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RESEARCH ARTICLE

ANTIOXIDANT AND ANTI-DIABETIC ACTIVITY OF *SALACIA CHINENSIS* STEM EXTRACT

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ABSTRACT

The present study aims to evaluate the antioxidant activity of *Salacia chinensis* specimen stem extract collected from five different locations. I*n- vitro* antidiabetic activity of aqueous stem extracts of *Salacia chinensis* of Karikan accession on α-amylase and α-glucosidase enzymes was studied. The stem extracts of *Salacia chinensis* (Hubili, Karikan, Bandal, Jogimat and Udipi locations from Karnataka) were evaluated for qualitative and quantitative antioxidant activity of aqueous, ethanol, chloroform, petroleum ether and acetone extracts by using DPPH as free radical. Different concentrations of aqueous stem extract of *Salacia chinensis* were subjected to α -amylase and α-glucosidase inhibitory assay. The absorbance was measured at 540 and 405 nm using UV-Vis Spectrophotometer and the percentage of α-amylase and α-glucosidase inhibitory activity and IC_{50} values of extract and fractions were calculated. The aqueous stem extract of *Salacia chinensis* showed significant radical scavenging activity. The aqueous stem extract of *Salacia chinensis* of Karikan accession exhibit dose –dependent increase in inhibitory effect on α-amylase enzyme(up to 87.30 %) which was comparable with standard drug acarbose (74.03 %) and α-glucosidase enzyme(up to 78.30 %) which was comparable with standard drug acarbose (81.60 %) whereas other concentrations shown lesser activity.

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INTRODUCTION

The *Salacia chinensis* Linn (Celestraceae family) commonly known as Dimal, Modhupal, Ingli, Chinese salacia, Lolly berry and saptarangi in Ayurveda, is a small erect, woody, climbing shrub, found almost throughout India including Andaman and Nicobar Island. The leaves are elliptic, narrowly ovate-round or obovate-elliptic, and glabrous; the petioles are 5-8 mm long. The fruit has one seed in it. Flowers have five petals and they are yellow or yellowish-green. The plant is well known for its medicinal properties for Diabetes Milletus, Rheumatism, Skin disease, Anti-inflammatory and Liver tonic. The root extract shows various activities like, antioxidant, anticaries, antiulcer, antidiabetic, hypoglycemic, antiobesity and skin lightening agent. The oxidative damage caused by free radicals is a major contributor to degenerative disease. Antioxidants are protective molecules which will prevent and repair damage caused by free radicals. Diabetes mellitus is a complex and a diverse group of disorders that disturb the metabolism of carbohydrate, fat and protein. The intestinal digestive enzymes α-amylase and α- glucosidase plays an important role in the digestion of carbohydrates. The therapeutic properties from medicinal plants reduces the post prandial glucose level in blood by the inhibition of α-amylase and α- glucosidase enzymes. The inhibition of α-amylase delays carbohydrate digestion and prolongs the overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption, consequently blunting post prandial glucose levels (Rhabaso-Lohert and Chaisson, 2004). The number of Diabetes mellitus cases has been increasing worldwide in recent years. As of 2015, an estimated 415 million people had diabetes worldwide, (Update 2015) with type 2 DM making up about 90% of the cases (Shi, 2014). The main objective of the present study was to evaluate the free radical scavenging activity and to investigate *in-vitro* α-amylase and α-glucosidase inhibitory activity of the aqueous stem extract of *Salacia chinensis.*

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MATERIALS AND METHODS

Sample collection

The Plants of *Salacia chinensis* were collected from different regions of Karnataka like Hubili, Karikan, Jogimat, Bandal and Udipi. These plants were identified in Queen Mary's College Chennai-600 004 and authenticated by Dr. P. Jayaraman, Plant Anatomy and Research Centre, Chennai-600 045.

Extract preparation

The healthy plants were shade dried, powdered and extracted with solvents namely methanol, acetone, chloroform, aqueous and petroleum ether at 1:3 w/v ratio (Merck, extra pure) for 1 min using an Ultra Turax mixer (13,000 rpm) and soaked overnight at room temperature. The sample was filtered through Whatman No. 1 paper, evaporated under vacuum in a rota-vator at 40 °C and redissolved in known volume of methanol, ethanol and water and stored at 18 °C until use.

