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ANTIOXIDANT AND ANTI DIABETIC POTENTIALS OF *SALACIA SPECIES*

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ABSTRACT

In Traditional Indian systems of Medicine, numbers of plants are prescribed for the treatment of diabetes and to improve anti oxidant status. In persistent hyperglycemia oxidative stress has been found mainly due to an increased production of free radicals and sharp reduction of antioxidants defenses and the tissue antioxidant status leading to the development of diabetic complications. Hence treatment of diabetic patients with an antioxidant and anti diabetic plant drug will be beneficial in attenuating these complications. With these objectives in the present work a comparative evaluation was made of both anti diabetic and antioxidant potentials of *salacia oblonga* (SO), *Salacia reticulate* (SR) and *Salacia chinensis* (SC) and discussed. The anti oxidant efficacy was evaluated through different systems of assay such as Lipid peroxidation assay, Nitric oxide radical scavenging assay and Reduce Glutathione assay. The Hydroalcoholic extracts of the stem of selected plants were found to possess different levels of antioxidant status. Anti diabetic efficacy was evaluated in the STS induced diabetic rats. However, considering all the assays it can be stated that SO exhibits better antioxidant activity and anti diabetic activity as compared to other two species screened.

Keywords: Antioxidant activity, Ant diabetic, *Salacia* species.

INTRODUCTION

Type 2 Diabetes mellitus is a chronic disorder arising due to absolute or relative deficiency of insulin secretion. The world Health organization (W.H.O) predicts that the number of cases worldwide with diabetes is presently 150 billion, which will double by the year 2025 [1]. This disorder has been currently managed by majority of the population through conventional drugs. However, either insulin or other modern drugs have not proven to be effective in modifying the course of diabetic complications. Due to cost and side-effect of modern drugs, herbal medicines are preferred by many people. A number of plant species have been used for the management of diabetes throughout the world. In the present work also three plant drugs namely *Salacia reticulata*, *S. oblonga* and *S. chinensis* which are commonly used in the treatment of diabetes were selected and screened for their anti oxidant and anti diabetes potentials [2].These plant drugs were used by the practitioners of Indian systems of medicine such as Siddha and Ayurveda. Besides these plant drugs were also used as anti diabetes drugs by folklore practitioners.

MATERIAL AND METHODS

Extraction

Stem of *Salacia reticulata* Wight. was collected from Petchiparai, Kanyakumari District, Tamil Nadu.Stem of *Salacia oblonga* Wall. collected from Coutrallam, Tirunelveli District and *Salacia chinensis* Linn. collected from Patchai Malai Hills Tiruchirapalli District of Tamil Nadu [3]The plants were identified and deposited at the herbarium of Centre for Advanced Research in Indian systems of Medicine, SASTRA University, Thanjavur. The collected plants materials were cleaned thoroughly with water and cut into small pieces and shade dried. The dried plant materials were coarsely powdered. The powdered plant material (200 g) was macerated in 2 liter 70% v/v ethanol for 72 hrs, filtered and concentrated in vacum at 40°C using a rotary evaporator. Extracts were subjected to further analysis as per standard textual procedures[2] the antioxidant potential of the selected plant drugs were measured employing different systems of assay, such as Lipid per oxidation assay, Nitric oxide radical scavenging assay and Reduce Glutathione assay.

Anti diabetic studies

Anti diabetic activity was evaluated in STZ induced Diabetic Albino Wistar rats, as per CPCSEA guide lines, after obtaining necessary approval from the Institutional Animal Ethical Committee (CPCSEA /IAEC/15/SASTRA). Diabetes was induced through intraperitoneal (i.p.) injection of buffered solution of streptozotocin (65 mg/kg bodyweight) Blood is collected on 3rd, 22nd, 28th, 35, 42& 60th days from the retro orbital sinus and blood glucose levels were estimated [7,8].

