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Natural Antidiabetic Potential of *Salacia chinensis* L. (Celastraceae) Based on Morphological, Phytochemical, Physico-chemical and Bioactivity: A Promising Alternative for *Salacia reticulata* Thw

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Abstract *Salacia reticulata* Thw. (Celastraceae) is widely used in traditional systems of medicine for the natural control of diabetics. However, *S. reticulate* is obtained from the wild and hence its popular use creates a huge pressure on its limited supply. Therefore, in the present study we evaluated the potential of an alternative natural antidiabetic candidate, Salacia chinensis (Celastraceae), by means of morphological, physico-chemical, phytochemical and bioactivity analyses. Gross morphological characters were compared based on taxonomically important vegetative and reproductive characters of leaf and petiole of both plants. Physico-chemical and phytochemical parameters were performed according to methods described by WHO. Total phenol content (TPC) and, total flavonoid content (TFC) were determined by using Folin–Ciocaltueand aluminum chloride methods, respectively. Radical scavenging activity was investigated by means of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) and $ABTS^+$ radical scavenging assays. Results were analyzed by the General Linear Model (GLM) of ANOVA followed by Duncan's Multiple Range Test (DMRT). LC_{50} values of brine shrimp toxicity were generated using probit analysis. The most distinguished morphological features were leaf length, width and leaf margin, which varied significantly between the two species. All tested physico-chemical parameters were within the acceptable levels. Qualitative phytochemical analysis and thin layer chromatographic profiles revealed the presence of all tested compounds and some common spots in both species, respectively. Moreover, both plants exhibited marked levels of radical scavenging activity, brine shrimp toxicity, TFC and TPC in varying levels. Results revealed that all monitored parameters displayed positive results in *S. chinensis*, thus partially justifying its use as an alternative natural antidiabetic source. This could promote the sustainable utilization of *S. reticulata* by easing its demand. Further, the generated findings could be effectively utilized for the standardization of *S. reticulata* and *S. chinensis* for upgrading the Sri Lankan pharmacopeia.

Keywords: *salacia reticulata, salacia chinensis, celastraceae, cytotoxicity, antioxidant activity, antidiabetic properties*

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1. Introduction

Diabetes mellitus is a serious chronic, metabolic disorder with significant impact on the health, quality of life, and life expectancy of patients [1]. The number of patients with diabetes is increasing annually and projected to rise up to 366 million in year 2030 [2,3]. However, existing treatments such as insulin or other modern pharmaceuticals simply modify the course of diabetic complications due to the multifactorial nature of the disease [4]. Therefore, many researchers are compelled to investigate multi-targeted and cost effective anti-diabetes remedies with higher efficacy in order to face this disastrous situation [3,5,6]. Among the potential candidates, *Salacia* species play a significant role in diabetic control all over the world and it has now become a subject of broad studies for diabetes management [7].

The genus *Salacia* consists of 407 species and is widely distributed in Sri Lanka, India, China, Vietnam, Indonesia, Brasil and other Asian countries [8,33]. Out of 407 *Salacia* species, *Salacia reticulata* Thw. (Celastraceae) has been identified as the most potent species for treating diabetes. Its pharmacological properties including blood glucose-lowering activity [9], inhibition of adipocyte differentiation [10], suppression of the accumulation of visceral fat [11] and metabolic disease prevention and

suppression of fat accumulation [12] have been scientifically validated. Further, the oral hypoglycemic activity, anti-rheumatic properties, curing of skin related ailments, hypoglycemic effects of Salacia reticulata extract has been investigated [12,13].

