

## Antidiabetic and Antioxidant Activity of *Scoparia dulcis* Linn.

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The hypoglycaemic activity of methanol extract of *Scoparia dulcis* was performed on both *in vitro* and *in vivo* models along with determination of total extractable polyphenol. Methanol extract of *Scoparia dulcis* contains 4.9% and water extract contains 3.2% of total extractable polyphenol. The antioxidant activity showed very promising result in both the tested methods that is 2,2-diphenyl-1-picrylhydrazyl and ferric ion reducing capacity. The antioxidant activity is directly correlated to the antidiabetic potential of drug. The two enzymes (amylase and glycosidase) found in intestine are responsible for the increasing postprandial glucose in body. *In vitro* model was performed on these enzymes and the results showed that methanol extract of *Scoparia dulcis* was effective to check the postprandial glucose level. The *in vivo* hypoglycaemic activity of methanol extract of *Scoparia dulcis* was performed on streptozotocin-induced diabetes mellitus showed significant inhibition of blood glucose level as compared to control and similar to that of standard glibenclamide. The overall data potentiates the traditional value of *Scoparia dulcis* as an antidiabetic drug.

**Key words:** Hypoglycaemic, polyphenol, antioxidant, postprandial

*Scoparia dulcis* is an annual erect herb distributed throughout tropical and subtropical regions of India, America, Brazil, West Indies, and Myanmar<sup>[1]</sup>. The whole plant is used for ailments like diarrhea, stomach-ache, kidney stones, kidney problems, and fever. *Scoparia dulcis* is a rich source of flavones, terpenes and steroids<sup>[2,3]</sup>.

After the recommendations made by World Health Organization (WHO) on diabetes mellitus (DM), the search for safe and effective hypoglycaemic agents has become an important area of research<sup>[4]</sup>. The search for novel hypoglycaemic compounds from medicinal plants is an important aspect of this and in view of the difficulties in testing *in vivo* studies, several *in vitro* tests have been developed in recent years<sup>[5]</sup>. The numbers of diabetic patients are rapidly rising in most parts of the world, especially in developing countries such as India, Thailand and Indonesia. Postprandial hyperglycaemia is one of the earliest abnormalities of glucose homeostasis associated with type 2 DM and is markedly exaggerated in diabetic patients with fasting hyperglycaemia. Postprandial hyperglycaemia is independent risk factor in the development of macrovascular complications of DM<sup>[6]</sup>.

A sudden increase in blood glucose levels, which causes hyperglycaemia in type 2 DM patients, occurs due to the hydrolysis of starch by pancreatic  $\alpha$ -amylase and the uptake of glucose by intestinal  $\alpha$ -glucosidases<sup>[7]</sup>. An effective strategy for type 2 diabetes management is the strong inhibition of intestinal  $\alpha$ -glucosidases and the mild inhibition of pancreatic  $\alpha$ -amylase<sup>[8]</sup>.  $\alpha$ -Amylase is present in both salivary and pancreatic secretion and is responsible for cleaving large malto-

oligosaccharides to maltose, which is then a substrate for intestinal glucosidase<sup>[9]</sup>.

As a number of antihyperglycemic agents have been found in plants, research into understanding the scientific basis for plant-based traditional medicines from various cultures has increased. Scientists explore clues to discover new therapeutic drugs for type 2 DM<sup>[10]</sup>. Streptozotocin is an antimicrobial agent and has also been used as a chemotherapeutic alkylating agent and reported as diabetogenic. Again, this insulinopenia syndrome, called 'streptozotocin diabetes', is caused by the specific necrosis of the pancreatic beta cells and streptozotocin has been the agent of choice for the induction of diabetes mellitus in animals ever since<sup>[11]</sup>.

Indian traditional medicines have a long history to treat DM by the herbs and herbal extracts. *Scoparia dulcis* is one of the traditional antidiabetic herbs. There were so many research work done on its antidiabetic properties. However, no one had studies its effect on enzymes  $\alpha$ -amylase and  $\alpha$ -glucosidase that regulate postprandial glucose absorption and metabolism and is responsible for postprandial hyperglycaemia. This study aimed to brief the antidiabetic properties of *S. dulcis* by correlating its antioxidant properties with *in vitro* and *in vivo* management of diabetes<sup>[12]</sup>.

