{2+2+1}}\,A_3\underline{G}_{(2)}$	$^{*}♂K_{(5)}\overline{C_{(5)}}\,A_3\underline{G}_{(2)}$

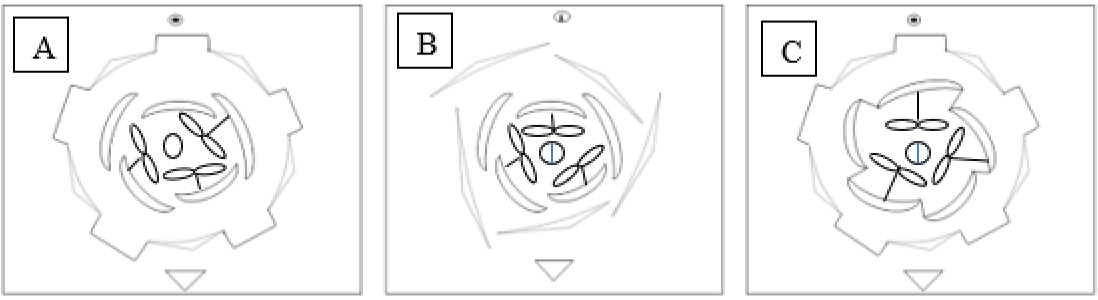

Figure 1: Flower diagrams of (A) S. *reticulata*, (B) S. *oblonga* and (C) S. *chinensis*

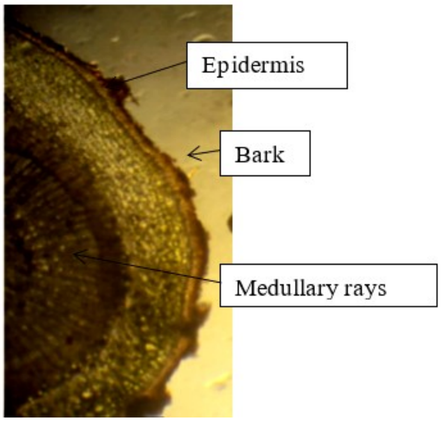

Plate 8: Transverse section of the stem of S. *chinensis* (10×40×1)

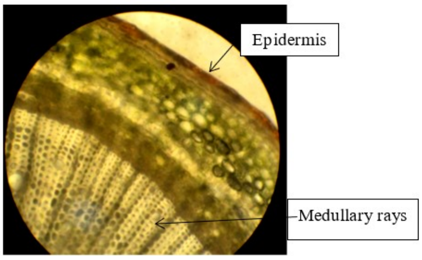

Plate 9: Transverse section of the stem of S. *oblonga* (10×40×1)

Powder Identification of *S. chinensis*, *S. oblonga* and *S. reticulata*

Tracheids, fibers, and crystals can be seen in the powders of *S. reticulata* (Plate 11). Fibers and crystals were present in *S. chinensis* (Plate 12) and crystals and tracheids were present in *S. oblonga* (Plate 13).

The present study has discovered an easy way to identify three *Salacia* plant materials microscopically and the method can also be

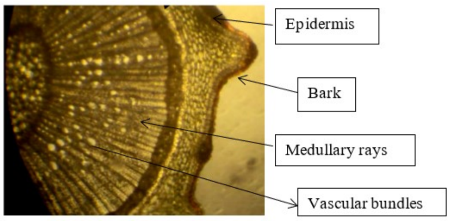

Plate 10: Transverse section of the stem of S. *reticulata* (10×40×1)

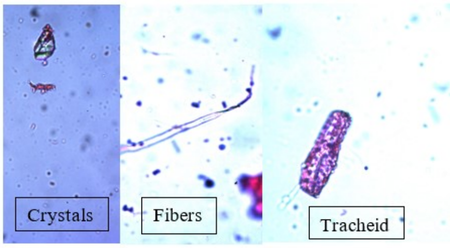

Plate 11: Stem powder microscopy of S. *reticulata* (10×40×1)