Even though *S. reticulata* possesses an array of uses and huge demand in national and international markets, it is obtained mainly from wild stocks, which creates a huge pressure on its supply. Therefore, searching for new antidiabetic potent species and scientific studies on phytochemical, physicochemical and bioactivity of such alternatives is paramount important in order to ensure their sustainable utilization. S. chinensis L. (Celastraceae) is one such candidate, which has been heavily used for the treatment of diabetes and many other diseases in Sri Lanka. Even though *S. chinensis* has been used since early times, its performance relative to *S. reticulata* is yet to be investigated. In this work, therefore, comparisons between the morphological, physicochemical, phytochemical, radical scavenging activity and cytotoxicity of *S. reticulata* and *S. chinensis* were conducted.

2. Material and Methods

2.1. Plant Materials

After obtaining permission from the relevant authorities, stem and leaf samples of *S. chinensis* and *S. reticulata were* collected from the Uwa Kosgama area of Sabaragamuwa province in Sri Lanka. These samples were collected from plants of approximately the same age, which were grown under similar soil and climatic conditions. Herbarium specimens of both species were prepared and authenticated by comparing with available classical texts and certified herbarium specimens available at the Royal Botanical Garden, Peradeniya, Sri Lanka.

2.2. Study of morphological Characters

Qualitative and quantitative morphological characters were determined by using previously published protocols. Ten plants were collected from each species and at least twenty leaf and petiole samples were used to record morphological data. A total of 18 morphological characters were recorded.

2.3. Preparation of Plant Materials

Collected leaf and stem samples were cut into pieces and dried under the shade at room temperature (28±2°C) for 3 days. Subsequently, they were sun-dried up to 6 days. Dried materials were coarsely crushed (100 g) using a grinder and sieved with 0.25 mm mesh to obtain a fine powder. Powdered materials were labeled and stored in air-tight container for furthers analysis.

2.4. Quantification of Moisture Content

Two grams of each sample were dried separately at 105°C to a constant weight. They were then cooled in desiccators for 30 min and their moisture content recorded.

2.5. Quantification of Total Ash

Two grams of air-dried plant sample were ignited at 500-600°C for 5 hours until they turned white. After cooling down in desiccators for 30 min, the total ash content was recorded.

2.6. Quantification of Water Soluble Ash

Previously ignited ash was boiled with 25 mL of distilled water for 5 min. Then it was filtered using a Whatman ash less filter-paper. Insoluble matter was washed with hot water and ignited for 15 min at 450 ºC. The residue was allowed to cool in desiccators for 30 min.

2.7. Quantification of Acid Insoluble Ash

The previously ignited ash was gently boiled with 25 mL of HCL. Insoluble matter was collected onto an ash less Whatman filter-paper and washed with hot water until the filtrate become neutral. The acid insoluble matter was transferred to the original crucible and ignited to a constant weight at 450 °C. The residue was allowed to cool in desiccators for 30 min.

2.8. Quantification of Extractable Matter

2.8.1. Quantification of Hot Extractable Matter

Four grams of coarsely powdered materials of each sample was separately refluxed with 100 mL of methanol for 1 h. The resulting mixture was filtered and the total volume was re-adjusted by adding methanol. Twenty-five mL of the filtrate was concentrated in a rotary evaporator (Buchi rotavapour, Type-R-114A29 B-480, Switzerland) at 45°C. The residue was dried at 105 °C for 6 h and allowed to cool for 30 min. The weight was recorded.

2.8.2. Quantification of cold extractable matter

Four grams of coarsely powdered materials were macerated with methanol (100 mL) and shaken for 6 hours. The resulting mixture was allowed to stand for 18 hours and then filtered. The filtrate (25 mL) was evaporated to dryness in a water bath. It was further dried at 105ºC for 6 hours. The final residue was allowed to cool in desiccators for 30 minutes and the weight was recorded.

2.9. Sample Preparation for Chemical Analysis

About 10 g of coarsely powdered plant material was extracted with 50 mL of methanol by using a Soxhlet apparatus. The extract was concentrated at 45 °C using a rotary evaporator (Buchi Rotavapour, Type-R-114A29 B-480, Switzerland).

