Anti-Glycation and Glycation Reversing Potential of *Salacia Reticulata* L. (Kothala Himbutu) Root, Stem, Leaf and Twig Extracts

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Abstract—*Glycation is a series of complex reactions between reducing sugars and proteins. This reaction ultimately produces multitude of detrimental advanced glycation end products (AGEs). Formation and accumulation of AGEs have been implicated in the development and progression of several diabetic complications, neurological diseases and aging. Thus, glycation inhibitors and glycation reversing agents offer a potential strategy as therapeutics for diverse diseases. Salacia reticulata L. is a scientifically well documented traditional anti-diabetic plant. However, anti-glycation and glycation reversing potential of this plant has not been studied. Present study reports anti-glycation and glycation reversing potential of Salacia reticulata.*

Freeze dried hot water extracts of Salacia reticulata root, stem, leaf and twigs were used in this study. Different concentrations of root, stem, leaf and twig extracts were subjected to anti-glycation and glycation reversing assays in vitro. Rutin was used as the positive control.

Root, stem, leaf and twig extracts of Salacia reticulata showed significant ($P < 0.05$) anti-glycation activity in a dose dependent manner. IC_{50} values for anti-glycation activity of root, stem, leaf and twigs extracts were 13.06 ± 0.69, 27.29 ± 0.93, 144.53 ± 1.12 and 171.90 ± 0.88 µg/ml respectively. Root extract showed significantly high ($P < 0.05$) anti-glycation activity compared to other extracts and rutin (IC_{50}: 21.88 ± 2.82 µg/ml). Glycation reversing potential of different parts of Salacia reticulata also showed significant ($P < 0.05$) and dose dependent relationship. EC_{50} values of root, stem, twig and leaf extracts were 101.60 ± 11.57, 116.67 ± 0.64, 180.53 ± 7.41 and 264.40 ± 9.30 µg/ml respectively. Potency of different parts of Salacia reticulata for anti-glycation and glycation reversing activities were root > stem > leaf > twig and root = stem > twig > leaf respectively.

It is concluded that all parts of Salacia reticulata possess both anti-glycation and glycation reversing activities. Further, this is the first study to report anti-glycation and glycation reversing potential of this plant.

Keywords— *Salacia reticulata*, anti-glycation, glycation reversing

I. INTRODUCTION

Diabetes mellitus is a chronic metabolic disease increasing in epidemic proportions throughout the world (King *et al*., 1998). It affected about 171 million people worldwide in 2000 and the number is projected to increase to at least 366 million by 2030 (Wild *et al*., 2004).

This chronic disease is characterized by hyperglycaemia due to defects in insulin secretion or insulin resistance (Wild *et al*., 2004). Prolong hyperglycaemia results in the formation of advanced glycation end products in body tissues (Reddy & Beyaz, 2006; Wautier & Guillausseau, 2001). The complex AGEs formed can leads to protein cross linking and contribute to the development and progression of several diabetic complications such as peripheral neuropathy, cataracts, impaired wound healing, vascular damage, arterial wall stiffening, decreased myocardial compliances (Ahmed, 2005; Thomas *et al*., 2005; Aronson, 2003; Wautier & Guillausseau, 2001), neurological diseases and aging (Reddy & Beyaz, 2006). Thus, glycation inhibitors and glycation reversing agents offer a potential strategy as therapeutics for diverse disease conditions.