2,2-diphenyl-1-picrylhydrazyl (DPPH),  $\alpha$ -amylase,  $\alpha$ -glucosidase and streptozotocin was purchased from Sigma Aldrich India Pvt. Ltd., Bangalore. Laboratory grade reagents were used in all experiments. UV spectrophotometer (UV 1700 Simazdu) was used in experiments. The plant was authenticated as *Scoparia dulcis* Linn. by Botanical Survey of India, Central

National Herbarium Howrah, Kolkata, India (Ref. No: CNH/I-I/38/2009/Tech II/137).

All the experiments were carried out using Wistar albino rats obtained from animal house, Birla Institute of Technology, Mesra, Ranchi, India and kept in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature  $24 \pm 2^\circ$  and relative humidity of 60-70%. A 10:14 light:dark cycle was followed. All animals were allowed to free access to water and fed with standard commercial rat chow pellets (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Ethics Committee (Reg. No:621/02/ac/CPCSEA) and were in accordance with the guidelines of the CPCSEA, Ministry of Forests and Environment, Government of India.

The antioxidant activity was determined by two methods i.e., reducing power and scavenging activity of DPPH. Total extractable polyphenol (TEP) of methanol extract of *Scoparia dulcis* (MESD) and water extract of *Scoparia dulcis* (WESD) were determined by taking 500 mg of each extracts and extracted sequentially with 40 ml methanol:water (50:50 v/v) and 40 ml acetone:water (70:30 v/v) at room temperature for 60 min in each treatment. Supernatants from these extractions were combined. They were concentrated to a small volume under reduced pressure at  $50^\circ$  and volume was made up to 10 ml with the same solvents (v/v). TEP were assayed spectrophotometrically by the Folin-Ciocalteu method using gallic acid as a standard and result expressed as gallic acid equivalents<sup>[13]</sup>.

The antioxidant activity of the *S. dulcis* extracts, based on the scavenging activity of the stable DPPH free radicals was determined by the method described by Kumaran and Karunakarn<sup>[14]</sup>. MESD and WESD (0.1 ml) were added to 3 ml of a 0.004% methanol solution of DPPH. Water (0.1 ml) was used as control. Absorbance was determined at 517 nm after 30 min.

The reducing power of *S. dulcis* extracts were determined according to the method described by Dawra *et al.*<sup>[15]</sup>. Different amounts of MESD and WESD (100-300 mg) were mixed with phosphate buffer (2.5ml, 0.02 M, pH 6.6) and potassium ferricyanide [ $K_4Fe(CN)_6$ ] (2.5 ml, 1%). The mixture was incubated at  $50^\circ$  for 20 min. Trichloroacetic acid (2.5 ml, 10%) was added to the mixture and centrifuged (3000 rpm) for 10 min. The upper layer of solution (2.5 ml) was

mixed with distilled water (2.5 ml) and  $FeCl_3$  (0.5 ml, 0.1%). A higher absorbance indicates a higher reducing power property<sup>[13]</sup>.

The *in vitro* enzymatic study was performed first on  $\alpha$ -amylase activity. Different concentration of the *S. dulcis* extract was mixed separately with 100 ml of the 2% potato starch solution in 200 ml beakers. To these mixtures 1 g of  $\alpha$ -amylase each were added and stirred vigorously at  $37^\circ$ . After 30 min 0.1 mol/l sodium hydroxide was added to terminate the  $\alpha$ -amylase activity. The glucose content of the solution was determined by glucose oxidase peroxidase method<sup>[6]</sup>.

