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Antihyperglycemic Activity of Root Bark of *Polyalthia longifolia* Var. pendula and Aerial Parts of *Sida rhombifolia* Linn. and Its Relationship with Antioxidant Property

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Polyalthia longifolia var. pendula (PL) and *Sida rhombifolia* Linn. (SR) were detected with free radical (DPPH) scavenging action by thin layer chromatography (TLC). The methanolic extract of *Polyalthia longifolia* var. pendula and *Sida rhombifolia* Linn. exhibited better antioxidant activity (IC_{50}: 10.77 and 42 µg/mL) than aqueous extract of *Polyalthia longifolia* var. pendula and *Sida rhombifolia* Linn. (IC_{50}: 44.36 and 62.68 µg/mL) in DPPH scavenging assay. All the plant extracts were found with low toxicity (LD_{50} >3 g/kg). The methanolic extract of *Polyalthia longifolia* var. pendula and *Sida rhombifolia* Linn. (200 mg/kg) produced significant reduction in blood glucose level at 2 h ($p < 0.05$), 4 h ($p < 0.01$) and 8 h ($p < 0.01$) in streptozotocin induced diabetic rats. In the oral glucose tolerance test (OGTT), the methanolic extract of *Polyalthia longifolia* var. pendula and *Sida rhombifolia* Linn. (200 mg/kg) decreased glycemia at 1 h and the effect persisted until 2 h ($p < 0.05$) after glucose loading. The antihyperglycemic effect of methanolic extract of *Polyalthia longifolia* var. pendula and *Sida rhombifolia* Linn. (200 mg/kg) was significant ($p < 0.01$). This effect is in agreement with the antioxidant property of the extracts, suggesting contribution of free radical scavenging action.

Key Words: *Polyalthia longifolia* var. pendula, *Sida rhombifolia* Linn, Antihyperglycemic, Antioxidant.

INTRODUCTION

Diabetes mellitus currently affects an estimated 143 million people worldwide and the number is growing rapidly. Several oral hypoglycemic agents are the primary forms of treatment for diabetes. However prominent side effects of such drugs are the main reason for an increasing number of people seeking alternative therapies that may have less severe or no side effects[1]. Investigations have related etiology of diabetes to oxidative stress[2]. Improvement in *in vitro* antioxidant status has been reported to sensitize the insulin receptor and stimulate the secretion of insulin from β-cell of islets of langerhans in pancreas of streptozotocin (STZ) induced diabetic rats[3]. Thus plant based herbal drugs and botanicals with free radical scavenging activity are emerging as the primary components of holistic approaches to diabetes management[4]. However a limited number of the plant species have been studied and validated for their hypoglycemic properties using diabetic animal models and in clinical studies using human subjects[5].

This triage of useful and effective plants is at the heart of traditional medicinal knowledge and is an extremely important source of therapeutic compounds in use today. *Polyalthia longifolia* var. pendula (Family: Annonaceae) is an evergreen plant, commonly known as Devdaru, cultivated all over India and the bark is traditionally used for fever, diabetes, hypertention and helminthiasis[6].

The *Sida rhombifolia* Linn. (Family- Malvaceae) is a small erect under shrub, commonly known as Mahabala, distributed as a weed throughout the tropical and subtropical countries and used for the treatment of heart disease, rheumatic problem, piles and all kind of inflammations[7]. The root bark of *Polyalthia longifolia* var. pendula (PL) is reported to contain steroid, quinoline and isoquinoline alkaloids[8] with antimicrobial and hypotensive activity[9]. The aerial parts of *Sida rhombifolia* Linn. (SR) contain isoquinoline alkaloids, quinazoline alkaloids, flavonoids and steroids[10], with hepatoprotective, anti-inflammatory, lipid lowering, cardiac depressant, antispasmodic, uterine stimulant and antibacterial activity[11]. The aerial parts of SR and root bark of PL have not earlier been investigated for their relationship with antioxidant and antihyperglycemic activity. The objective of the present investigation is to study the *in vitro* antioxidant activity of parts of these medicinal plants and evaluation of their antihyperglycemic effect in streptozotocin induced diabetic rats.

