



Original Research Article

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Antidiabetic and antioxidant activities of methanol extract of *Syzygium operculatum* (Roxb.) Niedz. bark

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ABSTRACT

Objective: To evaluate the antidiabetic and antioxidant effects of the methanol extract of *Syzygium operculatum* bark (MSOB) using *in vivo* and *in vitro* models.

Methods: Antidiabetic activity was assessed by using alloxan induced (120 mg/kg body weight) diabetic mice. The plant extract (200 mg/kg and 400 mg/kg body weight) was administrated *i.p.* to diabetic mice in comparison with standard metformin hydrochloride (150 mg/kg body weight). The antioxidant activity of the extract was evaluated by using a range of *in vitro* assays and results were compared to standards.

Results: The extract MSOB 200 mg/kg and 400 mg/kg reduced the blood glucose level 44.05% and 55.53% respectively where the standard drug metformin reduced 69.42% in dose of 150 mg/kg body weight. The extract MSOB showed significant 1,1-diphenyl-2-picrylhydrazyl scavenging activity, total phenol content, total flavonoid content and reducing power capacity compared to standards. The IC₅₀ values were found 300.34 µg/mL in 1,1-diphenyl-2-picrylhydrazyl radical scavenging assay where 40.31 µg/mL for ascorbic acid. The total phenol and flavonoid contents were 197.5 and 267.5 mg of gallic acid equivalent per gram, respectively.

Conclusions: The present study indicates that the extract of *Syzygium operculatum* bark is the potential sources of natural antioxidant and possesses significant antidiabetic activities.

1. Introduction

Diabetes is a major threat to global public health that is rapidly getting worse and the biggest impact is on adults of working age in developing countries[1]. Diabetes is the third leading fatal disorder after cancer and heart disease. In most developing countries at least one in ten deaths in adults aged 35 to 64 is attributable to diabetes and in some the figure is as high as one in five[2]. The new figure has been estimated that the number of people living with diabetes is expected to rise from 366 million in 2011 to 552 million and Bangladesh will have the seventh

highest number of diabetics in the world by 2030, if no urgent action is taken. Three new people at every ten seconds or almost ten million people per year are affected by diabetes[3]. The causes of diabetes are due to a combination of lifestyle and genetic factors[4]. Management of diabetes without any side effect is still a challenge for medical system. There are different groups of drugs for the treatment of diabetics, but most of them show drug interaction and adverse effects, so research is going on to find out safe and highly effective natural antidiabetic agents from plant species[5]. Recent evidence suggests that diabetes is associated with a high risk of cardiovascular disease[6]. Furthermore, after an acute coronary event diabetic subjects develop congestive heart failure more frequently and have a higher mortality rate than non-diabetic individuals[7-9]. Heart disease is a major complication and the leading cause of premature death among people with diabetes. At least 65 percent of people with diabetes die from heart disease or stroke[10]. Diabetes mellitus leads to multiple

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complications, such as blindness, kidney failure, amputations, strokes and heart attacks; it is considered to be a devastating illness[11]. It is usually accompanied by increased production of free radicals[12,13]. Although free radical production increases, somehow antioxidant production decreases in diabetes mellitus. It has been suggested that free radical production increases due to the increased oxidative stress in diabetes mellitus[14]. Generally, when cells use oxygen to generate energy, some free radicals are created. These by-products are generally reactive oxygen species (ROS) such as super oxide anion, hydroxyl radical and hydrogen peroxide that result from the cellular redox process. At low or moderate concentrations, ROS exerts beneficial effects on cellular responses and immune function but at high levels, free radicals and oxidants generate oxidative stress, a deleterious process that can damage cell structures, including lipids, proteins, and DNA. Primarily ROS plays an important role in the host defense mechanism against microorganisms, but the increased production of ROS is associated with the onset of a variety of diseases including cancers, inflammation, neurodegeneration, Parkinson's disease, atherosclerosis and pre-mature aging[15-20]. The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced *in situ*, or externally supplied through foods or supplements. These antioxidants act as free radical scavengers by preventing and repairing damages caused by ROS, and therefore can enhance the immune defense and lower the risk of health complications caused by oxidative stress. Increased level of ROS in diabetes could be due to their increased production and decreased destruction by nonenzymatic and enzymatic catalase, glutathione peroxidase and superoxide dismutase antioxidants. Currently available synthetic antioxidants like butylated hydroxy toluene and gallic acid esters have been suspected to prompt negative health effects like liver damage and mutagenesis. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity[21,22]. In recent years, there is an increasing interest in finding antioxidant activity of the natural products as oxidative stress is found to be responsible for several health complications.