2.10. Phytochemical Screening

The phytochemical screening tests for alkaloids, flavonoids, saponins, steroid glycosides and tannins were performed according to the method described by in previous studies [13,42].

2.11. Thin Layer Chromatography (TLC)

The thin layer chromatography was performed according to the method described in WHO guidelines [14] with some modifications. Pre-prepared methanol extracts

of each sample were dissolved in 10 mL of methanol separately and 8 µL of the solutions of each extract was spotted on TLC plates (Pre-coated silica gel 60 A 20 × 20 cm; 0.2 mm thickness) and dried. The mobile phase consisted of cyclohexane, dichloromethane, ethyl acetate and methanol at 3: 2: 0.3: 0.4volume ratio. Generated spots were observed under UV 366 nm. The plates were then sprayed with vanillin-sulfuric acid, dried at 105 °C for 5 min.

2.12. Determination of Total Flavonoid Content

Total flavonoid content was determined according to the method described by Chang *et al.*, [16] using 2% aluminum chloride. The assay was carried out in 96-well micro plates. A stock solution (2 mg/mL) was prepared by dissolving a known amount of extract in dimethyl sulfoxide (DMSO) solvent and methanol. The pre-plate absorbance of 100 µL of a 4times diluted stock solution was read using a micro plate spectrophotometer (Shimadzu, UV Mini 1240, Japan).A similar volume(100 µL) of 2% aluminum chloride solution was added and the resulting mixture was incubated at room temperature for 10 min. The final absorbance was obtained at 415 nm. Quercetin was used as the standard.

2.13. Determination of Total Polyphenolic Content (TPC)

The total phenolic content was determined using a modified Folin–Ciocaltue method. The assay was carried out in a 96-well micro plate. A stock solution (2 mg/mL) was prepared by dissolving a known amount of extract in dimethyl sulfoxide (DMSO) solvent and distilled water. Twenty µL of a 4 times diluted stock solution was mixed with 110µL of Folin - Ciocalteu reagent, and the pre plate absorbance was read at 765 nm. Seventy µL of 10%of saturated sodium carbonate solution was added and the resulting mixtures were incubated at room temperature for 30 min. The absorbance was read at 765 nm. Gallic acid (1mg/mL) was used as the standard.

2.14. DPPH (1, 1-diphenyl-2-picryl-hydrazyl) Radical Scavenging Assay

DPPH radical scavenging assay was performed according to the method described previously [17]. One hundred five µL of methanol, 50 µL of sample extracts of different concentrations (31.25, 15.62, 7.81, 3.90, 1.95, 0.97 µg/mL) and 45 µL of DPPH were mixed in 96-well micro plate and were incubated at room temperature for 10 minutes. The absorbance was read at 517 nm. Trolox was used as the standard antioxidant.

2.15. ABTS+ Radical Scavenging Assay

The $ABTS^+$ radical scavenging activity was determined according to the method described by Re *et al.*, (1999) in 96-well micro-plate. ABTS radical generation was performed by incubating 10 mg $ABTS^+$ in 2.5 mL of 2.5 mM of potassium persulphate solution at room temperature for 16 hours. Freshly prepared $ABTS^+$ solution was then diluted 7 times with a pH 7.4phosphatebuffer saline (PBS).Two hundred µL of reaction solutions consisting of 95 µL PBS, 50 µL samples extracts of different concentrations, and 55 µL of $ABTS^+$ solution were incubated at room temperature for 10 minutes and the absorbance was read at 734 nm. Trolox (1mg/mL in PBS) was used as the standard antioxidant.