EXPERIMENTAL

The root bark of PL and aerial parts of SR were collected in bulk from tribal region of Mayurbhanj district, Orissa. The specimens were identified at the Regional Plant Resource Center, Bhubaneswar, Orissa. A voucher specimen (SPS/SOA/105) has been deposited in our herbarium for further information.

Preparation of plant extract: The plant parts were oven dried at a temperature of 40 ºC for 48 h, they were prepared according to traditional methods. Dried coarse powder of root bark of PL (100 g) was extracted with methanol (1 L) in cold maceration for 72 h. The extract was filtered and distilled on water bath; a reddish brown syrupy mass was obtained (yield: 11.2 %). The aqueous extract of PL was prepared by infusing 100 g coarse powder in 1 L of boiling water for 0.5 h (yield: 8.6 %). The methanolic extract (yield: 7.5 %) and aqueous extract (yield: 4.7 %) of aerial parts of SR were prepared by same method as PL. The liquid extracts were filtered and evaporated to dryness in rotary evaporator. The plant extracts were chemically tested for the presence of different phytoconstituents using standard methods[12].

***In vitro* DPPH radical scavenging activity:** Free radical scavenging detection on thin layer chromatography (TLC) plates was performed using the diphenylpicryl-hydrazyl (DPPH) method[13]. One mg of the extract was weighed into a small test tube and 10 mL of methanol was added. The mixture was shaken together and with the help of capillary, spotted on the aluminum-coated plate *ca.* 10 mm away from the bottom of the plate. The point of the spot was clearly labelled and the plate was allowed to dry in air and developed in a trough containing the mobile phase (*n*-hexane:ethyl acetate, 8:2 and toluene:ethyl acetate, 7:3). The above was allowed to dry and viewed in UV light at 365 and 254 nm. The fluorescent points were marked and the slide was sprayed with DPPH (1 µg/mL) solution in methanol. After this, the plate was left to dry and yellow colouration produced was noted. The evaluation of radical scavenging abilities of the extracts were carried out by UV spectrophotometric measurement using ascorbic acid as reference compound. Decrease in absorbance from that of freshly prepared 76 µL solution of DPPH in methanol in presence of different concentrations of plant extracts (12.5, 25, 50, 75 and 100 µg/mL) was continuously recorded at 515 nm at 25 ºC. All the experiments were carried out in triplicate. The IC_{50} values were calculated following development of regression equations.

Scavenging of superoxide radical (potassium superoxide assay): The scavenging activity towards the superoxide radical was performed by using alkaline DMSO method[14]. Potassium superoxide and dry DMSO were allowed to stand in contact for 24 h and the solution was filtered immediately before use. The filtrate (200 µL) was added to 2.8 mL of an aqueous solution containing NBT (56 µM), EDTA (10 µM) and potassium phosphate buffer (10 mM). The reaction mixture was started by illuminating the reaction mixture with different extract for 150 s. Immediately after illumination, the absorbance was measured at 560 nm. Ascorbic acid was used as positive control.

The male Wistar albino rats (250-280 g) and albino mice of both sexes (20-25 g) were purchased from the animal house of Siksha 'O' Anusandhan University, Bhubaneswar, Orissa, India. They had free access to standard rat pellets and water *ad libitum* and maintained under standard condition at a temperature of 25 ± 2 ºC, with a 12/12-light/dark cycle and 35-60 % humidity. The conditions in the animal house and the study protocol were approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) vides registration no. 1171 / C/ 08 / CPCSEA.

Oral glucose tolerance test: The animals were fasted overnight and divided into six groups of six rats in each group. Glucose (2 g/kg) was administered orally 0.5 h after oral administration of PL1, PL2, SR1 and SR2. Results were compared with glibenclamide (10 mg/kg) and control (water 2 mL/kg). Blood glucose level was assessed by the glucose-oxidase method at 0 (before glucose challenge), 0.5, 1.0, 1.5, 2.0 h after glucose administration[15].

