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Original Article

Antihyperglycemic activity of the bark methanolic extract of *Syzygium mundagam* in diabetic rats

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ARTICLE INFO

Article history:

Received 28 March 2016
 Revised 6 November 2016
 Accepted 1 December 2016
 Available online 3 January 2017

Keywords:

Oxidative stress
 Diabetes
 Antioxidants
 Glucose tolerance
 Hyperglycemia
 Hypoglycemia

ABSTRACT

The present study was designed to investigate the free radical defence and antihyperglycemic property of *S. mundagam*. Petroleum ether, ethyl acetate, methanol and hot water extracts of bark were determined for the total phenolic, tannin, flavonoid content and antioxidant property using DPPH, ABTS⁺, phosphomolybdenum, FRAP, superoxide, nitric oxide and metal chelating assays. The antioxidant response was best observed in ABTS⁺ (109686.87 $\mu\text{M TE/g}$ extract), phosphomolybdenum (268.54 g AAE/100 g extract) and superoxide radical scavenging assays (84.30%). Bark methanol extract was found highly efficient in scavenging the free radicals than other extracts. The higher phenolic content (54.44 g GAE/100 extract) could be attributed to this effect. The glucose homeostasis was observed till 180th min in glucose loaded rats treated with the bark methanol extract. The extract could also induce potent hypoglycaemia in STZ induced diabetic rats. The antioxidant defence system could be one of the prime mechanisms of *S. mundagam* leaf and bark extracts that needs to be studied further for the exact molecular action leading to antidiabetic effect.

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1. Introduction

Diabetes mellitus (DM) is grabbing an alarming interest among the scientists and researchers globally. It is one of the most prevalent diseases with the highest number of patients in India. Type-2 diabetes occurs when there is a primary defect in insulin resistance, coupled with a relative insulin deficiency. The major complications of DM include aggravated atherosclerotic disease of the heart, myocardial infarction, heart failure, predisposition to infection, limited joint mobility, hardening of the skin and cataract formation.¹ The pathogenesis of type-2 diabetes is not fully understood. Animal models of type-2 diabetes have been proved to be useful to study the pathogenesis of and to find a new therapy for, the disease.² Oxidative stress and free radicals makes the diabetic condition more severe. Various mechanisms have been suggested to contribute the formation of these reactive oxygen-free radicals in diabetic condition. Glucose oxidation is believed to be the main source of free radicals. Glucose in enediol form get oxidized in a transition-metal dependent reaction to an enediol radical anion that is converted into reactive ketoaldehydes and to

superoxide anion radicals.^{3,4} The superoxide anion radicals can lead to production of extremely reactive hydroxyl radicals, superoxide anion radicals which can also react with nitric oxide to form reactive peroxynitrite radicals.^{5,6} Hyperglycemia is also found to promote lipid peroxidation of low density lipoprotein (LDL) by a superoxide-dependent pathway resulting in the generation of free radicals.^{7,8} Living organism has its own enzymatic and non-enzymatic antioxidant defence system against free radicals. When the balance between these antioxidants and pro-oxidants collapses, there becomes a need of supplementing antioxidants in diet or as medicine which could also block the progression of diabetes. Plants could be the best source to fulfil the need as medicine and food against oxidative stress induced diabetes. The adverse effect by continuous use of synthetic drugs has encouraged the use of plant based medicine which could provide maximum healing with minimum or no side effect. Traditional medical practitioners have identified several such plants which are needed to be validated scientifically. The most common drug metformin recommended as a hypoglycaemic drug was developed from *Galega officinalis*. Thus, it is obvious that the plants with such wonder drugs are in urgent search to treat diabetes but this fact is yet to gain importance among people throughout the world.

Syzygium sp. are well known for their medicinal properties especially for its antidiabetic potential.^{9–12} Hence, the study

Peer review under responsibility of Alexandria University Faculty of Medicine.