Quantitative analysis of Free radical scavenging activity of *Salacia chinensis*

The antioxidant activity was determined by Selvaraj *et al.,* 2014 using DPPH, as a free radical. Stem extract of 100µl were mixed with 2.7ml of methanol and then 200µl of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control (Lee *et al*., 2005). Subsequently, at every 5 min interval, the absorption maxima of the solution were measured using a UV double beam spectra scan (Chemito, India) at 517nm. The antioxidant activity of the sample was compared with known synthetic standard of (0.16%) of Butylated Hydroxy Toluene (BHT). The experiment was carried out in triplicate. Free radical scavenging activity was calculated by the following formula:

$$\text{Inhibition} = \frac{[(\text{Absorbance of control (Ac 517)} - \text{Absorbance of sample (As517)}) \times 100}{(\text{Absorbance control (Ac517)})]}$$

α-amylase inhibitory assay

The α-Amylase inhibitory activities of the given plant samples were carried out according to Nickavar *et al.,* 2009.

α – glucosidase inhibitiory assay

The α-glucosidase inhibition was determined according to Matsui *et al.,* 2007.

RESULTS AND DISCUSSION

Antioxidant activity

The antioxidant activity of the stem extract of *Salacia chinensis* was carried out from plants collected from five different locations namely Hubili, Karikan, Bandal, Jogimat and Udipi using DPPH (1, 1-Di-Phenyl-2-Picryl Hydrazyl) to identify the free radicals. The Qualitative analysis and the results were shown in table-1. Among the five different solvent extracts of *Salacia chinensis,* the aqueous stem extract recorded the most effective DPPH radical scavenging activity followed by ethanol, acetone, petroleum ether and chloroform extracts. Among the five different accessions of *Salacia chinensis,* the Karikan accession recorded the most effective DPPH radical scavenging activity followed by Udipi, Hubili, Bandal and Jogimat accessions. The antioxidant positive samples were subjected for further quantitative analysis. Quantitative analysis of free radical scavenging activity was carried out on stem extracts of *Salacia chinensis* of aqueous, ethanol,acetone, chloroform and petroleum ether (Table-2). Among the five different solvent extracts of *Salacia chinensis* the aqueous stem extract (86.80%) recorded the most effective DPPH radical scavenging activity followed by ethanol (81.90%), acetone (65.50%), petroleum ether (55.70%) and chloroform (44.26%). The stem extracts in different solvents recorded different extent of antioxidant activity. The aqueous stem extract of *Salacia chinensis* recorded the higher percentage of free radical scavenging activity than ethanol followed by acetone, petroleum ether and chloroform.

Table 1. Antioxidant activity of *Salacia chinensis* stem extract

S.No	Solvents	Hubili	Bandal	Karikan	Udipi	Jogimat
1	Ethanol	+	+	++	+	+
2	Aqueous	++	+	+++	++	+
3	Acetone	+	-	+	+	-
4	Chloroform	-	-	-	-	-
5	Petroleum ether	-	-	+	+	-

Key.- absent + mild ++ moderate +++ present in high amount

Table 2. Antioxidant activity in stem extracts of *Salacia chinensis*

Places	BHT	Ethanol	Aqueous	Acetone	Petroleum Ether	Chloroform
Hubili	98.40 ± 0.20^{a}	77.00 ± 0.20^{d}	81.53 ± 0.35^{d}	60.37 ± 0.45^{d}	43.40 ± 0.50^{c}	37.70 ± 0.50^{c}
Karikan	98.40 ± 0.20^{a}	81.90 ± 0.60^{e}	86.80 ± 0.50^{e}	65.50 ± 0.40^{e}	55.70 ± 0.50^{d}	41.80 ± 0.70^{d}
Udipi	98.40 ± 0.20^{a}	68.00 ± 0.30^{c}	72.90 ± 0.20^{c}	50.00 ± 0.20^{c}	55.70 ± 0.70^{d}	44.26 ± 0.44^{e}
Jogimat	98.40 ± 0.20^{a}	61.50 ± 0.40^{b}	68.00 ± 0.40^{b}	31.10 ± 0.40^{bs}	37.70 ± 0.60^{b}	24.50 ± 0.60^{b}
Bandal	98.40 ± 0.20^{a}	57.27 ± 0.65^{a}	64.50 ± 0.70^{a}	29.43 ± 0.50^{a}	35.20 ± 0.46^{a}	19.60 ± 0.30^{a}
F value	0.000	1485.707	1217.622	5009.343	912.048	129.445
P value	0.001**	< 0.001**	< 0.001**	< 0.001**	< 0.001**	< 0.001**

**It denotes significant at 1% level. Different alphabets among concentrations denotes significant at 5% level using DMRT.

Table 3. Anti-diabetic activity of stem extract of *S. chinensis* by α -amylase inhibitory acti ivity

Sample	Conc.(mg/ml)	Inhibition %
Salacia chinensis	10	16.40 ± 0.72^{a}
	20	35.90 ± 0.56^{b}
	30	57.20 ± 0.60^{c}
	40	71.80 ± 0.53^{d}
	50	87.30 ± 0.36^{e}
Acarbose (Standard)	0.1	74.03 ± 0.77
IC_{50} value	27.906	
F value	7445.972	
P value	<0.001**	

**It denotes significant at 1% level. 2. Different alphabets among concentrations denotes significant at 5% level using DMRT.