Acute toxicity studies: The lethal dose (LD_{50}) of the two plant extracts was assessed by using albino mice of either sex, weighing 20-25 g to determine the dose. The animals were fasted overnight prior to the experimental procedure. Different doses of the extracts were administered by the intra-peritoneal route. The LD_{50} was calculated according to Miller and Tainter[16]. 1/10th of lethal dose was taken as a screening dose. Results were expressed as mean ± SD. The data were statistically analyzed by one-way ANOVA, followed by Dunnet's t-test. P values less than 0.05 and 0.01 were considered significant.

Antihyperglycemic activity: The Wistar albino rats were kept fasting for 24 h and thereafter diabetes was induced by intra-peritonial injection of STZ (Sigma Chemicals Co. USA) freshly dissolved in citrate buffer (pH 4.5) immediately before use. Streptozotocin was given at a dose of 65 mg/kg body weight. In order to avoid the STZ induced hypoglycemic mortality, 5 % glucose solution was given for 24 h to STZ treated rats.

In acute study, the rats were divided into six groups of six each. Group I served as diabetic control (Normal saline water 2 mL/kg p.o.), Group II received glibenclamide (10 mg/kg p.o.), Group III, IV, V and VI were given orally methanolic and aqueous extract of PL and SR at a dose of 100, 200, 200 and 400 mg/kg p.o. respectively. Animals were fasted for 16 h prior to drug administration allowing access only to water. Blood samples were collected from the orbital plexus of the rats at 0, 1, 2, 4, 8 and 24 h after extract administration. Samples were analyzed for blood glucose by using glucose-oxidase method. Data were analyzed using ANOVA followed by Dunnet's t-test. The p values less than 0.05 and 0.01 were considered to be significant.

RESULTS AND DISCUSSION

The phytochemical screening of methanolic and aqueous extract of PL revealed the presence of alkaloids, gum, triterpenoids and tannins. The methanolic and aqueous extract of SR showed the presence of alkaloids, lipid, steroids, flavonoids, triterpenoids and tannins.

Several concentrations, ranging from 12.5-100 µg/mL of the methanolic and aqueous extract of PL and SR were tested for their antioxidant activity in DPPH model. It was observed that the free radicals were scavenged by the extracts in a concentration dependent manner upto the given concentration in this model. The maximum percentage inhibition of PL1, PL2, SR1 and SR2 were found to be 96.31, 81.15, 89.14 and 84.74, respectively at 100 µg/mL concentration (Figs. 1-4). The IC_{50} values of these extracts were calculated as 10.77, 44.36, 42.00 and 62.68 µg/mL, respectively. However, these extracts also showed encouranging response in inhibiting superoxide radicals (61, 55, 58 and 31 %), in comparison to ascorbic acid (62 %).

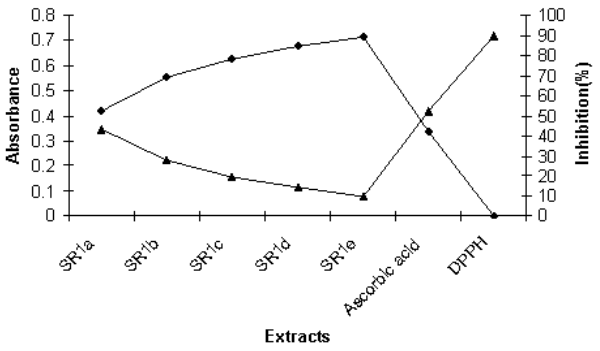

Fig. 1. DPPH scavenging action of methanolic extract of *Sida rhombifolia* (SR1). a: 12.5 µL of SR1; b: 25 µL of SR1; c: 50 µL of SR1; d: 100 µL of SR1. (▲) Absorbance, (◆) Inhibition %