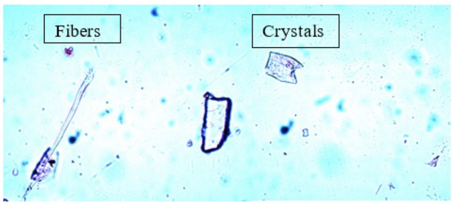

Plate 12: Stem powder microscopy of S. *chinensis* (10×40×1)

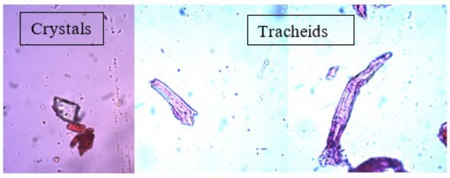

Plate 13: Stem powder microscopy of S. *oblonga* (10×40×1)

Table 4: R_f values for *S. chinensis*, *S. oblonga* and *S. reticulata* at 366 nm

1). *S. chinensis*	-	-	-
2). *S. oblonga*	0.64 (dark pink)	0.59 (blue)	0.32 (pink)
3). *S. reticulata*	0.58 (dark pink)	0.52 (blue)	-

Table 5: R_f values for *S. chinensis*, *S. oblonga* and *S. reticulata* at 254 nm before and after spraying Vanillin sulfuric

1). *S. chinensis*	Before spraying	After spraying
	0.7	0.7
	-	-
2). *S. oblonga*	Before spraying	After spraying
	0.6	0.6
	0.26	0.26
3). *S. reticulata*	Before spraying	After spraying
	0.63	0.63
	-	-

engaged to detect the extent of adulteration in powdered raw medicinal plant materials as well.

Thin Layer Chromatography (TLC) of *S. chinensis*, *S. oblonga* and *S. reticulata*

S. chinensis, *S. oblonga* and *S. reticulata* were run in 1, 2 and 3 number positions respectively (Plate 14).

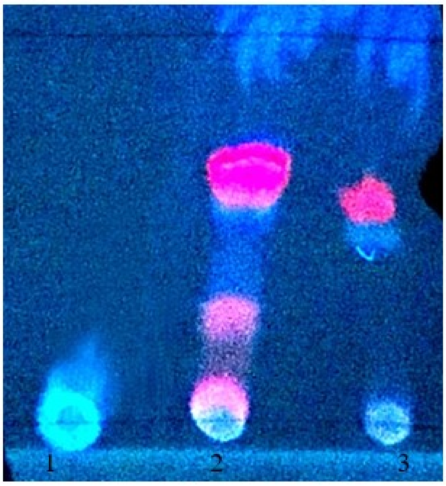

Plate 14: TLC fingerprint of S. *chinensis*, S. *oblonga* and S. *reticulata* at the wavelength of 366 nm

Table 6: Qualitative phytochemical analysis of stem extracts of *S. chinensis, S. oblonga* and *S. reticulata*

Class of compound	Plant species		
	S. reticulata	*S. oblonga*	*S. chinensis*
Saponins	+	+	+
Terpenoids	+	+	+
Alkaloids	-	-	-
Tannins	+	+	+

According to Plate 14 and R_f values at 366 nm and at 254 nm before and after spraying Vanillin sulfuric (Table 4 and Table 5), *S. reticulata, S. oblonga* and *S. chinensis* were chemically differentiated too.

Qualitative Phytochemical Screening of *S. chinensis, S. oblonga* and *S. reticulata*

The phytochemical screening indicated the presence of saponins, terpenoids, and tannins in these three *Salacia* species, whereas alkaloids and flavonoids were not detected in this study (Table 6).

Plants have secondary metabolites that are used for the remedial of several human diseases and healing. The phytochemical analysis of the plants has a commercial concern in both research institutes and pharmaceutical companies for the development of new drugs for the treatment of many diseases (Wadood *et al.*, 2013). According to Morikawa *et al* (2015), *Salacia* species played a momentous role in diabetic control all over the world for thousands of years and it has now become a subject of wide range of studies for diabetes control and management too. This ability of *Salacia* species is may be due to the presence of these detected bioactive compounds. Hence, further studies are needed to quantify these phytochemicals to copping with different diseases too.