Salacia reticulata L. belongs to the genus *Salacia* and found in the submontane forests in Sri Lanka and India. In Sri Lankan and Indian traditional Ayurvedic medicine this plant is used in the treatment of variety of diseases. The roots and stems of *Salacia reticulata* have been extensively

used in the management of diabetes (Chandrasena, 1935). Traditional knowledge of anti-diabetic activity of roots and stems of this plant has been scientifically proven by many researches using *in vitro*, *in vivo* and clinical studies (Jayawardena *et al*., 2005; Kajimoto *et al*., 2000; Kumara *et al*., 2000; Shimoda *et al*., 1998; Yoshikawa *et al*., 1997; Yoshikawa *et al*., 1998; Serasinghe *et al*., 1990). However, extremely limited studies have been showed that bark and root extracts can reduce HbA1c levels in diabetes patients (Jayawardena *et al*., 2005; Kajimoto *et al*., 2000). Further, there are no studies on anti-diabetic activity of leaves and twigs of *Salacia reticulata*. We have previously shown that root, stem, leaf and twigs extracts of *Salacia reticulata* have anti-oxidant activity (Ranasinghe *et al*., 2007). As lot of oxidative reactions are known to participate in the process of AGEs formation (Reddy & Beyaz, 2006) different parts of *Salacia reticulata* may have anti-glycation and glycation reversing activities. Present study reports anti-glycation and glycation reversing potential of root, stem, leaf and twigs extracts *Salacia reticulate in vitro*.

II. METHODOLOGY

A. Materials

Leaves, stem, roots and twigs were collected from *Salacia reticulata* plantations of Eco-Tech Company Ltd at Nathandiya, Sri Lanka.

B. Chemicals and reagents

Bovine serum albumin (BSA), D-glucose and trichloroacetic acid (TCA) were purchased from Sigma-Aldrich, USA. All the other chemicals used for the preparation of buffers and solvents were of analytical grade.

C. Preparation of hot water extracts

Plant materials collected were air dried in an air-conditioned room (25 ± 2 °C) for 6 days. Then, air dried samples were ground to fine powder using a laboratory grinder. Two grams from powdered root, stem, leaves and twigs samples were extracted in 50 ml of hot water for 20 min. Extracts were then filtered, centrifuged at 6000 rpm for 10 min and freeze-dried (Christ-Alpha 1-4 Freeze dryer, Biotech International, Germany). Freeze-dried extracts were used in anti-glycation and glycation reversing assays.

D. Anti-glycation assay

The anti-glycation assay was performed according to the method of Matsuura *et al*. (2002) with some modifications. Freeze-dried extracts of *Salacia reticulata* root, stem, leaf and twigs (n=3 each) at 6 different concentrations (7.8, 15.6, 31.2, 62.5, 125.0 and 250.0 µg/ml) were used in the assay. Reaction mixtures containing 800 µg BSA, 400 mM glucose and different concentrations of *Salacia reticulata* extracts in a reaction volume of 1 ml in 50 mM phosphate buffer (pH 7.4) containing 0.02 % sodium aside (w/v) were incubated at 60 °C for 40 h. After cooling, aliquots of 600 µl were transferred to 1.5 ml eppendorf tubes and 60 µl of 100 % (w/v) TCA was added, stirred, centrifuged at 15,000 rpm at 4 °C for 4 min and supernatants were removed. The resulting AGEs-BSA precipitate was dissolved in 3 ml of phosphate buffer saline (pH 10) and fluorescence intensity was measured at an excitation wave length of 370 nm and emission wave length of 440 nm using a spectroflurometer (Amino-Bowman®, Thermo Spectronic, USA). Rutin was used as the standard (positive control). Anti-glycation activity (inhibition %) of each *Salacia reticulata* extract and rutin was calculated using the following equation.

$$\text{Inhibition (\%)} = [(F_c-F_b)-(F_s-F_{sb}) / (F_c-F_b)] * 100$$

Where, F_c is the florescence of incubated BSA and glucose (control), F_b is the florescence of incubated BSA alone (blank), Fs is the florescence of the incubated BSA and glucose with *Salacia reticulata* extracts or the positive control (rutin) and F_{sb} is the florescence of incubated BSA with the *Salacia reticulata* extracts or the positive control.