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concentrated on the search for the plants from the genus *Syzygium* which could be another demanding source of antidiabetic drug. *Syzygium mundagam* of the family Myrtaceae, is endemic to Western Ghats. The plant is one of the antidiabetic ingredients in formulations with tulsī.¹³ The fruits of the tree are eaten by the Paniya and Kuruma tribes of Wayanad.¹⁴ Hence the attempt has been made to investigate the antioxidant property of *S. mundagam* that would also help in controlling diabetic conditions.

2. Material and methods

2.1. Collection of plant materials

The leaves and bark of *S. mundagam* were collected during October 2011 from Chanthanathodu, Wayanad district of Kerala, Western Ghats, India. The authenticity of *S. mundagam* was confirmed (CMPR 7932) by Dr. M. Prabhukumar, Scientist and Head-in-charge, Plant Systematic and Genetic Resources Division, Centre for Medicinal Plant Research, Arya Vaidya Sala, Kottakkal, India. Freshly collected leaves and bark were cleaned to remove adhering dust and then dried under shade. The dried sample was powdered and used for further studies.

2.2. Chemicals

Ferric chloride, 2,2-diphenyl-1-picryl-hydrazyl (DPPH), potassium persulfate, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) disodium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), ferrous chloride, 2,4,6-tripyridyl-s-triazine (TPTZ), polyvinyl polypyrrolidone (PVPP), ethylenediamine tetracetic acid (EDTA) disodium salt streptozotocin, nicotinamide were obtained from Himedia (Mumbai, India), Merck (Hyderabad, India) and Sigma (Thane, India). All other reagents used were of analytical grade.

2.3. Successive solvent extraction

The powdered plant material (100 g) was extracted in Soxhlet extractor successively with petroleum ether, ethyl acetate and methanol (300 mL). Finally, the material was macerated using hot water with occasional stirring for 24 h and the aqueous extract was filtered. Each time before extracting with the next solvent, the material was dried in hot air oven below 40 °C. The different solvent extracts were concentrated by rotary vacuum evaporator and air dried. The dried extract obtained with each solvent was weighed. The percentage yield was expressed in terms of air dried weight of plant material.

2.4. Quantification of total phenolics, tannins and flavanoids

The total phenol content of leaf and bark extracts was determined according to the method described by Siddhuraju and Becker.¹⁵ The results are expressed as gallic acid equivalents. The extracts incubated with polyvinyl polypyrrolidone (PVPP) were used for tannin estimation.¹⁶ The tannin content of the sample was calculated as follows:

$$\text{Tannin (\%)} = \text{Total phenolics (\%)} - \text{Non tannin phenolics (\%)}$$

The flavonoid contents¹⁷ were quantified as it acts as major antioxidants in plants reducing oxidative stress. The amount of flavonoid was calculated in rutin equivalents.

2.5. Total antioxidant activity assay by radical cation 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS⁺)

ABTS radical cation decolorization assay was done to determine the total antioxidant activity of the samples according to the method of Re et al.¹⁸ The total antioxidant activity (TAA) was expressed in μM trolox equivalence/g extract.

2.6. Ferric reducing antioxidant power (FRAP) assay

The antioxidant capacities of phenolic extracts of samples were estimated according to the procedure described by Pulido et al.¹⁹ The FRAP value is expressed as mmol Fe (II) equivalent/mg extract.

2.7. Phosphomolybdenum assay

The antioxidant activity of samples was evaluated by the phosphomolybdenum method.²⁰ The results were reported in ascorbic acid equivalents per gram extract (AEAC).

2.8. Radical scavenging activity using DPPH method

The DPPH radical scavenging activity of extracts was determined by the method of Blois.²¹ More significantly the IC₅₀ of the extracts were also calculated.

2.9. Metal chelating activity

The chelating of ferrous ions by leaf and bark extracts was estimated by the method of Dinis et al.²² The metal chelating activity was determined in percentage of inhibition.