2.16. Brine Shrimp Toxicity Assay

Brine shrimp toxicity assay was carried out as described by Michael *et al.*, [18] with slight modifications. *Artemia salina* eggs were incubated in artificial sea water (salt 38 g/L and adjusted to pH 8.5 using 1N NaOH, 25-30 ºC) under constant aeration for 48 h. After hatching, active nauplii were transferred to 20 well plates containing 2 mL of samples of different concentrations (0.1 ppm, 1 ppm, 5 ppm, 10 ppm, 20 ppm) in 1% DMSO and left for 18 h at 28 °C under the light. Each treatment consisted of 12 nauplii, along with the negative control containing 2.0 mL of 1% DMSO in artificial sea water and a positive control containing potassium permanganate. A set of four wells per dose was prepared. The number of dead larvae was counted under a light microscope (Olympus CX31, Japan).

2.17. Statistical Analysis

Results of physico-chemical parameters antioxidant activity and cytotoxicity were analyzed by the general linear model (GLM). ANOVA was conducted using the Duncan's Multiple Range Test (DMRT) and LC_{50} values of brine shrimp toxicity assay were generated using probit analysis (version SAS 9.2) and presented as means ± SE with 95% confidential level.

3. Results and Discussion

3.1. Morphological Variations

The current study compares the morphological, physico-chemical, phytochemical and bioactivity of an alternative antidiabetic candidate *S. chinensis* with that of an established species *S. reticulata.* Pertaining to the morphological analysis, the results indicated that the leaf type, leaf arrangement, leaf shape and leaf petiole are similar for both plant species (Table 1). This was highly expected considering their close taxonomy (similar family and genus). However, some of the quantitative measurements, particularly leaf size and leaf petioles, are smaller in *S. chinensis* than in *S. reticulata*. These results are in agreement with the report previous studies [8].

Since all leaf and stem samples were obtained from plants of approximately the same age and from similar agro-climatic and soil conditions, these results could be considered as true genetic variations. In this regard, distinguished vegetative and reproductive morphological characters have been successfully applied for the identification of *Munronia pinnata* [19], *Ruelia tiberosa* and *Withania somnifera* [19].

3.2. Physico-chemical Parameters

Physico-chemical parameters play an important role in quality control and standardization based on stability, purity and phytochemical composition of an herbal drug [20]. Since the ash represent the physiological (derived from plant itself such as calcium oxalate and silicate) and

non-physiological (accumulated from external environment such as sand, soil, adulterants etc.) impurities, determination of these parameters are paramount important in order to maintain the quality control of herbal medicines and for detecting adulteration or improper handling of drugs [20,21]. Precise measurement of moisture content is required to determine the actual weight of drug material. Low moisture also suggests better stability against degradation of the product.

Table 1. Comparative morphological characters of leaf and petiole of *Salacia reticulata* and *Salacia chinensis*

Character		Plant species	
		Salacia reticulata	***Salacia chinensis***
01	Leaf type	Simple	Simple
02	Leaf surface	Coriaceous, Glabrous, Shining above	Thinly Coriaceous, Glabrous and Slightly glossy above
03	Leaf arrangement	Opposite	Opposite
04	Leaf shape	Elliptic or Oval	Elliptic or Elliptic-lanceolate
05	Leaf apex	Obtuse or usually shortly Acuminate	Acute to shortly Acuminate
06	Leaf base	Obtuse	Sub obtuse
07	Leaf margin	Very shallowly Crenate-serrate	Entire or Shallowly Crenate-serrate
08	Leaf venation	Paler and with prominent reticulations beneath	Reticulate veins beneath
09	Petiole/Rachis types	Petiolate	Petiolate
10	Leaf length (cm)	11.58	7.87
11	Leaf width (cm)	5.48	3.25
12	Length /width ratio	2.12	2.45
13	Leaf petiole length (mm)	7.50	5.9
14	Leaf tip length (mm)	4.35	4.0
15	Number of venation	14-18	12-16

All quantitative results are means of 20 replicates per plant and ten individuals per each species were used.