In our study we used the bark extract of *Syzygium operculatum* (*S. operculatum*), a medium-sized tree found in the southern hilly region of Bangladesh. The local name in Bangladesh is Bottejham. For several diseases and ailments the different parts of this plant are used traditionally by the local people as relieving agent. For example, bark is used as tonic and astringent to the bowels, given in dysentery, bronchitis and ulcer. The bark of this plant has been used as an ingredient in various beverages, common tea for gastrointestinal disorders and as an antiseptic for dermatophytic disorders for many years[23]. Recent article has shown that the leaf

of this plant possesses great glucose lowering capacity[24]. Previous attention to phytochemical led to the isolation of oleanane type triterpene from its bark[25] and the presence of sterol, flavanone, chalcone, triterpene acid, *b-sitos-terol* and ursolic acid as the main constituents in the methanol extract of the *S. operculatum* bark (MSOB)[26,27]. However, to the best of our knowledge, the chemical constituents of MSOB have not been studied yet as well as its antidiabetic and antioxidant activities. Hence, in the present study, MSOB was examined for its antidiabetic and antioxidant activities.

2. Materials and methods

2.1. Collection of plant material

The bark of *S. operculatum* was collected from Khagrachari Krishi Gobeshona, Khagrachari Hill District, Chittagong Division, Bangladesh. It was authenticated and identified by Dr. Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Chittagong, Bangladesh.

2.2. Preparation of extract

After cleaning, the barks were dried in shade at room temperature for 14 days and then kept in an oven at 40-45 °C for 72 h and pulverized in a mechanical grinder. The extract was prepared by cold extraction process. In this process the coarse powder was submerged in methanol (75%) since methanol is the most common solvent for extracting most of the constituents present in herbal materials. A total of 220 g of dried powder was cold extracted with sufficient amount of methanol. Dried powder was kept in methanol for 15 days with occasional shaking and stirring. Final content was filtered by using cotton and Whatman No.1 filter paper to get the pure extract. Finally we got the solid extract from filtrate by evaporating the solvent in water bath at 30-35 °C.

2.3. Drugs and chemicals

Metformin hydrochloride (Square Pharmaceuticals Ltd., Bangladesh), alloxan monohydrate (Sisco Research Laboratories Pvt. Ltd., Mumbai, India), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) (Sigma Chemical, USA), methanol (Sigma Chemical, USA), ascorbic acid (SD Fine-Chem Ltd., India), Folin-Ciocalteu reagent (E-Merck, India), sodium carbonate (E-Merck, India), gallic acid (Sigma Chemicals, USA), aluminium chloride (SD Fine-Chem Ltd., India), potassium acetate (E-Merck, India) were procured and used in the experiment. All chemicals in this experiment were of analytical reagent grade.

2.4. Antidiabetic activity

2.4.1. Selection of animal

The study was conducted by collecting 20 Swiss albino male mice of 4-6 weeks of age weighing 25-30 g from International Center for Diarrheal Diseases Research, Bangladesh. They were housed in suitable cages (ten mice per cage) at an ambient temperature of 25-27 °C with 12 h light and dark cycles having proper ventilation in the room. The animals were allowed to acclimatize to the laboratory environment for one week and then randomly divided into groups for experiment. Guidelines of Institutional Animal Ethics Committee were followed to carry out this study[28].

2.4.2. Experimental induction of diabetes

Alloxan was used for the development of diabetes. Alloxan destroyed the β -cell of pancreas, thus glucose level increased rapidly and reached to diabetic level within 7 days[29,30]. Animals were administered freshly prepared alloxan (120 mg/kg body weight *i.p.*) daily for one week. After one week, mice with moderate diabetes having hyperglycemia were considered diabetic for further experimentation (Figure 1).