2.10. Assay of superoxide radical (O₂⁻) scavenging activity

The ability to inhibit formazan formation by scavenging superoxide radicals by the extracts was studied by the method of Beauchamp and Fridovich.²³ The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\% \text{ inhibition} = (\text{Control OD} - \text{Sample OD} / \text{Control OD}) \times 100$$

2.11. Nitric oxide radical scavenging activity

The procedure is based on the method, where sodium nitroprusside in aqueous solution at physiological pH, spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of nitric oxide (extract and standard at 100 μg) compete with oxygen leading to reduced production of nitrite ions. The absorbance of the chromophore formed was read at 546 nm.²⁴ The percentage inhibition of nitric oxide was calculated using the formula:

$$\% \text{ inhibition} = [(\text{Control OD} - \text{Sample OD}) / \text{Control OD}] \times 100$$

2.12. Animals

Healthy Wistar albino rats (100–150 g) and Swiss albino mice (20–25 g) of either sex and of approximately the same age were used in this study. They were fed with standard chow diet and water ad libitum and were housed in polypropylene cages under controlled temperature.

2.13. Acute toxicity

Acute oral toxicity studies were performed²⁵ according to the OECD (Organization for Economic Co-operation and Development)

guidelines. Male mice (n = 6/each dose) were selected for acute toxicity study. The animals were fasted overnight with free access to water. Extract (suspended in 0.6% carboxy methyl cellulose) was administered orally at a dose of 5 mg/kg. The general behaviours such as motor activity, tremors, convulsions, Straub reaction, aggressiveness, piloerection, loss of lighting reflex, sedation, muscle relaxation, hypnosis, analgesia, ptosis, lacrimation, diarrhoea and skin colour were observed for 3 days. If mortality observed in 4/6 or 6/6 animals, the dose administered was considered as toxic dose. However, if the mortality was observed in only one mouse, then the dose was repeated with higher doses such as 100, 200, 500, 1000 and 2000 mg/kg.

2.14. Oral glucose tolerance test (OGTT)

In this method, the rats were fasted for 12 h and blood was taken from the tail end 30 min before administration of *Syzygium mundagam* bark (SMBM) extract (100 and 200 mg/kg). Glibenclamide was administered as standard drug (10 mg/kg). The control group kept untreated. Thirty minutes later, the rats from all groups were given glucose (2 g/kg) orally. The groups were divided as: group I: control, group II and III: 100 and 200 mg/kg SMBM extract and group IV: glibenclamide. Blood were collected from the tail vein just prior to glucose administration (0 min), 30, 60, 120 and 180 min after glucose loading.²⁶ The blood glucose level was determined at each time intervals and compared with control.

2.15. Anti-hyperglycemic activity

Hyperglycemia was induced in overnight fasted animals by a single intraperitoneal injection of 60 mg/kg streptozotocin (STZ), 15 min after the i.p. administration of 120 mg/kg nicotinamide.²⁷ After 1 h 20% glucose was orally administered to avoid the hypoglycaemic condition in diabetic rats. Before the injection initial blood glucose was noted. On 10th day, blood glucose levels were checked and the rats with glucose levels >250 mg/dL were grouped for the study. Diabetic rats were treated with oral doses of SMBM extracts (100 and 200 mg/kg) and standard glibenclamide (10 mg/kg) and after 1 h of drug administration blood glucose levels were observed for 5 h at an interval of 1 h. The diabetic rats kept untreated were grouped as controlled. The groups were divided as: group I: STZ control, group II and III: 100 and 200 mg/kg SMBM extract and group IV: glibenclamide.

2.16. Statistical analysis

The results were expressed as Mean \pm SD/SEM. The data were statistically analyzed using SPSS version 17.0 (SPSS, ANNOVA statistical software, TULSA, USA) by means of one way ANOVA followed by Duncan's test for antioxidant studies (determined in Mean \pm SD) and Dunnett's test for *in vivo* studies (determined in Mean \pm SEM). Mean values were considered statistically significant when $p < 0.05$, $p < 0.01$ and $p < 0.001$.