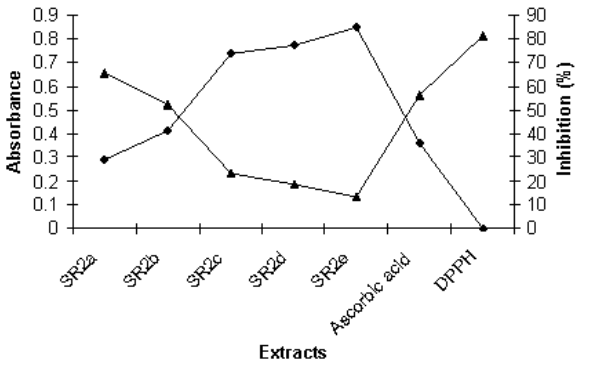

Fig. 2. DPPH scavenging action of aqueous extract of *Sida rhombifolia* (SR2). a: 12.5 µL of SR2; b: 25 µL of SR2; c: 50 µL of SR2; d: 100 µL of SR2. (▲) Absorbance, (◆) Inhibition %

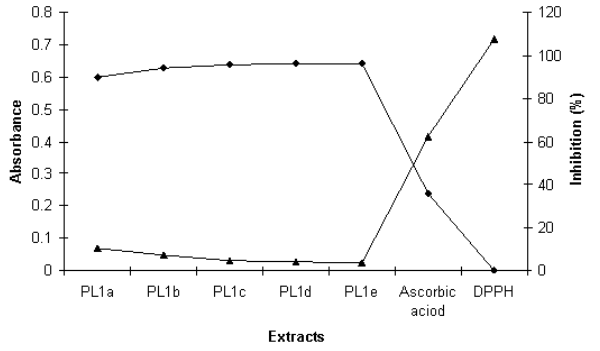

Fig. 3. DPPH scavenging action of methanolic extract of *Polyalthia longifolia* var. pendula (PL1). a: 12.5 µL of PL1; b: 25 µL of PL1; c: 50 µL of PL1; d: 100 µL of PL1. (▲) Absorbance, (◆) Inhibition %

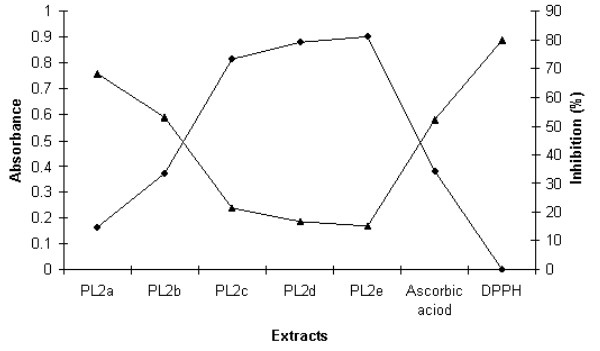

Fig. 4. DPPH scavenging action of aqueous extract of *Polyalthia longifolia* var. pendula (PL1). a: 12.5 µL of PL2; b: 25 µL of PL2; c: 50 µL of PL2; d: 100 µL of PL2. (▲) Absorbance, (◆) Inhibition %

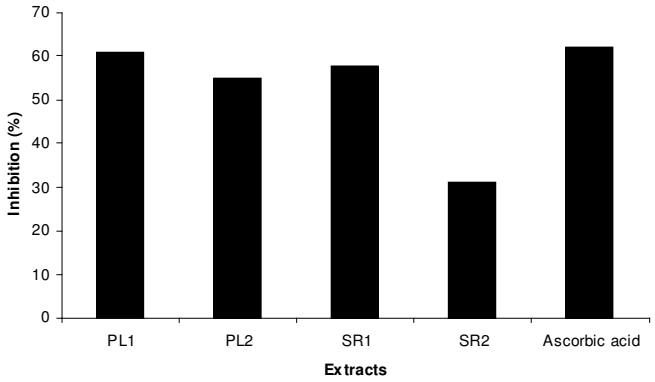

Fig. 5. Superoxide anion radical scavenging action of methanolic extract of *Sida rhombifolia* (SR1, 100 µg/mL), aqueous extract of *Sida rhombifolia* (SR2, 100 µg/mL) and methanolic extract of *Polyalthia longifolia* var. angustifolia (PL1, 100 µg/mL) compared to ascorbic acid (100 µg/mL)

The LD_{50} values for PL1 and PL2 were not less than 3 and 4 g/kg. The LD_{50} of SR1 and SR2 were found to be not less than 5 and 6 g/kg, respectively. These results suggest the less probability of toxicity of these plants.