Animals were divided into 9 groups having 6 animals in each group

Group I - Control - Normal Saline (0.5ml)

Group II - Disease Control - Normal Saline (0.5ml) + STZ (65mg/kg bw)

Group III - Standard - Glibenclamide(5mg/kg bw) +STZ (65mg/kg bw)

Group IV - Test -SO (300mg/kg bw) + STZ (65mg/kg bw)

Group V - Test - SO (500mg/kg bw) + STZ (65mg/kg bw)

Group VI - Test - SR (300mg/kg bw) + STZ (65mg/kg bw)

Group VII - Test -SR (500mg/kg bw) + STZ (65mg/kg bw)

Group VIII - Test - SC (300mg/kg bw) + STZ (65 mg/kg bw)

Group IX - Test - SC (500mg/kg bw) + STZ (65mg/kg bw)

Antioxidant Activity of *Salacia species*

Animals

Male Wistar albino rats (160 - 180 g) were kept in the Central Animal House at 26±2 ^{0} C and relative humidity (40% - 60%) and 12:12 light and dark cycle maintained throughout the experimental period. Animals were provided with standard rodent diet and water *ad libitum*.

Randomly selected rat was fasted overnight, and the liver was removed by CO_2 inhalation method. Isolated liver was washed in ice cold saline and homogenized in 0.15 M potassium Chloride. The homogenate was then used for TBARS and Reduced Glutathione estimation.

Lipid Peroxidation Assay

The degree of lipid peroxidation was evaluated by estimating the thiobarbituric acid reactive substances (TBARS) using the standard method [1] (Ohkawa *et al*., 1979). Briefly different concentrations of the extract were added to the liver homogenate (0.5 ml). Lipid peroxidation was initiated by adding 100 microliter of 15mM of $FeSO_4$ solution and then incubated at 37^{o}C for 30 minutes. After 30 minutes, 1.0 ml of 10% TCA was added and centrifuged for 10 minutes, to the supernatant obtained 1.0 ml of Thiobarbituric acid

was added. The tubes were then boiled for 20 minutes and the pink colour developed was read at 535 nm [14].

Reduced Glutathione Assay

Reduced Glutathione was estimated by Ellman's method (Tripathi *et al*., 1998). Briefly different concentrations of the extract were added to the liver homogenate (0.5 ml). Then 0.5 ml of 5% TCA in 0.1 mM EDTA was added, After 10 minutes all the tubes were centrifuged at 2000 g for 10 minutes. To 1.5 ml of supernatant added 2.5 ml of Phosphate buffer and the colour was developed by adding 0.01% of DTNB. The colour developed was read at 412 nm [15].

Nitric Oxide Radical Scavenging

Nitric oxide radical scavenging activity was measured spectrophotometrically (Govindharajan *et al*., 2003). 1.0 ml of Sodium nitroprusside (5 mmol) in phosphate buffer was mixed with different concentrations of the extract (100 – 500 microgram/ml in phosphate buffer). The tubes were then incubated at 25^0C for two hours. At the end of second hour 1.5 ml of reaction mixture was removed and diluted with 1.5 ml of Greiss reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% of naphthyl ethylenediamine dihydrochloride) The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphtylethylene diamine dihydrochloride was measured at 546 nm [16].

Calculation of % of Inhibition

(Control OD – Test O.D)/ Control OD) * 100
(or)
100 – (Test OD/Control OD) * 100

Statistical Analysis

All the data were presented as the Mean ± SD.

RESULTS AND DISCUSSION

Taking 0% inhibition in the mixture without plant extract, regression equations were prepared from the concentrations of the extracts and percentage inhibitions of free radical formation. IC_{50} values were calculated from these regression equations. IC_{50} value is inversely related to the activity. The extracts were found to have different levels of antioxidant activity in different systems. Table 1, 2 and 3 demonstrates the comparative data of free radical scavenging activity exhibited by extracts of the selected antidiabetic plants evaluated through chemical methods. Of the selected medicinal plants highest activity was revealed by *Salacia oblonga.*

Table 1: Anti oxidant efficacy of *Salacia oblonga* extracts

Concentration (µg/ml)	% inhibition of LPO	% inhibition of Oxidation of GSH	% inhibition of Nitric oxide formation
25	8.6 ± 1.2	15.8 ± 2.6	12.6 ± 2.6
50	18.9 ± 2.5	25.6 ± 1.2	22.6 ± 3.9
75	34.9 ± 3.6	33.8 ± 2.5	35.8 ± 5.9
100	55.2 ± 2.8	45.9 ± 2.6	44.5 ± 4.8
125	68.9 ± 2.9	58.9 ± 1.6	55.9 ± 3.5
IC_{50}	98.2	105.9	112.8