As shown in Table 2, all tested physicochemical parameters including moisture percentage, total ash content, and water soluble ash content, acid insoluble ash content of crude extracts obtained from different parts of *S. reticulate* and *S. chinensis* were within the acceptable level according to the WHO guidelines. Significantly higher ($p < 0.05$) values for all tested physico chemical parameters (Table 2) were observed in leaf than in stem extracts. This result is in agreement with the previous study of Tachakittirungrod et al. [22] with *A. pannuculata*. Similar patterns have also been observed by Dharmadasa e*t al. [23]*, who compared the physicochemical parameters of leaf, stem and root extracts of *Munronia pinnata* and its substitute *Andrographis paniculata.* They observed the same increasing order of leaf > stem > root in both plants extracts. Furthermore, comparatively higher extractable matter contents were apparent in the extracts of all plant parts (leaf and stem) obtained through hot over the cold extraction method to show the higher efficiency of the former (Table 2).

Table 2. Physico-chemical parameters of different parts of S*alacia reticulata, Salacia chinensis* and market sample of S*alacia reticulata*

Parameter	Part of the plant				
	SRL	**SRS**	**SCL**	**SCS**	**SRM**
TA	6.55 ± 0.01^{A}	2.051 ± 0.03^{D}	4.60 ± 0.00^{B}	1.64 ± 0.01^{E}	2.86 ± 0.03^{C}
WSA	1.72 ± 0.00^{A}	0.64 ± 0.00^{C}	1.21 ± 0.00^{B}	0.54 ± 0.04^{D}	0.46 ± 0.00^{E}
AIA	0.80 ± 0.00^{A}	0.17 ± 0.00^{D}	0.40 ± 0.00^{B}	0.09 ± 0.00^{E}	0.35 ± 0.00^{C}
MC	12.62 ± 0.01^{A}	9.54 ± 0.02^{D}	12.15 ± 0.00^{B}	9.86 ± 0.02^{C}	9.53 ± 0.01^{D}
HEM	17.04 ± 0.07^{A}	11.77 ± 0.07^{C}	14.20 ± 0.05^{B}	7.89 ± 0.07^{E}	10.13 ± 0.05^{D}
CEM	13.50 ± 0.02^{A}	9.34 ± 0.06^{C}	12.08 ± 0.02^{B}	5.71 ± 0.05^{E}	8.10 ± 0.03^{D}

Results are means of three replicates ± SE, Means followed by same letter in each column are significantly different at 0.05 level TA=Total Ash; WSA=Water soluble ash; AIA= acid insoluble ash; MC=Moisture Content; HEM = hot extraction method; CEM = cold extraction method; SRL=*Salacia reticulata* leaf, SRS=*Salacia reticulata* stem, SCL=*Salacia chinensis* leaf, SCS=*Salacia chinensis* stem, SRM=Market sample of *Salacia reticulata* stem.

3.3. Phytochemical Screening

Phytochemical screening techniques involve botanical identification, extraction with suitable solvents, purification, and characterization of the active constituents with pharmaceutical importance [24]. In addition, most of the therapeutic peoperties of herbal drugs are mainly dependenton major phytochemical groups present in different parts of the plants [25,26,27]. Hence, a preliminary investigation on this aspect is important for the quantitative estimation and location of pharmacologically active chemical compounds [28]. As shown in Table 3, the major phytochemicals including alkaloids, flavonoids, saponins, steroid glycosides and tannins are presentin all the leaf and stem samples of *S. reticulata* and *S. chinensis* analyzed in this study.