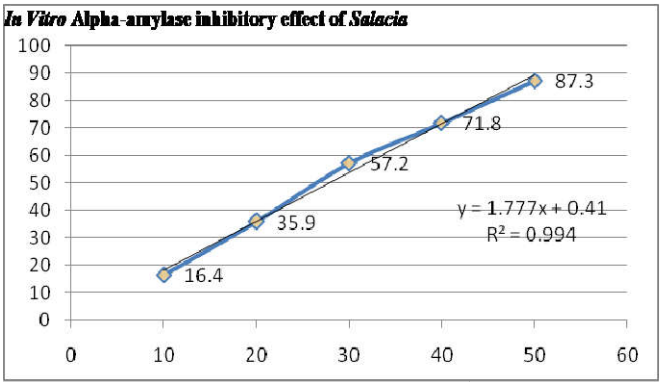
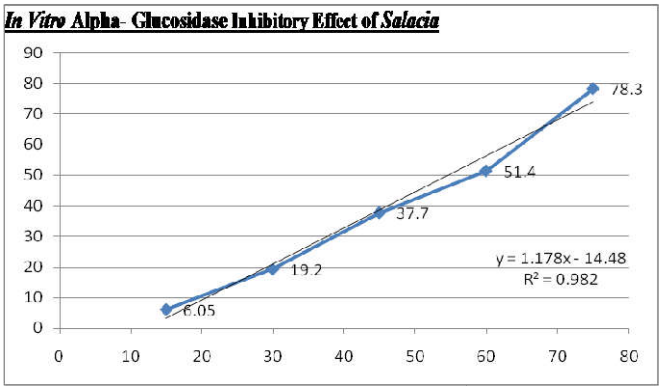


Table 4. Anti-diabetic activity of stem extract of *Salacia chinensis* by α- glucosidase inhibitory activity

Sample	Conc.(mg/ml)	Inhibition %
Salacia chinensis	15	6.12 ± 0.22[a]
	30	19.20 ± 0.79[b]
	45	37.70 ± 0.82[c]
	60	51.40 ± 1.23[d]
	75	78.30 ± 0.56[e]
Acarbose (standard)	0.4	81.6 0± 0.87
IC_{50} value	54.736	
F value	3757.127	
P value	<0.001**	



Anti-diabetic activity: The antioxidant activity of Karikan accession showed higher activity when compared to other locations therefore anti-diabetic activity was evaluated only in this accession.

α- amylase inhibitory effect of stem extract of *Salacia chinensis:* The α- amylase activity of stem extracts of *Salacia chinensis* of Karikan accession were investigated at the concentrations of 10,20,30,40 and 50 mg/ml. The maximum α-amylase inhibition activity (Table-3) was at the concentration of 50 mg/ml showed 87.30% and standard drug agarbose at 0. 1 mg/ml concentration showed maximum inhibition of α-amylase activity as 74.03%. The stem extracts of *Salacia chinensis* (Karikan accession) and acarbose (standard) showed an IC_{50} value of 27.906 and 0.0653 mg/ml respectively in the α- amylase inhibition assay.

α- glucosidase inhibitory effect of stem extract of *Salacia chinensis:* The α-glucosidase activity of stem extracts of *Salacia chinensis* of Karikan accession were investigated at the concentrations of 15,30,45,60 and 75 mg/ml were analysed for α- glucosidase activity. The maximum α- glucosidase inhibition activity (Table-4) was at the concentration of 75 mg/ml showed 78.30% and standard drug agarbose extract at 0.4 mg/ml showed maximum inhibition of α- glucosidase activity at 81.60 %. The stem extracts of *Salacia chinensis* and acarbose (standard) showed an IC_{50} value of 54.736 and 0.1876 mg/ml respectively in the α- glucosidase inhibition assay.similar results were obtained on *Salacia sps* leaf extract, which exhibited anti-diabetic potential (Priya *et al.,* 2016).

Conclusion

The aqueous stem extract of *Salacia chinensis* showed maximum antioxidant activity both qualitatively and quantitatively. The aqueous stem extract of *Salacia chinensis* collected from Karikan accession efficiently inhibits both enzymes in dose dependent manner. The antidiabetic action of *Salacia chinensis* stem can also be attributed to the intestinal α-amylase and α-glucosidase inhibitory activity. Further studies are required to elucidate whether *Salacia chinensis* have antidiabetic potential by *in vivo* studies for validating the traditional value.

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