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Evaluation of Anti-Diabetic Property of Extracts of Different Plant Parts of *Salacia chinensis Linn*

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Abstract

Plants from the Western Ghats Mountains (a global biodiversity hot-spot) in western India are increasingly gaining importance for their newfound disease curative properties. One such example is an extract of the plant, *Salacia chinensis Linn.*, the compound Salacinol which (along with related compounds) is increasingly being used in the treatment of diabetes. Of late, demand for this extract has increased at a rapid pace, leading to widespread overharvesting of Salacia roots (the plant part predominantly used for extraction) and consequent population decline by over 50%. Such overexploitation in a global biodiversity hotspot threatens the ecological sustainability of this fragile ecosystem and global health care, as well as local livelihoods. One strategy to relieve the harvest pressure on wild population while attempting to cater to the ever increasing demand of raw material by the pharmaceutical industry is to utilize alternative plant parts and raise viable commercial cultivation. With a view to developing a sustainable harvest strategy, this paper presents an assessment of anti-diabetic activity of alternative plant parts (stems, seeds, leaves). We present the results of in-vitro evaluation of α- glucosidase inhibition activity by *S. chinensis* extracts with respect to parameters like plant part, age of plant and effective solvent system. Promising α-glucosidase enzyme inhibition results were obtained from crude extracts of stems and seeds. The highest inhibition levels demonstrated by aqueous extracts of roots and stems were 80.43 ± 1.14 % (IC 50-22.17 µg/ml) and 81.2 ± 0.41 % (IC_{50}- 22.23 µg/ml) respectively, whereas for successive aqueous extracts of seeds inhibition levels were 56.0 ± 1.30 % (IC_{50}-79.04 µg/ml). By demonstrating that stems and seeds of *S. chinensis* can be used as an alternative to roots, our study has the potential to form the basis for a sustainable path forward for the harvesting of this plant for medicinal purposes.

Keywords: *Salacia chinensis*; α-glucosidase inhibition; Anti-diabetic property; Sustainable harvesting; Western Ghats

Introduction

Salacia chinensis Linn. (Synonym- Salacia prinoides) (Figure 1), Family-Hippocrateaceae, is an evergreen climbing shrub or a small tree occurring in India, Sri Lanka, China, Malaysia, Java and Phillipines [1-3]. In Maharashtra (India), it occurs in pockets mainly around the Sahyadri – Konkan corridor area of the northern Western Ghats [1]. Locally, it is referred to as 'Saptarangi', 'Saptachakri' or 'Ingali' [1,3]. *S. chinensis* has gained importance as a rich repository of chemical constituents and is known to contribute to various medicinal properties. Phytochemical profiling reveals the presence of constituents such as salacinol [4], kotalanol, neokotalanol, neosalacinol [5], foliasalaciosides, foliachinenosides [6], mangiferin [7] proanthocyanidin [8], triterpenoids like lupanes, hopanes and freidelanes [9] and eudesmane-type sesquiterpenes [7]. Extracts of *S. chinensis* have been shown to exhibit medicinal/curative properties with respect to several health conditions such as type 2 diabetes [4], tumors [10] mutagenicity [11], hepatitis, arthritis, cardiac disorders, mental disorders [12], and insulin resistance [13]. In addition, they have been demonstrated to improve glucose tolerance [14] and regulate kidney carbohydrate metabolism [15]. However, within traditional medicinal systems, the use of S. chinensis has been historically restricted to its use as an anti-diabetic agent; Salacinol, kotalanol, mangiferin, neosalacinol and neokotalanol have been shown to be major inhibitors of α-glucosidase enzyme [4,5], the enzyme that breaks down ingested carbohydrates and starches. These plant compounds therefore, act by reducing the absorption of glucose into the blood stream.

Figure 1: Details of *Salacia chinensis Linn* harvest. 1a. Shrubby habit of *S. chinensis Linn.;* 1b. A twig showing leaves and fruits; 1c. Stem and roots (plant age>20 years) ;1d. Stem and roots harvested from captive plantation (age 4 yrs); 1e. Root showing concentric rings.

The International Diabetes Federation (IDF) predicts the growth of diabetic patients from 366 million in 2011 to 552 million in 2030 [16]. Current strategies for the management of type 2 diabetes have

limitations; for instance insulin therapy has been known to result in insulin resistance [17], anorexia nervosa, brain atrophy and fatty liver during chronic treatments [18]. In contrast, the use of Salacia under prescribed dosage is considered completely safe [19]. Commercial demand for plant material of *Salacia* (*S. chinensis, S. oblonga, S. reticulata*) currently exceeds 100 metric tons per year; 95% of this demand is supplied from the wild (uncultivated) populations of the Western Ghats. Currently, only the roots of the plant are utilized for commercial harvesting of medicinal products, effectively resulting in the destruction of the individual plant. So far, the potential of alternative plant parts as sources of the medicinal compounds remains unexplored. Our paper addresses this gap by evaluating the α-glucosidase inhibitory activity of non-root parts of the plant, including stem, leaf and seed in addition to root. Variation in inhibitory activity by age of the plant and solvent system are also studied.