Table 3. Phytochemical screening of different parts of *Salacia reticulata, Salacia chinensis* and market sample of *Salacia reticulata*

Metabolite	Part of the plant				
	SRL	SRS	SCL	SCS	SRM
Saponins	+	+	+	+	+
Alkaloids	+	+	+	+	+
Tannins	+	+	+	+	+
Flavonoids	+	+	+	+	+
Steroid Glycosides	+	+	+	+	+

(+) = Presence (–) = Absence, SRL=*Salacia reticulata* leaf, SRS=*Salacia reticulata* stem, SCL=*Salacia chinensis* leaf, SCS=*Salacia chinensis* stem, SRM=Market sample of *Salacia reticulata* stem

More detailed phytochemical analysis by thin layer chromatography (TLC) was conducted to evaluate any significant difference between the two plant species. This is a vital analytical technique that is widely used for herbal material authentication and drug standardization process due to its simplicity, effectiveness and low cost. In this work, most of the spots observed in TLC profiles (Figure 2) were both present in the leaf and stem extracts of *S. reticulate* and *S.chinensis*. The presence of common spots may be due to their similar family and the genus classification. The thin layer chromatographic profiles observed under UV 366 nm showed a higher number of spots in extracts from the leaf (12) than in the stem (2). However, some spots including the prominent, bright, light blue ones (R_f– 0.67) were present in the stem and leaf extracts of both fresh and market samples of *S. reticulata*, while it was not observed in the *S. chinensis* leaf extract. After spraying the coloring reagent (vanillin sulfuric acid), 11 spots for leaf and 8 spots for stem were observed. A green spot (R_f - 0.66) was observed only in the leaf extracts of both species where as a prominent, dark purple spot (R_f - 0.81) was characteristic solely of the stem extracts of *S. reticulata*, as it was absent in the stem extract of *Salacia chinensis*. These species-specific markers could be used for authenticating *S. reticulata* and *S. chinensis*. In this connection, the examination of thin layer chromatographic profiles for plant classification has been successfully employed for the authentication of *Gyrinopswall*a [29], *Munronia pinnata* and *Andrographis paniculata* [23], *Withania somnifera* and *Ruelia tuberose [19]*. Moreover, quantification and identication of quinonemethides and other triterpenoid constituents from *Salacia reticulate* have been carried out [41,43].

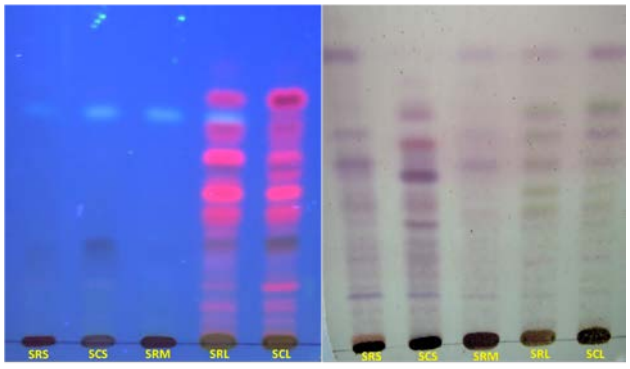

Figure 1. TLC finger prints of methanolic extracts of *Salacia reticulata, Salacia chinensis* and market sample of *Salacia reticulata* observed under UV 366 nm (Left) and after spraying vanillin sulfuric (Right), SRS=*Salacia reticulata* stem, SCS=*Salacia chinensis* stem, SRM=Market sample of *Salacia reticulata* stem, SRL=*Salacia reticulata* leaf. SCL= *Salacia chinensis* leaf; solvent system: Cyclohexane: Dichloromethane: Ethyl acetate: methanol 3: 1: 0.3: 0.4)

3.4. Antioxidant Activity

An antioxidant is a substance that prevents [30]. The antioxidant activity of plants is mainly contributed by the varying amounts of active molecules/ ingredients present in different parts of the plant. Estimation of antioxidant capacity of biological substances is vitally important for managing oxidative stress-related physiological disorders [31]. Single Electron Transfer (SET) assays determine the potential of antioxidant to transfer one electron to reduce a species, including metals, carbonyls and radicals. These assays are based on deprotonation and ionization potential [32]. In the present study, attempts were made to compare total phenol content, (TPC), total flavonoid content (TFC) and radical scavenging activity by means of DPPH and $ABTS^+$ radical scavenging assays of methanolic extracts of leaf and stem of *S. reticulata*, *S. chinensis* and market sample of *S. reticulata*.