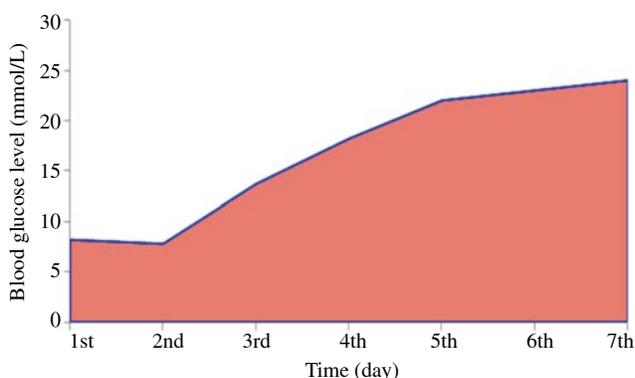


Figure 1. Development of diabetes due to blood glucose level increased after administered alloxan.

2.4.3. Experimental studies

A total of 20 Swiss albino male mice were randomly divided into five groups with four mice in each group. It was important to identify individual animal of a group during the treatment. To denote individual animal, they were marked or coded on their tails. The groups (2 to 5) were assigned for testing antihyperglycemic effects. Group 1 received only normal food and water. Group 2 was selected for diabetic control, which received neither metformin nor plant extract. Group 3 was standard treatment group in which metformin was administered intraperitoneally at single dose of 150 mg/kg body weight daily for one week. Groups 4 and 5 received plant extract at a single dose of 200 and 400 mg/kg body

weight daily for one week. Blood glucose level was measured using glucometer by taking blood sample from tail vein every day after 24 h drug and plant extract administration.

2.4.4. Preparation of dosage of active drug and plant extract

Metformin hydrochloride (a biguanide derivative) was in microcrystalline form and freely soluble in saline water. The dose was prepared in solution form using saline water in such a concentration that each 0.1 mL solution contained metformin hydrochloride according to the dose of 150 mg/kg body weight. Two different doses of MSOB were selected for treatment group. The extract solution was prepared using dimethyl sulfoxide, such that each 0.1 mL of solution contained the dose 200 mg/kg and 400 mg/kg body weight.

2.4.5. Acute toxicity test

The acute toxicity test for MSOB was carried out to evaluate any possible toxicity using the method of Lorke[31]. Different doses of extract were administered orally into groups of 12 mice. The given maximum dose was 500 mg/kg body weight, while the control group only received distilled water. The number of deaths was counted at 48 h after treatment.

2.5. Antioxidant activity

2.5.1. DPPH radical scavenging assay

Free radical scavenging abilities of the test samples can be determined by measuring the change in absorbance of DPPH by Braca *et al*[32]. The bark extract of *S. operculatum* was dissolved in methanol and 2 mL of extract at various concentrations (31.25, 62.50, 125.00, 250.00 and 500.00 μ g/mL) was added to 3 mL of a 0.004% methanol solution of DPPH. After 30 min, absorbance of the resulting solution was measured against a blank at 517 nm. The percentage DPPH radical scavenging activities (% SCV) were calculated by comparing the results of the test with the control using following formula:

$$\% \text{ SCV} = [(A_0 - A_1) / A_0] \times 100$$

Where, SCV = radical scavenging activity, A_0 = absorbance of the control and A_1 = absorbance of the test (extracts / standard). Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted % SCV versus concentration curve. Test carried out in duplicate and ascorbic acid was used as standard.

2.5.2. Total phenolic contents

Phenolic compounds are a class of antioxidant agents,

which was determined by using the method of Shahidi and Wanasundara[33]. To measure total phenolic content, 1 mL of plant extract (200 µg/mL) of MSOB was mixed with 5 mL of the Folin-Ciocalteu reagent and 4 mL of 7.5% sodium carbonate. The mixture was shaken thoroughly. Then it was allowed to stand for 20 min at 25 °C. The absorbance of the solution was measured at 760 nm using a spectrophotometer against blank. The total content of phenolic compounds in MSOB is expressed as the number of equivalents of gallic acid following calculation from the equation:

$$A = (c \times V) / m$$

Where, A = total content of phenolic compounds (mg/g) plant extract in gallic acid equivalents (GAE), c = the concentration of gallic acid established from the calibration curve (mg/mL), V = the volume of extract (mL), and m = the weight of pure plant methanol extract (g).

2.5.3. Total flavonoid contents

The total flavonoid content was determined by Takahashi *et al.*[34] using gallic acid as a reference compound. About 1 mL of plant extract (200 µg/mL) of MSOB was added to 3 mL of methanol and 200 µL of 10% aluminium chloride solution, 200 µL of 1 mol/L potassium acetate solution and 5.6 mL of distilled water was added into the test tube. The test tube was incubated for 30 min at room temperature to complete the reaction. Then the absorbance of the solution was measured at 420 nm using a spectrophotometer against blank. Total content of flavonoid compounds in MSOB was calculated by the equation of GAE following equation:

$$C = (c \times V) / m$$

Where, C = total content of flavonoid compounds (mg/g), c = the concentration of gallic acid established from the calibration curve (mg/mL), V = the volume of extract (mL), and m = the weight of pure plant methanol extract (g).