These *Salacia* species may be rich with useful chemicals and, anatomical and morphological features would aid to thrive in natural habitats. Therefore, it is necessary to pay attention to crop wild relatives as they contain valuable traits that are beneficial for crop improvement programs.

Conclusion

The aim of this study is to reveal the inter-specific relationships of selected *Salacia* species based on the variation in the qualitative phytochemical screening and phenotypic approach. Due to their similar appearance, they are deceitfully used in traditional medicinal performance. *S. chinensis, S. oblonga* and *S. reticulata* can be considere the three different species in the same genus according to morphological analysis and anatomical analysis. These three species revealed different R_f values in TLC profile too. Preliminary phytochemical screening revealed the presence of saponins, terpenoids, and tannins in all taxa which are medicinally important bioactive compounds that may be potential for novel drug discovery. These findings may also lead to further isolation, purification, characterization of the active compounds from the extract of the various plant parts from different *Salacia* species using chromatographic and spectroscopic techniques too.

The results of the present study indicate a strong need for sustainable conservation and scientific validation of available knowledge regarding medicinal plants before they are applied industrially. Moreover, the establishment of commercial cultivation of *Salacia* species is also encouraged.

References

Arunakumara, K.K.I.U. & Subasinghe, S. (2010). *Salacia reticulata* Wight: A Review of Botany, Phytochemistry and Pharmacology. *Tropical Agricultural Research and Extension*, 13(2): pp 41-47.

Biradar, S. R. & Rachetti, B.D. (2013). Extraction of Some Secondary Metabolites Thin Layer Chromatography from Different Parts of *Centella Asiatica* L. (URB). *American Journal of Life Sciences*, 1, pp 243-247. https://doi.org/10.11648/j.ajls.20130106.11

De Zoysa, M. & Inoue, M. (2008). Forest Governance and Community based Forest Management in Sri Lanka: Past, Present and Future Perspectives. *International Journal of Social Forestry*, 1(1): pp 27-49.

Dhanasiri, G., Reddy, M.S., Naresh, B. & Cherku P.D. (2013). Micropropagation of *Salacia reticulata*: An Endangered Medicinal Plant. *Plant Tissue Culture and Biotechnology*, 23(2): pp 221-229.

Dissanayake, M. D. & Clayton, W. D.(ed.). (1996). *A Revised Handbook to the Flora of Ceylon*: *Volume X*. Rotterdam: Balkema. pp 426 ISBN, 464168091.

Flammang, A.M., Erexson, G.L., Mecchi, M.S. & Murli, H. (2006). Genotoxicity Testing of a *Salacia oblonga* Extract. *Food and Chemical Toxicology*, 44: pp 1868–1874.

Gunatilleke, I.A.U.N. & Gunatilleke, C.V.S. (1990). Distribution of Floristic Richness and its Conservation in Sri Lanka. *Conservation Biology*, 4: pp 21-31.

IUCN Standards and Petitions Subcommittee. (2012). Guidelines for Using the IUCN Red List Categories and Criteria. Version 3.1. Prepared by the Standards and Petitions Subcommittee.

Jennifer, A.W., Choe, Y.S., Noss, M.J., Baumgartner, C.J. & Mustad, V.A. (2007). Extract Of *Salacia oblonga* Lowers Acute Glycemia in Patients With Type 2 Diabetes. *The American Journal of Clinical Nutrition*, 30: pp 86-124.

Kalpan, R.D. (2001). The Science of Plant Morphology: Definition, History and Role in Modern Biology. *American Journal of Botany*, 88(10): pp 1711-1741.

Karunaratne, V. (2013). Safeguarding the Medicinal Value of Sri Lankan Flora. *Journal of the National Science Foundation of Sri Lanka*, 41 (3): pp 173-174.