E. Glycation reversing assay

Reaction mixture containing 800 µg BSA and 400 mM glucose in 1 ml of 50 mM phosphate buffer (pH 7.4) containing 0.02 % sodium aside (w/v) was incubated at 60 °C for 40 h. After cooling, aliquots of 600 µl were transferred to 1.5 ml eppendorf tubes and 60 µl of 100 % (w/v) TCA was added, stirred, centrifuged at 15,000 rpm at 4 °C for 4 min and supernatants were removed. The resulting AGEs-BSA precipitates were dissolved in 50 mM phosphate buffer (pH 7.4) and added with *Salacia reticulata* extracts (15.6, 31.2, 62.5, 125.0 and 250.0 µg/ml; n=3 each) in a final reaction volume of 1 ml for incubation at 60 °C for 40 h. After cooling, 60 µl of 100 % (w/v) TCA was added, stirred and centrifuged at 15,000 rpm at 4 °C for 4 min. The resulting precipitates were dissolved in 3 ml of phosphate buffer saline (pH 10) and fluorescence

intensity was measured at an excitation wave length of 370 nm and emission wave length of 440 nm using a spectroflurometer. Percentage glycation reversing was calculated using the following equation.

$$\text{Glycation reversing (\%)} = [(F_c-F_b)-(F_s-F_{sb}) / (F_c-F_b)] * 100$$

Where, F_c is the florescence of incubated BSA and glucose (control), F_b is the florescence of incubated BSA alone (blank), Fs is the florescence of the incubated BSA, glucose and *Salacia reticulata* extracts and F_{sb} is the florescence of incubated BSA with the *Salacia reticulata* extracts.

F. Statistical analysis

Data represented as mean ± SD (n=3). Data of each experiment were statistically analyzed using SAS version 6.12. One way analysis of variance (ANOVA) and the Duncan's Multiple Range Test (DMRT) were used to determine the differences among treatment means. P < 0.05 was regarded as significant.

III. RESULTS

Anti-glycation and glycation reversing activities of *Salacia reticulata* root, stem, leaf and twigs extracts are given in Table 1 and Table 2 and Fig 1 and Fig 2.

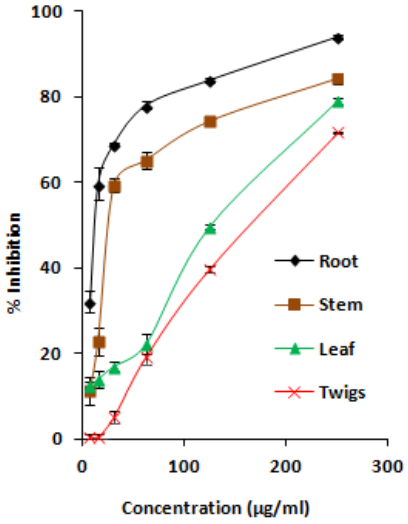

Fig 1. Percentage of inhibition anti-glycation activity of *Salacia reticulata* root, stem, leaf and twigs extracts. IC_{50} values: root: 13.06 ± 0.69^{d} µg/ml; stem: 27.29 ± 0.93^{c} µg/ml; leaf: 144.53 ± 1.12^{b} µg/ml; twigs: 171.90 ± 0.88^{a} µg/ml. IC_{50} values superscripted by different letters are significantly different at p < 0.05.