2.5.4. Reducing power capacity

The reducing powers of bark extracts of *S. operculatum* were carried out by using the method of Oyaizu[35]. Different concentrations of the extracts (500.00, 250.00, 125.00, 62.50, 31.25 µg/mL) were prepared. To all the extracts in test tubes 2.5 mL of sodium phosphate buffer followed by 2.5 mL of 1% potassium ferricyanide was added. The contents were vortexed well and then incubated at 50 °C for 20 min. After incubation, 2.5 mL of 10% trichloroacetic acid was added to all the test tubes and centrifugation was carried out at 3000 r/min for 10 min. To 2.5 mL of supernatant, 2.5 mL of distilled water was added. To this about 0.5 mL of 0.1% ferric chloride was added to each test tube and incubated at 35 °C for 10 min. The absorbance was read at 700 nm. The reducing power of the extract was linearly

proportional to the concentration of the sample. Ascorbic acid was taken as reference standard. Phosphate buffer (pH 6.6) was used as blank solution.

2.6. Statistical analysis

All the values in the test are expressed as mean ± SEM. We used the One-way analysis of variance, followed by *post-hoc* Dunnett's test with the SPSS programme (SPSS 16.0, USA). The statistical method applied in each analysis was described in each figure. Results were considered to be significant when $P < 0.05$.

3. Results

3.1. Antidiabetic activity

The effect on blood glucose level of alloxan induced diabetic mice after treatment with bark extract of *S. operculatum* is shown in Figure 2. The maximum reduction of blood glucose level for bark extracts 200 mg/kg and 400 mg/kg were 44.05% and 55.53% respectively after one week treatment compared to diabetic control. The result was comparable with the activity of standard drug metformin which reduced blood glucose level 69.42% at the same time compared to diabetic control in the dose of 150 mg/kg body weight. In the study for antidiabetic activity, it was found that extract 200 mg/kg showed significant activity in 6th and 7th day ($P < 0.01$) and 400 mg/kg showed significant activity in 5th, 6th and 7th day ($P < 0.01$) where standard drug metformin showed significant activity in 4th, 5th, 6th and 7th day ($P < 0.01$).

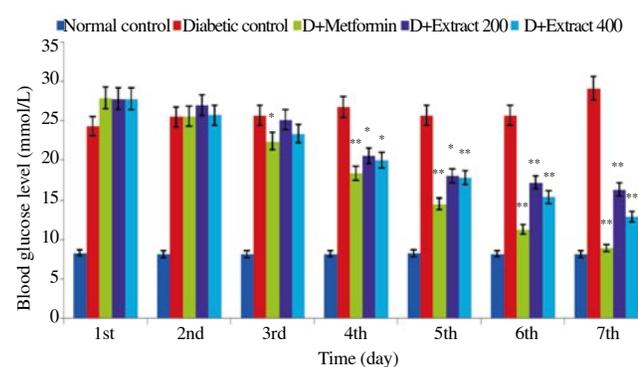


Figure 2. Effect on blood glucose level of alloxan induced diabetic mice after treatment with *S. operculatum* bark extract.

D: Diabetic. Values are mean ± SEM (n=4). Where significant values are * $P < 0.05$ and ** $P < 0.01$ by Dunnett's test as compared to diabetic control.

3.2. Acute toxicity test

Acute toxicity assay did not show any toxic effects for the animal within the due course of study.

3.3. Antioxidant assays

3.3.1. DPPH radical scavenging assay

Concentration providing 50% inhibition (IC₅₀) was calculated from the graph plotted as inhibition percentage versus concentration. The results of DPPH radical scavenging assays on plant extracts and ascorbic acid is shown in Figure 3. The IC₅₀ of the standard and MSOB were 40.31 µg/mL and 300.34 µg/mL respectively. So, comparison with the ascorbic acid, it was clear that plant extracts possessed antiradical activity.