The inhibitory activity of  $\alpha$ -glucosidase enzyme was determined by incubating a solution of starch (2% w/v sucrose) 1 ml with 0.2 M Tris buffer pH 8.0 and various concentration of plant extract for 5 min at  $37^\circ$ . The reaction was initiated by adding 1 ml of  $\alpha$ -glucosidase enzyme (1 U/ml) to it followed by incubation. Then the mixtures were heated in boiling water bath to stop the reaction. The amount of liberated glucose was measured by glucose oxidase peroxidase method<sup>[6]</sup>.

The  $IC_{50}$  value of enzymatic activity of the plant extract was determined by performing the assay as above with varying concentration of the plant extract ranging from 20 to 100  $\mu$ g. The  $IC_{50}$  value was determined from plots of percent inhibition vs log inhibitor concentration and calculated by non-linear regression analysis from the mean inhibitory values<sup>[17]</sup>. Acute oral toxicity was performed in mice by following organization for economic cooperation and development (OECD) guidelines<sup>[18]</sup>.

The whole study was divided in four groups, each group contain six animals. Streptozotocin (60 mg/kg) was injected to rats in each group of animals following 18 h fast. After 48 h the blood glucose level of rats were determined by glucose oxidase method. Only those animals which showed hyperglycaemia (blood glucose levels=200-500 mg/dl), were considered diabetic and taken for further experimentation. The group I received vehicle only (Tween-80, 1%) and served as control. Group II and group III received MESD 200 and 400 mg/kg therapeutic dose, respectively and group IV received glibenclamide 1.0 mg/kg. The study was carried out after repeated (once a

day) administration of the extract for 21 consecutive days. Basal glycaemia was determined in overnight fasted animals. The vehicle (1% tween 80), MESD (200 and 400 mg/kg) and glibenclamide (1.0 mg/kg) were orally administered to the animals of group I-IV for 21 consecutive days. Blood samples collected on the 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> days were analyzed for the determination of blood glucose level<sup>[19]</sup>.

The results were presented as mean±SEM. One-way Anova with Dunnett's post-test was performed using Graph Pad Prism version 3.00 for windows. Graph Pad Software, San Diego California USA,  $P < 0.05$  were considered significant.

*S. dulcis* MESD contains 4.9% and WSED 3.2% of total extractable polyphenolic compound. The antioxidant activity of TEP of MESD and WSED showed promising result in both methods such as DPPH and reducing power determination method. DPPH activity was directly proportional to TEP contents (fig. 1). The MESD showed higher absorbance as compared to WSED. The reducing power was found higher in MESD (0.650±0.011 in 300 mg/ml) as compared to WSED (0.533±0.017 in 300 mg/ml) (fig. 2). The TEP contents and antioxidant activity results were strongly supported MESD to be more active antidiabetic agent as compared to WSED. That's why MESD was selected for further *in vivo* and *in vitro* antidiabetic activities.

These activities showed *Scoparia dulcis* can be used as an antioxidant and some author directly correlate to the antioxidant activity with antidiabetic activity.

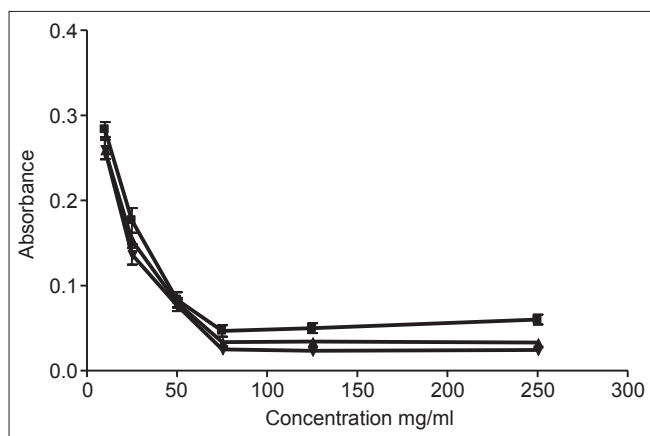


Fig. 1: DPPH scavenging activity. Plot of absorbance vs. concentration of DPPH scavenging activity of gallic acid (-■-), total extractable polyphenol of methanol extract of *Scoparia dulcis* (MESD) (-▲-) and water extract of *Scoparia dulcis* (WSED) (-▼-).