Table 2: Anti oxidant efficacy of *Salacia reticulata* extracts

Concentration (µg/ml)	% inhibition of LPO	% inhibition of Oxidation of GSH	% inhibition of Nitric oxide formation
25	12.5 ± 2.1	25.3 ± 3.5	15.2 ± 2.5
50	20.6 ± 2.8	38.9 ± 4.8	28.9 ± 3.8
75	38.9 ± 5.2	52.9 ± 3.6	45.9 ± 5.9
100	49.8 ± 3.6	66.8 ± 5.7	62.5 ± 6.2
125	56.9 ± 3.8	72.1 ± 2.5	71.5 ± 3.8
IC_{50}	198.5	112.5	155.2

Table 3: Anti oxidant efficacy of *Salacia chinensis* extracts

Concentration (µg/ml)	% inhibition of LPO	% inhibition of Oxidation of GSH	% inhibition of Nitric oxide formation
25	12.5 ± 1.2	19.5 ± 3.6	20.5 ± 4.5
50	29.8 ± 2.6	28.9 ± 4.5	38.9 ± 5.6
75	48.9 ± 3.6	35.6 ± 6.5	48.6 ± 5.8
100	59.8 ± 5.6	49.8 ± 5.9	59.8 ± 2.5
125	69.5 ± 5.8	68.9 ± 6.8	72.5 ± 2.6
IC_{50}	**302.5**	**385.6**	**325.6**

Table 4: Glucose levels in the STZ induced diabetic rats.

Group	Initial	3rd Day	22nd Day	28th Day	35th Day	42nd Day	60th Day
	Glucose levels were estimated in the units mg/dl						
Control	100 ± 9.30	109 ± 8.0	110 ± 8.4	115 ± 6.2	117 ± 8.1	114 ± 8.6	124 ± 6.5
Disease Control	112 ± 10.6	112 ± 6.1	246 ± 15.4	254 ± 11.4	253 ± 25.5	258 ± 16.2	259 ± 15.0
STD	117 ± 8.1	120 ± 5.6	184 ± 19	158 ± 21	143 ± 14	147 ± 20	150 ± 8.50
Treated (300mg/SO)	106 ± 15	120 ± 5.2	237 ± 14	226 ± 14	191 ± 14	178 ± 19	158 ± 26
Treated (500 mg/SO)	101 ± 9.3	111 ± 10	166 ± 20	182 ± 19	176 ± 16	146 ± 9.1	144 ± 11
Treated (300 mg/SR)	102 ± 17	121 ± 11	225 ± 23	292 ± 31	233 ± 24	178 ± 31	177 ± 10
Treated (500 mg/SR)	122 ± 9.6	167 ± 15	186 ± 17	236 ± 24	204 ± 11	160 ± 12	152 ± 7.9
Treated (300 mg/SC)	113 ± 9.0	122 ± 9.7	200 ± 16	243 ± 22	192 ± 14	175 ± 14	169 ± 21
Treated (500 mg/SC)	114 ± 11	153 ± 31	169 ± 39	185 ± 41	157 ± 27	130 ± 23	133 ± 26

CONCLUSION

The present study revealed that all the selected plants exhibited satisfactory scavenging effect in all the radical scavenging assays and the use of these plants in the management of diabetes would exert several beneficial effects by virtue of their antioxidant activity. Amongst the three medicinal plants, *Salacia chinensis* revealed poorest antioxidant activity. However, considering all the results, it can be concluded that *Salacia oblonga* possess good antioxidant activity.

This study has revealed that hydroalcoholic extract of stem of *Salacia oblonga* not only prevents hyperglycemica but also acts as an anti oxidant. A 60 days treatment with *Salacia oblonga* would maintain the blood glucose level to the near normalcy. Similarly, the blood glucose lowering effect of *Salacia oblonga* (300 mg/kg.bw) and standard drug Glibenclamide (5mg/kg. b.wt.) was also evaluated by Glucometer Strip method. It is observed that blood glucose levels were significantly reduced and maintained in the blood stream during the treatment period. This experimental study revealed that *salacia oblonga* extract and *salacia chinensis* at higher dose maintains blood glucose levels in STZ induced diabetic rats.

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