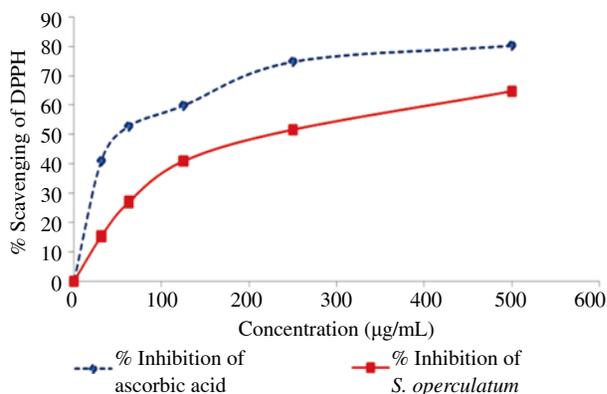


Figure 3. DPPH radical scavenging activity of *S. operculatum* bark extract and ascorbic acid.

3.3.2. Total phenolic contents

The total content of phenolic compounds in the extract is expressed as the number of GAE. The phenolic content was calculated by using an equation of standard curve obtained from various concentration of gallic acid (Figure 4). The total phenol contents in MSOB were 197.5 mg GAE/g of extract (Table 1).

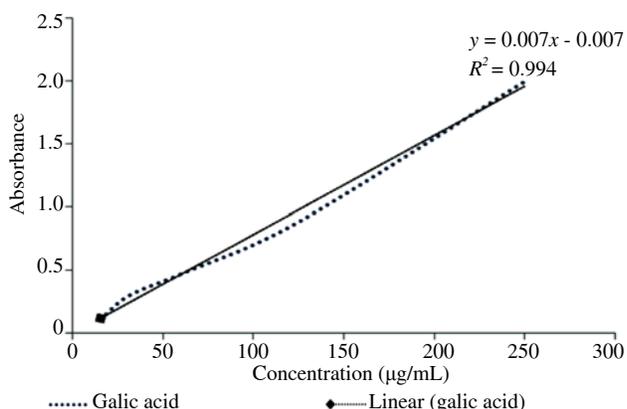


Figure 4. Calibration curve of gallic acid for total phenol content determination.

Table 1

Total phenolic contents of *S. operculatum* extract with necessary data.

Sample	Concentration (µg/mL)	Absorbance	m (g)	c (mg/mL)	A	Mean (mg/g)
MSOB	200	0.295	0.0002	0.039	195	197.5
	200	0.305	0.0002	0.040	200	

3.3.3. Total flavonoid contents

Total content of flavonoid compounds in the plant extracts was

calculated by the equation of GAE (Figure 5). The total flavonoid content in MSOB was 267.5 mg of GAE/g of extract (Table 2).

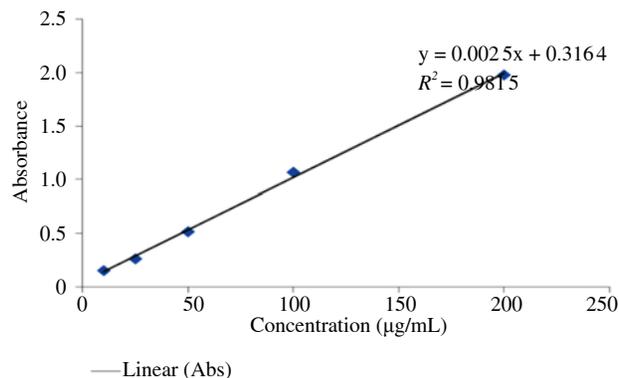


Figure 5. Calibration curve of gallic acid for total flavonoid content determination.

Table 2

Total flavonoid content of *S. operculatum* extract with necessary data.

Sample	Concentration (µg/mL)	Absorbance	m (g)	c (mg/mL)	C	Mean (mg/g)
MSOB	200	0.248	0.0002	0.056	280	267.5
	200	0.213	0.0002	0.051	255	

3.3.4. Reducing power capacity

For the measurement of the reductive ability, it has been found that the Fe³⁺-Fe²⁺ transformation occurred in the presence of extract samples which was postulated previously by Oyaizu[35]. The reducing powers of MSOB and standard ascorbic acid are shown in Figure 6. Ascorbic acid was taken as reference standard. The values indicated that sample had strong reducing power capacity.