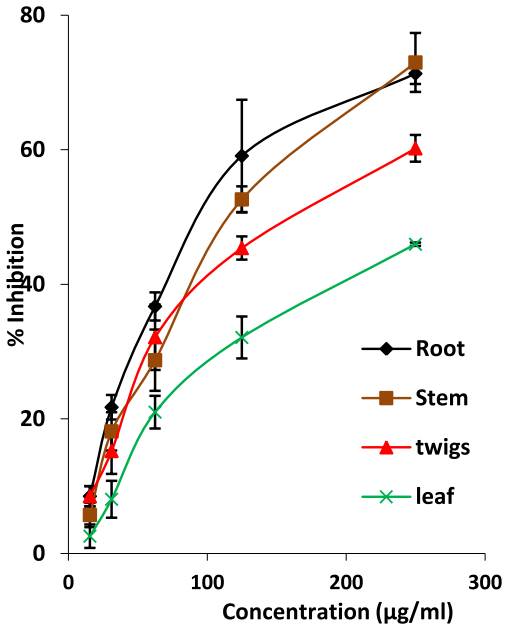

Fig 2. Percentage of inhibition glycation reversing activity of *Salacia reticulata* root, stem, leaf and twigs extracts. EC_{50} values: root: 101.60 ± 11.57^{c} µg/ml; stem: 116.67 ± 0.64^{c} µg/ml; twig: 180.53 ± 7.41^{b} µg/ml; leaf: 264.40 ± 9.30^{a} µg/ml. IC_{50} values superscripted by different letters are significantly different at p < 0.05.

Table 1. Anti-glycation activity of different parts of Salacia reticulata

Salacia reticulata extract	Concentration (µg/ml)						IC_{50} (µg/ml)
	7.8	**15.6**	**31.2**	**62.5**	**125.0**	**250.0**	
Root	32.09 ± 3.47	59.55 ± 5.42	68.96 ± 0.18	77.92 ± 1.17	83.79 ± 0.57	93.81± 0.74	13.06 ± 0.69[a]
Stem	11.25 ± 4.46	22.85 ± 4.69	59.37 ± 2.38	65.14 ± 2.68	74.28 ± 1.24	84.23 ± 1.72	27.29 ± 0.93[b]
Leaf	12.44 ± 1.24	13.95 ± 2.77	16.93 ± 1.54	22.07± 3.31	49.57 ± 0.86	78.86 ± 1.02	144.53 ± 1.12[c]
Twigs	0.57 ± 0.63	0.49 ± 0.82	5.08 ± 1.41	19.10 ± 1.78	39.69 ± 0.65	71.57± 0.15	171.90 ± 0.88[d]

Data represented as mean ± SD. IC_{50} values superscripted by different letters are significantly different at $p < 0.05$.

Root, stem, leaf and twigs extracts of *Salacia reticulata* showed significant ($P < 0.05$) anti-glycation activity in a dose dependent manner. IC_{50} values for anti-glycation activity of root, stem, leaf and twigs extracts were 13.06 ± 0.69, 27.29 ± 0.93, 144.53 ± 1.12 and 171.90 ± 0.88 µg/ml respectively. Root extract showed significantly high ($P < 0.05$) anti-glycation activity compared to other extracts and rutin (IC_{50}: 21.88 ± 2.82 µg/ml). Potency of different parts of *Salacia reticulata* for anti-glycation activity was root > stem > leaf >twigs.

Glycation reversing potential of different parts of *Salacia reticulata* also showed significant ($P<0.05$) and dose dependent relationship. EC_{50} values of root, stem, twigs and leaf extracts were 101.60 ± 11.57, 116.67 ± 0.64, 180.53 ± 7.41 and 264.40 ± 9.30 µg/ml respectively. Potency of different parts of *Salacia reticulata* for glycation reversing activity was root = stem >twig> leaf.

Table 2. Glycation reversing activity of different parts of *Salacia reticulata*

Salacia reticulata extract	Concentration (µg/ml)						EC_{50} (µg/ml)
	15.6	**31.2**	**62.5**	**125.0**	**250.0**		
Root	8.50 ± 1.50	21.73 ±1.83	36.71 ± 2.09	59.09 ± 8.35	71.32 ± 1.54	101.60 ± 11.57[c]	
Stem	5.71 ± 1.81	18.15 ± 2.84	28.72 ± 4.55	52.62 ± 1.94	72.98 ± 4.38	116.67 ± 0.64[c]	
Twigs	2.56 ± 1.74	8.05 ± 2.74	21.00 ± 2.43	32.10 ± 3.12	45.96 ± 0.25	180.53 ± 7.41[b]	
Leaf	8.50 ± 1.50	15.23 ± 3.41	32.14 ± 4.87	45.40 ± 1.73	60.22 ± 1.99	264.40 ± 9.30[a]	

Data represented as mean ± SD. EC_{50} values superscripted by different letters are significantly different at $p < 0.05$.