The two medicinal plants were effective in the OGTT. Plasma glucose level reached a peak of 143.16 ± 3.60 mg/dl at 1 h after oral glucose loading (2 g/kg) in control rats (Table-1). The PL1 at a dose of 250 mg/kg produced a significant decrease of glycemia at 1.0, 1.5 and 2.0 h ($p < 0.01$) after glucose loading. ThePL2 at a dose of 250 mg/kg, produced significant decrease of glycemia at 1 h ($p < 0.01$) and the glucose lowering effect persisted until 2 h ($p < 0.05$) after glucose loading. The SR1 at a dose of 250 mg/kg produced a significant decrease of glycemia at 1 h ($p < 0.01$), 1.5 h ($p < 0.01$) and 2 h ($p < 0.05$). The SR2 at a dose of 250 mg/kg also produced significant decrease of glycemia at 1 h ($p < 0.01$) and persisted until 2 h ($p < 0.05$).

Methanolic and aqueous extract of PL and SR produced a dose-dependent antihyperglycemic effect in STZ induced diabetic rats in acute study. The PL1 and SR1 at a dose of 250 mg/kg produced significant reduction in blood glucose levels at 2, 4 and 8 h ($p < 0.01$) respectively. The PL2 and SR2 at a dose of 250 mg/kg produced a significant reduction in blood glucose levels at 2 h ($p < 0.05$), 4 h ($p < 0.01$) and 8 h ($p < 0.01$).The methanolic extract of PL and SR exhibited better antioxidant activities than their aqueous extracts in DPPH and superoxide scavenging assay. The activities were comparable to that of ascorbic acid, which was used as standard antioxidant compound. The methanolic extract of PL and SR showed increased scavenging activity against free radicals than aqueous extract. It may be due to presence of more antioxidant principles. Our phytochemical study on extracts of PL and SR has shown that flavonoids and tannins are abundant in these plants. Flavonoids and tannins have been reported to be antioxidative action in biological system, acting as scavenger of singlet oxygen and free radicals[17]. Hence the presence of these compounds in the extracts of the plants may be contributory to their antioxidant activity. Several authors reported that flavonoids, tannins, alkaloids, terpenes and steroids are known to be bioactive antidiabetic principles[18]. The antihyperglycemic effects of these plant extracts may be due, in part to their flavonoids, alkaloids,

TABLE- 1
EFFECT OF METHANOLIC AND AQUEOUS EXTRACT OF OF *P. longifolia* var. pendula (PL) AND *S. rhombifolia* linn. (SR) ON ORAL GLUCOSE LOADED HEALTHY NORMAL RATS

Groups (n = 6)	Dose	Plasma glucose level (mg/dl)				
		Base value	Time after glucose administration			
			0.5 h	1.0 h	1.5 h	2.0 h
Control	2 mL/kg	93.00 ± 3.22	136.50 ± 3.95	143.16 ± 3.60	128.00 ± 3.28	98.83 ± 3.6
Glibenclamide	10 mg/kg	91.50 ± 3.60	111.83 ± 4.53**	100.50 ± 3.61**	90.60 ± 2.94**	84.66 ± 2.58**
PL1	250 mg/kg	90.33 ± 2.90	113.50 ± 4.37**	103.83 ± 331**	94.33 ± 2.42**	86.16 ± 4.7*
PL2	250 mg/kg	91.33 ± 3.70	116.60 ± 4.17**	106.00 ± 3.22**	96.16 ± 3.31**	91.83 ± 3.54*
SR1	250 mg/kg	89.00 ± 4.09	115.83 ± 4.53*	104.50 ± 3.61**	93.60 ± 2.94**	90.83 ± 2.56*
SR2	250 mg/kg	89.33 ± 3.90	118.50 ± 4.92**	109.66 ± 6.02**	98.00 ± 2.82**	91.30 ± 3.93*
F-value		1.02	24.44	92.41	120.51	11.75