Khandelwal, K.R. (2008). *Practical Pharmacognosy*, 19^{th} Edition. Nirali Prakashan, Pune. pp 149- 160.

Ministry of Forestry and Environment (1999).*Biodiversity Conservation in Sri Lanka: A framework of Action*. Ministry of Forestry and Environment, Sri Lanka.

MOE.(2012). The National Red List 2012 of Sri Lanka. *Conservation Status of the Fauna and Flora*. Ministry of Environment, Colombo, Sri Lanka. pp 236.

Morikawa, T., Akaki, J., Ninomiya, K., Kinouchi, E., Tanabe, G., Pongpiriyadacha, Y. & Muraoka, O. (2015). Salacinol and Related Analogs: New Leads for Type 2 Diabetes Therapeutic Candidates from the Thai Traditional Natural Medicine *Salacia chinensis*. *Nutrients*, 7(3): pp 1480-1493.

Osaki, S., Oe, H. & Kitamura, S.(2007). α-Glucosidase Inhibitor from Kothala-himbutu (*Salacia reticulata* WIGHT). *The Journal of Nutrition*, 71: pp 981-984.

Peeris, C.V.S (1975). *The Ecology of Endemic Tree Species of Sri Lanka in Relation to their Conservation*. Ph.D. Thesis, University of Aberdeen, UK.

Pushpakumara, D.K.N.G., Kotagama, H.B., Marambe, B., Gamage, G., Silva, K.A.I.D., Gunaratne, L.H.P., Wijesundara, C. & Karaluvinne, S.S.D.K. (2002). Prospects of Pharmaceutical Prospecting to Finance Biodiversity

Conservation in Sri Lanka. *Sri Lankan Journal of Agricultural Economics*, 4(1): pp 39-71.

Ratnasooriya, W.D., Jayakody, J.R.A.C. & Premakumara, G.A.S. (2003). Adverse Pregnancy Outcome in Rats Following Exposure to a *Salacia reticulata* (Celastraceae) Root Extract. *Brazcilian Journal of Medical and Biological Research*, 36: pp 931-935.

Sharmal, S., Rathi, N., Kamal, B., Pundir, D., Kaur, B. & Arya, S. (2010). Conservation of Biodiversity of Highly Important Medicinal Plants of India through Tissue Culture Technology: A Review. *Agriculture and Biology Journal of North America*, 1(5): pp 827-833.

Wadood, A., Ghufran, M., Jamal, S. B., Naeem, M., Khan, A., Ghaffar R. & Asnad. (2013). Phytochemical Analysis of Medicinal Plants Occurring in Local Area of Mardan. *Biochem Anal Biochem*, 2:4. http://dx.doi.org/10.4172/2161-1009.1000144.

Yoshikawa, M., Pongpiriyadacha, Y., Kishi, A., Ka-geura, T., Wang, T., Morikawa, T. & Matsuda, H. (2003). Biological Activities of *Salacia Chinensis* Originating in Thailand: The Quality Evaluation Guided by a-Glucosidase Inhibitory Activity. *Yakugaku Zasshi*, 123: pp 871–880.

Yoshikawa, M., Murakami, T., Shimada, H., Matsuda, H., Yamahara, J., Tanabe G. & Muraoka, O. (1997). Salacinol, Potent Antidiabetic Principle with Unique Thiosugar Sulfonium Sulfate Structure from the Ayurvedic Traditional Medicine *Salacia reticulata* In Sri Lanka And India. *Tetrahedron Letters*, 38: pp 8367–8370.

Yoshikawa, M., Shimoda, H., Nishida, N., Takada M. & Matsuda, H. (2002). *Salacia reticulata* And its Polyphenolic Constituents with Lipase Inhibitory and Lipolytic Activities have Mild Antiobesity Effects in Rats. *The Journal of Nutrition*, 132(7): pp 1819–1824.