Materials and Methods

Study area

Healthy individuals of *S. chinensis* were harvested from wild populations at Amboli (15°57′45″ N, 73°59′52″ E, altitude 690 m above MSL) and cultivated areas from Ghisar village (18°17′6.3″ N, 73°32′47.1″ E, altitude 855 m above MSL), Maharashtra, India, in the year 2012 and 2013. Morphological characteristics of the samples were compared with botanical voucher specimens at the Botanical Survey of India, Pune (Accession no. 51570) and the samples were confirmed as *Salacia chinensis*.

Chemicals and solvents

α -Glucosidase was freshly isolated from the small intestine of a healthy laboratory rat (*Albino wistar*). Sucrose (RM 3063, Himedia, India), acarbose (Glucobay, Bayer Pharma, India), glucose reagent (11208102, AGAPPE diagnostics, India), sodium phosphate, monobasic, anhydrous (NaH_2PO_4, RM3964, Himedia, India), Di-sodium hydrogen phosphate dihydrate (Na_2 HPO_4. $2H_2O$, RM257, Himedia, India), methanol (Loba chemie, India), distilled water (Loba chemie, India) and microwell plate: Tarson 96 well polystyrene, non-treated (Cat. no. T941196, Tarsons, India) were used for the assay.

Equipment

The absorbance was measured by using a micro-plate reader (Versamax, Molecular devices).

Extraction

Harvested plant material was shade dried. Dried roots, stems, seeds and leaves were subjected to extraction. Extraction was carried out using 100 g of plant material (100 g) in 400 ml of methanol (for methanolic extraction), and 100 g of plant material in 400 ml of water (for direct aqueous extractions). The mixtures were refluxed for 1 hour. (1 hour time duration was determined as optimal by comparing extraction yields for 1, 2 and 3 hours durations and observing no increase in the extract beyond 1 hour) The procedure was repeated twice. For successive aqueous extraction, the spent raw materials of methanolic extraction were subjected individually to aqueous extraction with a 1 hour reflux. The extracts were then filtered, concentrated and dried under vacuum.

Enzyme inhibition assay

α -Glucosidase inhibition activity assay was performed as per Vogel and Vogel [20] with a few modifications. In brief, the pre-incubation mixture contained 80 mM phosphate buffer pH 7.0 and vehicle buffer or positive control or test sample. Following pre-incubation (37°C, 30 minutes), substrate (sucrose 37 mM-0.316 g dissolved in 25 ml of 80 mM phosphate buffer) was added to a final concentration of 23.125 mM, followed by re-incubation at 37°C for 50 minutes. The reaction was arrested by transferring the reaction mixture to a boiling water bath for 2 minutes and then cooling to room temperature. 250 µl of glucose reagent was added to 50 µl of reaction mixture and was incubated at 25°C for 10 minutes. The absorbance was measured at 510 nm in a micro-plate reader (Versamax, Molecular devices) and enzyme inhibition percentage was measured using the formula,

% inhibition={Absorbance (control)-Absorbance (test)} X 100/ Absorbance (control) Inhibition concentration 50 (IC_{50}) value was calculated using log-probit analysis.

Results

The enzyme inhibition activity demonstrated by different plant parts and by age of plant as well as solvent system is shown in (Table 1). Table 2 shows crude extract yield values for different plant parts (roots, stems, seeds and leaves) by age of the plant as well as cultivated status (i.e. whether wild or cultivated). Figure 2 and 3 represent the effect of age on IC_{50} value of successive aqueous extracts of *Salacia* roots and aqueous extracts of *Salacia* stems respectively.

Plant parts	Age of plant material(years)	Solvent	Inhibition by 100 µg/ml concentration of extract (%)*
Roots harvested from captive plantation	3	Methanol	49.48 ± 1.29
		Successive aq. Extraction	78.98 ± 2.13
		Aqueous	65.31 ± 0.61
	4	Methanol	67.16 ± 0.79
		Successive aq. Extraction	80.99 ± 0.27
		Aqueous	71.90 ± 0.92
Roots harvested from wild population	> 20	Methanol	71.00 ± 0.4
		Successive aq. Extraction	83.00 ± 0.12

		Aqueous	80.43 ± 1.14
Stems harvested from captive plantation	3	Methanol	39.73 ± 3.48
		Successive aq. Extraction	71.00 ± 0.20
		Aqueous	69.95 ± 1.79
	4	Methanol	54.25 ± 1.11
		Successive aq. Extraction	73.70 ± 0.56
		Aqueous	73.44 ± 0.51
Stems harvested from wild population	> 20	Aqueous	81.20 ± 0.41
Seeds harvested from wild population	-	Methanol	13.38 ± 0.30
		Successive aq. Extraction	56.00 ± 1.3
Leaves harvested from captive plantation	3	Methanol	20.79 ± 1.94