IV. DISCUSSION

Protein glycation (Maillard reaction) is a series of complex reactions between carbonyl groups of reducing-sugars with amino groups of proteins. Amino groups of proteins react initially with reducing sugars to form Schiff bases followed by their Amadori rearrangement products. These Amadori products undergo a rearrangement reaction giving a multitude of end products that are known as AGEs (Reddy & Beyaz, 2006). Some of the AGEs are intensely coloured compounds and have typical fluorescence characteristics (Reddy & Beyaz, 2006). Therefore, in this study anti-glycation and glycation reversing activities were measured in terms of inhibition of Maillard fluorescence formation.

Findings of this study clearly showed that all parts of *Salacia reticulata* possess anti-glycation activity. Interestingly, root extract had significantly high anti-glycation activity compared to the positive control, rutin. Therefore, especially root extracts can be used in the management of AGEs associated chronic diseases. This plant is a well known anti-diabetic plant in Sri Lankan traditional knowledge and Indian system of Auyrveda (Chandrasena, 1935). Anti-diabetic activity of this plant has been explained through variety of mechanisms. However, anti-diabetic activity with respect to anti-glycation activity has been very poorly documented. Therefore, findings of this study showed a novel way of explaining the anti-diabetic mechanisms of this plant.

Glycation reversing is the reversing of already formed AGE cross links. It is an approach to attenuate AGE related complications. Such protein crosslink breakers might be useful as therapeutics for regulation of complications resulting from diabetes, neurological diseases and aging (Reddy & Beyaz, 2006). However, only few AGE crosslink breakers known to date and there are reports of their limited efficacy in *in vivo* studies (Reddy & Beyaz, 2006). Therefore, it is vital to explore compounds with AGEs reversing ability to manage AGE related complications. Findings of this study clearly showed that all parts of *Salacia reticulata* possess glycation reversing activity. These novel anti-diabetic properties further add values for this traditional medicinal plant as an anti-diabetic plant with multiple mechanisms. This is the first report of simultaneous presence of anti-glycation and glycation reversing activities of this plant.

Interesting and valuable findings of this study are that the presence of anti-glycation and glycation reversing activities in leaves and twigs. Therefore, leaves and twigs which can be repeatedly harvested in short cycles unlike root and stem can be used as a good natural source with anti-glycation and glycation reversing activities.

Different AGE inhibitors suppress AGE formation at different stages of glycation. Aspirin inhibits protein glycation at the early stage of glycation process by acetylating free amino groups of protein. Therefore, it causes to block the attachment of reducing sugars (Malik & Meek, 1994). The inhibitory activities of vitamin B1 and B6 derivatives such as pyridoxamine and thiamine pyrophosphate (Reddy & Beyaz, 2006; Booth *et al*., 1996) have mainly been attributed to their abilities to scavenge reactive carbonyl compounds. We have previously shown that all parts of *Salacia reticulata* possess anti-oxidant activity (Ranasinghe *et al*., 2007). Therefore, anti-glycation and glycation reversing activities may be due to the presence of anti-oxidative compounds. However, it is difficult to decide exactly at which stage of glycation process or in what way the intervention by *Salacia reticulate* extracts is exerted to reduce the glycation reaction. Further experiments are necessary to identify active compound/s, *in vivo* efficacy and mode of actions.

V. CONCLUSIONS

It is concluded that all parts of *Salacia reticulata* possess both anti-glycation and glycation reversing activities. Roots and stems were the most biologically active parts of the plant. Further, leaves and twigs, which can be repeatedly harvested in short cycles unlike roots and stems can be used as a good natural source with anti-glycation and glycation reversing activities. This is the first study to report anti-glycation and glycation reversing potential of this plant.

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