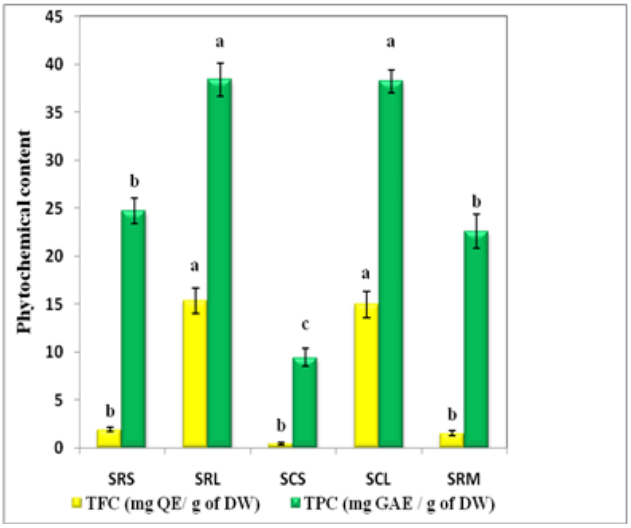

Figure 2. Phytochemical content of Salacia reticulata, Salacia chinensis & market sample of Salacia reticulata

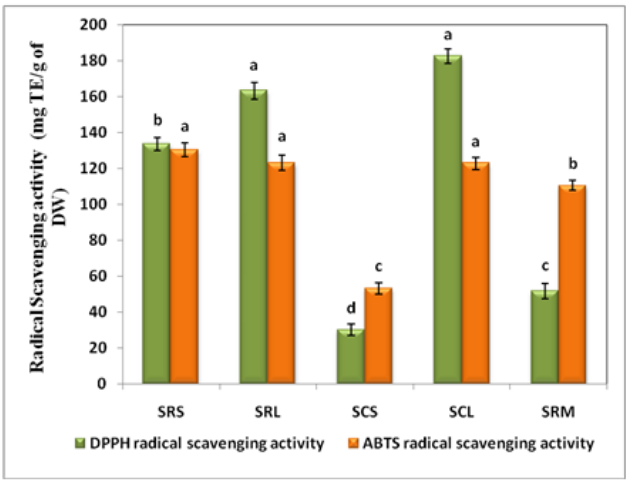

Figure 3. Radical scavenging activity of Salacia reticulata, Salacia chinensis& market sample of Salacia reticulata (SRS=Salacia. reticulata stem, SRL=Salacia reticulata leaf, SCS=Salacia chinensis stem, SCL=Salacia chinensis leaf; MS=Market sample of Salacia reticulata stem)

As demonstrated in Figure 2, all the tested extracts exhibited marked TPC and TFC. However, higher TPC and TFC were observed in the leaf and stem extracts of *S. reticulata* compared to *S. chinensis* and the market sample of *S. reticulata*. Moreover, TFC and TPC were significantly ($p<0.05$) higher in extracts from the leaf than in the stem of both species. The highest TFC (15.35±2.36 mg TE/g) and TPC (38.42±2.74 GAE/g) were reported from leaf extracts of *S. reticulata*. The order of increase of TFC and TPC was SRL >SCL >SRS>SRM >SCS. Interestingly, the radical scavenging activity determined by DPPH assay also demonstrated the significantly higher ($p<0.05$) values for SCL (182.37±4.01mg TE/g DW) followed by SRL (163.3±4.73mg TE/g DW), SRS