Results expressed as mean ± SD (n = 6). The data were statistically analyzed by one-way ANOVA, followed by Dunnet,s t-test. P values less than 0.05 were considered significant. Rats of all groups were loaded with glucose (2 g/kg p.o.) 0.5 h after extracts, glibenclamide and water (p. O.); *p < 0.05; **p < 0.01 (compared to control). PL1: Methanolic extract of *Polyalthia longifolia* var. angustifolia; PL2: Aqueous extract of *Polyalthia longifolia* var. angustifolia; SR1: Methanolic extract of *Sida rhombifolia*; SR2: Aqueous extract of *Sida rhombifolia.*

TABLE-2
ANTIHYPERGLYCEMIC EFFECT OF METHANOLIC AND AQUEOUS EXTRACT OF *P. longifolia* var. pendula (PL) AND *S. rhombifolia* Linn. (SR) IN STREPTOZOTOCIN-INDUCED DIABETIC RATS AFTER SINGLE DOSE (ACUTE STUDY)

Groups (n = 6)	Plasma glucose level (mg/dl)						
	Base value	Time after glucose administration					
		1 h	2 h	4 h	8 h	24 h	F-value
Control	241.80±8.47	245.50±7.58	241.00±7.94	240.50±5.60	239.33±9.75	242.50±3.93	0.60
Glibenclamide	255.70±11.10	241.16±12.15*	209.00±14.13**	173.40±9.56**	155.70±7.70**	247.70±5.75	81.96**
PL1	255.66±11.06	243.33±12.92	216.50±12.5**	184.33±11.69**	164.16±9.49**	253.00±6.26	69.86**
PL2	241.50±10.60	233.00±10.67	225.66±9.93*	190.16±11.70**	181.00±11.60**	240.70±8.28	37.84**
SR1	246.80±9.68	236.00±8.80	218.00±12.4**	192.80±11.72**	179.83±11.26**	238.33±9.00	39.24**
SR2	239.70±12.00	233.00±11.90	221.33±11.00*	201.16±13.87**	190.50±15.13**	247.16±9.28	9.61**

Results expressed as mean ± SD (n = 6). The data were statistically analyzed by one-way ANOVA, followed by Dunnet,s t-test. P values less than 0.05 were considered significant. *p < 0.05, **p < 0.01 compared to control. PL1 (250 mg/kg): Methanolic extract of *Polyalthia longifolia* var. pendula; PL2 (250 mg/kg): Aqueous extract of *Polyalthia longifolia* var. pendula; SR1 (250 mg/kg): Methanolic extract of *Sida rhombifolia*; SR2 (250 mg/kg): Aqueous extract of *Sida rhombifolia*.

tannins and terpenes. In streptozotocin induced diabetic rats the methanolic and aqueous extracts of PL and SR at the dose of 200 mg/kg body weight improved the oral glucose tolerance causing significant reduction in plasma glucose. These results support the beneficial use of these plants in treatment of type 2 diabetes. These extracts were effective, perhaps even better than glibenclamide since they were not hypoglycemic but only antihyperglycemic. Moreover, the LD_{50} value for PL1 and PL2 were not less than 3 and 4 g/kg, respectively. The SR1 and SR2 were without side effects even at a dose of 5 g/kg. These results support the absence of adverse effects of these plants.

Conclusion

The antihyperglycemic effects observed in the diabetic animals were in proportionate with their *in vitro* antioxidant activity. Thus the glucose lowering activity of the plant extracts may be attributed to their free radical scavenging action. Further studies are desirable to identify the active compounds.

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