Relationship of extracts total extractable phenolic content to antidiabetic activity, previous research has shown that plant phenolic extracts and purified phenolic compounds can inhibit the *in vitro* activity of  $\alpha$ -amylase<sup>[20]</sup>.

The percent  $\alpha$ -amylase inhibition of MESD showed positive results. It inhibits the activity of  $\alpha$ -amylase in conversion of starch into maltose by slowing its activity. It showed 50% inhibition at the concentration of 65.91  $\mu$ g/ml (Table 1). The percentage inhibition of  $\alpha$ -glucosidase by MESD showed 50.8% inhibition at the concentration of 80  $\mu$ g/ml (Table 1) and the  $IC_{50}$  value was found to be 80.35.

*In vivo* study of MESD was done on streptozotocin-induced diabetic in rats. The effect of the treatment with extract and glibenclamide on blood glucose level in diabetic rats after post-treatment days was shown in Table 2. The study was conducted by collecting blood on 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> days after induction of diabetes and analyzed for the determination of blood glucose level. All the values were found significant in both treatment dose (200 and 400 mg/kg) as compared to control and standard glibenclamide (Table 2). These

TABLE 1: THE PERCENT  $\alpha$ -AMYLASE AND  $\alpha$ -GLUCOSIDASE INHIBITION

Concentration ( $\mu$ g/ml)	$\alpha$ -Amylase		$\alpha$ -Glucosidase
	MESD	Acarbose	MESD
20	38.7	39.2	38.0
40	42.8	44.6	42.1
60	48.2	52.6	46.4
80	53.8	58.6	50.8
100	58.9	66.7	54.3
$IC_{50}$	65.91	53.21	80.35

MESD=Methanol extract of scoparia dulcis

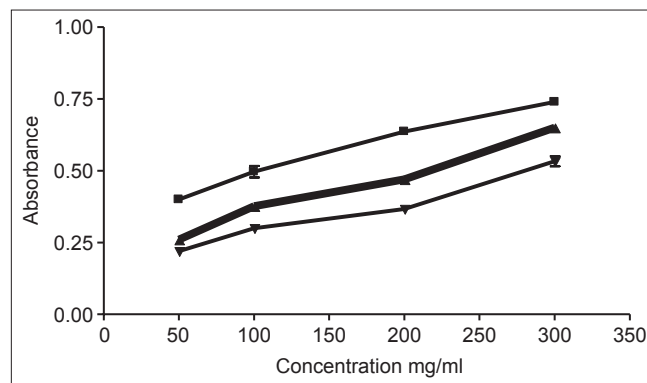


Fig. 2: Reducing power activity. Plot of absorbance vs. concentration of reducing power activity of gallic acid (-■-), total extractable polyphenol of methanol extract of *Scoparia dulcis* (MESD) (-▲-) and water extract of *Scoparia dulcis* (WSED) (-▼-).

**TABLE 2: BLOOD GLUCOSE LEVEL IN STREPTOZOTOCIN DIABETIC RAT**

Treatment	Blood glucose profile (mg/dl)			
	0	7 <sup>th</sup>	14 <sup>th</sup>	21 <sup>st</sup>
Control (vehicle)	342.20±15.01	421.00±24.31	395.00±24.31	419.00±24.44
MESD 200 mg/kg	331.00±21.46	316.80±13.60*	302.80±16.60*	279.40±13.55*
MESD 400 mg/kg	319.00±16.46	275.80±16.60*	259.80±16.60*	231.40±13.55*
Glibenclamide 1 mg/kg	311.00±9.44	251.00±17.84*	231.00±15.84*	219.00±11.79*

Effect of repeated oral administration of extracts and glibenclamide on blood glucose level in streptozotocin diabetic rat. The values represent in±SEM n=6, \*<0.05. MESD=Methanol extract of *Scoparia dulcis*.

findings suggested that methanol extract of *Scoparia dulcis* has hypoglycaemic activities, which validates its traditional use in Odisha, India.

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