(133.6±3.49mg TE/g DW), SRM (51.66±4.24mg TE/g DW) and SCS (30.02±3.19mg TE/g DW). On the other hand, the radical scavenging activity determined by ABTS assay showed an apparently different trend of higher activity in SRS (130.38±3.86 TE/g of DW) followed by SRL (122.95±4.23 TE/g of DW), SCL (122.77±3.27 TE/g of DW), SRM (110.38±2.78 TE/g of DW), and SCS (53.21±3.26 TE/g of DW) respectively. ABTS and DPPH are two most commonly used antioxidant methods. The DPPH free radical (DPPH) does not require any special preparation, while the ABTS radical cation (ABTS_+) must be generated by enzymes or chemical reactions (Arnao, 2000). Both techniques are characterized by excellent reproducibility under certain assay conditions, but they also show significant differences in their response to antioxidants as observed in our data. To account for such differences, the ABTS+ radical scavenging assay is generally used to study the antioxidant activity of both lipophilic and hydrophilic samples. This is because the $ABTS^{+}$ can be dissolved in both aqueous and organic media. In contrast, DPPH can only be dissolved in organic media, especially in ethanol. The antioxidant activity of the extract and the yield is therefore highly dependent on the selected solvent [33]. This becomes an important limitation when interpreting the role of hydrophilic antioxidants. -In any case, results of the current study are in agreement with Yoshino et al., [34], who investigated the presence of marked radical scavenging activity in leaf and stem extracts of *S. reticulata*. Moreover, the results on the radical scavenging activity, total phenol content and total flavonoid content are in agreement with Chavan et al. [35], who investigated the presence of marked radical scavenging activity, total phenol content and total flavonoid content of *S. chinensis*.

3.5. Brine Shrimp Toxicity Assay

Finally, comparisons between the brine shrimp toxicity (BST) of stem and leaf extracts of *S. reticulate* and *S. chinensis* were also performed. This assay is considered a simple, reliable and convenient method for the preliminary assessment of toxicity and it has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, pesticides and cytotoxicity testing of plant materials [36,37]. Due to its good correlation with cytotoxicity, it is commonly used as a preliminary tool for screening cytotoxicity in plant crude extracts [38]. The LC_{50} value represents the concentration of the chemical that produces death in half of the subjects after a certain exposure period.

Table 4. Cytotoxicity of different parts of *Salacia reticulata, Salacia chinensis* and market sample of *Salacia reticulata*

Sample	LC_{50} (mg/mL)	Fiducial CI 95%		R^2
		Lower value	Upper value	
SRS	1.31[a]	0.56	2.54	0.94
SRL	0.74[a]	0.29	1.42	0.88
SCS	2.60[a]	1.32	4.88	0.87
SCL	0.88[a]	0.37	1.65	0.86
SRM	1.68[a]	0.73	3.31	0.97

Results are means of four replicates with five dilutions (200 naupalii) per sample; Means followed by same letter in each column are significantly not different at 0.05 level.

As demonstrated in Table 4, leaf and stem extracts of *S. reticulata* and *S. chinensis* exhibited potent brine shrimp toxicity. The order of potency was SRL > SCL > SRS > SRM > SCS (LC_{50} values were 0.74 mg/mL, 0.88 mg/mL, 1.31 mg/mL 1.68 mg/mL and 2,60 mg/mL respectively). The higher cytotoxicity in leaf extracts of both species might be due to the presence of higher flavonoid and antioxidants. These conform with the previous findings [39,44,45] and who compressively investigated cytotoxicity of leaf stem and root extracts of 5 morpho types of *Munronia pinnata*.

4. Conclusion

The present investigation compared for the first time the distinguished morphological, physicochemical, phytochemical properties, cytotoxicity and *in vitro* antioxidant activity of different parts of *S. reticulata* with its potent substitute *S. chinensis*. These extensive evaluations confirmed the marked similarities between the two plant species. Therefore, it could be partially justified that *S. chinensis* could be used as a natural antidiabetic substitute for *S. reticulata*, which could lead to the sustainable utilization of the latter. Moreover, the establishment of commercial cultivation of S. *chinensis* is also encouraged.

Conflicts of Interests

Authors declare that there is no conflict of interest.

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