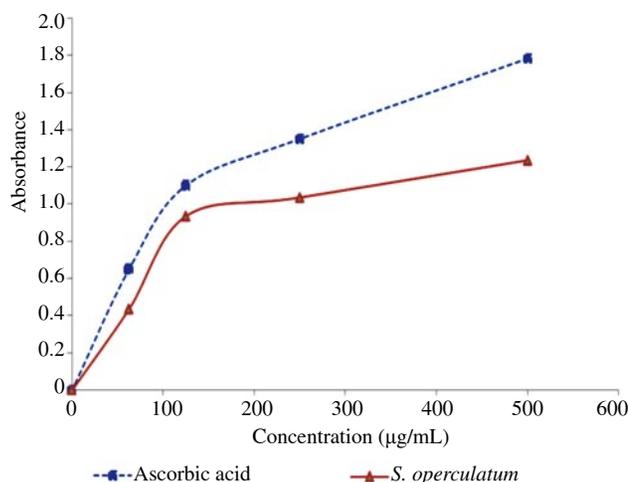


Figure 6. Reducing power of *S. operculatum* bark extract with ascorbic acid.

4. Discussion

A lot of modern medicines have been discovered as a result of the scientific follow-up of the traditional herbal preparations. Nowadays, most of the medicines, cosmetics, chemicals etc. are

synthetically prepared, in many cases their origin is natural and more specifically of plants sources. Taking the same concepts, we have assessed methanol extract of the *S. operculatum* bark for its antioxidant and antidiabetic effect by using different *in vitro* and *in vivo* methods. The data of present experiment show that the plant possesses a significant antidiabetic properties and it may be an important source of natural antioxidant.

In the case of antidiabetic activity of the extract, it exerted the significant antihyperglycemic activity in compare to the standard drug metformin. The attributed antihyperglycemic property of this plant may be due to their capability to repair the role of pancreatic tissues by causing a boost in insulin output or diminishment in the intestinal absorption of glucose as administration of alloxan in experimental animals leads to a fall in insulin release thereby a drastic decrease in plasma insulin concentration leading to stable hyperglycemic states[36]. The maximum reduction of blood glucose level for bark extracts 200 mg/kg and 400 mg/kg were 44.05% and 55.53% respectively after one week treatment compared to diabetic control. The results were comparable with the activity of standard drug metformin which reduced blood glucose level 69.42% at the same time compared to diabetic control in the dose of 150 mg/kg body weight. These observations suggest that the experimental extract might acquire insulin like effect on peripheral tissues either by promoting glucose consumption, metabolism or inhibiting hepatic gluconeogenesis. Generally there is very tiny biological knowledge of the specific modes of action in the treatment of diabetes, but the article reviews show that this plant contains substance like sterol, flavanone, chalcone, triterpene acid, b-sitos-terol, ursolic acid, etc., which are frequently implicated as having antidiabetic effects[37,38]. Ursolic acid is a natural pentacyclic triterpenoid present in this plant. Ursolic acid is well known to possess a wide range of biological functions, including anti-inflammatory, anti-oxidative, anti-mutagenic, anti-carcinogenic and antihyperglycemic effects. Recent study showed that ursolic acid improves insulin resistance and is able to lower blood glucose level by promoting the glucose uptake and utilization in muscle cells, increasing liver glycogen synthesis and enhancing pancreatic β -cell function[38]. We do not know the precise molecular mechanism involved here to get this effect but the phytochemical findings give us some space to believe that it may be a good source for antidiabetic effects.

The previous phytochemical study also indicates that this plant may be a potent source of natural antioxidant. On experimenting it was found that the IC_{50} value of the plant extract was 300.34 μ g/mL in DPPH radical scavenging assay where it is 40.31 μ g/mL for ascorbic acid used as a standard. The total phenol and flavonoid contents were 197.5 mg GAE/g and 267.5 mg GAE/g respectively.

In reducing power capacity assessment also the extract exhibited strong reduction potency. The result obtained in the present study indicates that *S. operculatum* bark extract exhibits potent free radical scavenging and antioxidant activity in addition with reducing power capacity on concentration dependent manner. These findings suggest that the plant could be a good source of natural antioxidant that can be used as therapeutic agents in disease related to oxidative stress.

It was our preliminary attempt only, and samples need further detailed investigation by fractionation and isolation of compounds to know exactly about the possible molecular mechanism for these effects. Hence if further investigations are done forward, the plant may be found as an important agent for using as medicinal agents for diabetes and antioxidant thus lowering the chances of various oxidative stress related abnormalities.

Conflict of interest statement

We declare that we have no conflict of interest.

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