Table 1: α - Glucosidase inhibition activity of various plant parts across age for extracts from different solvent systems. *Average of three readings

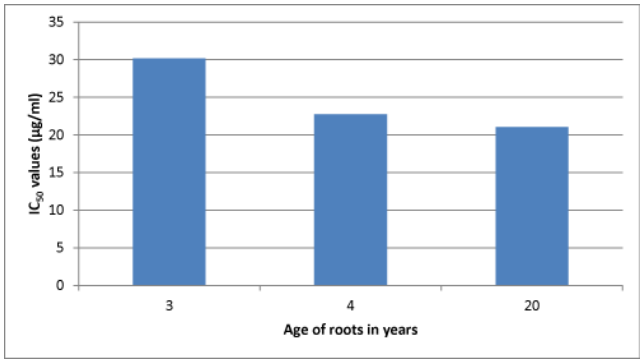

Figure 2: Effect of age on IC_{50} (µg/ml) of successive aqueous extracts of roots.

Highest α-Glucosidase enzyme inhibition activity was obtained from plant roots, followed by stems, seeds and leaves (Table 1). Comparing enzyme activity across plant parts yielded promising findings. Successive aqueous extracts of stems and seeds (both 100 µg/ml) exhibited inhibition values of 73.44% and 56.00%, respectively, values that are comparable to those obtained from root extracts (80.99%).

Discussion

Enzyme inhibition activity of successive aqueous extracts of roots was significantly higher than the activity of direct aqueous extracts and methanolic extracts (p value<0.001). However, there was no significant difference between inhibition activities of successive aqueous extracts and direct aqueous extracts of stems (p value>0.05). Methanolic extracts of stems had significantly lower enzyme inhibition activity (p value<0.001) than the direct aqueous and successive aqueous extracts. Even among plantation of young age (3 years), significant enzyme inhibitory activity was observed in root and stem extracts. As the age of plant increased, IC_{50} value decreased, indicating higher enzyme inhibition (Figures 2 and 3).

Direct aqueous extraction resulted in a better yield of crude extracts in comparison of the other solvents used (Table 2).

Solvent System	**Cultivated (3-4 yrs) ***			**Wild Population (>20 yrs)**		
	Stems (%)	Roots (%)	Leaves (%)	Stems (%)	Roots (%)	Seeds (%)
Methanol	2.9-5.0	4.7-8.3	7.0	-	10.1-10.8	10.0
Successive Aqueous	4.5-6.3	6.2-12.3	9.7	-	7.3-8.8	4.0
Direct Aqueous	6.9-9.3	8.7-12.4	-	10.8	11.5	-

Table 2: Crude extract yields using different solvent systems. * Readings indicate average extraction yields.

IC_{50} values of stem extracts and root extracts were found to be in the range of 22-55 µg/ ml and 12-58 µg/ ml, respectively. According to Quality Standards of Indian Medicinal Plants [21], medicines from *Salacia chinensis* within their prescribed limits (1-3 gm per day) are safe. These values are much smaller, and therefore safer, than the LD50 value of 2000 mg/kg suggested by Kannan et al. [22].

Traditionally, roots of *Salacia* are used in production of anti-diabetic medicines. There are no reports suggesting strategies for sustainable harvest of roots of this species. Furthermore agronomic practices for *Salacia chinensis* cultivation are yet to be developed, thereby exerting enormous pressure on wild populations of this plant for the supply of raw material. As a result, the very existence of *S.*

chinensis is under threat. This underlines the urgency to develop protocols for repeated harvests and package of cultivation practices [23].

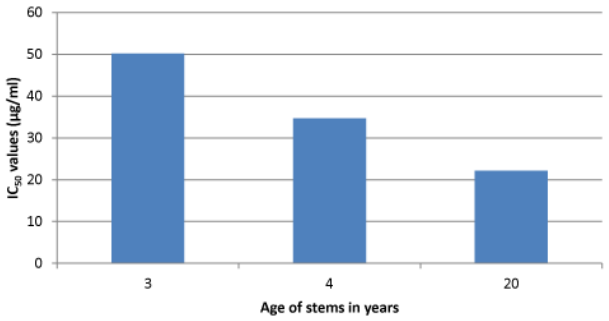

Figure 3: Effect of age on IC_{50} (µg/ml) of aqueous extracts of stems.

Our study has the potential for informing such practices by demonstrating the potential of stems and seeds. Future direction should focus on strategies to facilitate the use of these non-root plant parts as renewable source of raw material by developing sustainable agronomic practices like coppicing, pruning to increase harvestable biomass. Systematic cultivation will help farmers, cultivators to gain insights into how to harvest, how much to harvest and when to harvest. Industry can be benefited by assured supply of authentic raw material through contractual farming schemes of the government.

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