3. Results

3.1. Quantification of total phenolics, tannins and flavanoids

The total phenolics, tannins and flavonoids contents of different extracts of leaf, and bark of *S. mundagam* is shown Table 1. The extractable phenolics were found to be higher in methanol extract of bark (54.44 g GAE/100 g extract) while it was low in bark petroleum ether (1.61 g GAE/100 g extract). The total tannins estimated from the total phenolics were high in ethyl acetate extract of bark (47.96 g GAE/100 g). Leaf methanol extract also exhibited a good

level of total phenolics and tannin. Flavonoid content of bark was higher both in ethyl acetate and methanol (12.78 and 12.48 g RE/100 g extract respectively).

3.2. Total antioxidant activity assay by radical cation 2,2'-azinobis (3-ethylbenzothiozoline-6-sulphonic acid) (ABTS⁺)

The ability of extracts to neutralize the ABTS⁺ cation radicals is presented in Table 2. Among the extracts, bark methanol extract showed the best potential to scavenge ABTS radicals (109686.87 μ M TE/g extract). Other extracts depicted far lower activity where ethyl acetate extract of bark and methanol extract of leaf were comparatively better in their scavenging property. Petroleum ether extract of leaf (1592.99 μ M TE/g extract) was least active.

3.3. Ferric reducing antioxidant power (FRAP) assay

Ferric reducing potential of *S. mundagam* was estimated from their ability to reduce TPTZ - Fe (III) complex to TPTZ - Fe (II) and shown in Table 2. The methanol (6612.54 mM Fe (II)/mg extract) extract of bark revealed higher ferric reducing activity. The activity of other extracts ranged between 7.60 (leaf petroleum ether) to 3514.72 mM Fe (II)/mg extract (leaf methanol).

3.4. Phosphomolybdenum assay

The molybdenum reducing ability of bark methanol extract was found to be higher (268.54 g AAE/100 g extract) where the petroleum ether extract showed lower activity (1.85 g AAE/100 g extract) (Table 2). Except ethyl acetate extract of bark, other extracts showed moderate to low potential of reducing the radical.

3.5. Radical scavenging activity using DPPH[•] method

The methanol extract of bark (4.8 μ g/mL) and leaf (5.1 μ g/mL) were highly active in counteracting the DPPH radicals generated in the methanol solution (Fig. 1). The ethyl acetate extract of both parts were also equally capable of scavenging the radical. The IC₅₀ of plant extracts were comparable to standards quercetin, rutin, BHA and BHT.

3.6. Metal chelating activity

The metal ion chelating ability of *S. mundagam* extracts were estimated and are shown in Fig. 2. In contrast to other assays, petroleum ether extract of leaf (73.9%) and bark (58.0%) exhibited best activity. The observed activity was well higher than the standard BHT which showed a chelation of 48% (Fig. 2).

3.7. Assay of superoxide radical (O₂⁻) scavenging activity

The ability of *S. mundagam* to scavenge the superoxide radicals generated in the reagent solution to inhibit formazan is directly proportional to the inhibition percentage of plant extract. The ability of ethyl acetate (84.1%) and methanol extract (84.3%) of bark to scavenge the radicals was much superior to other extracts (Fig. 3).

3.8. Nitric oxide radical scavenging activity

S. mundagam extracts could also display 25.6% nitric oxide radical scavenging ability being higher in methanol extract of bark (Fig. 4). The extracts were found to be less active compared to standard BHT (92.4%).

Table 1
Total phenolics, tannins and flavonoids content of *S. mundagam* leaf and bark.

Samples	Extracts	Total phenolics (g GAE/100 g extract)	Tannins (g GAE/100 g extract)	Flavonoids (g RE/100 g extract)	
<i>S. mundagam</i>	Leaf	Petroleum ether	1.88 ± 0.39	0.38 ± 0.50	6.38 ± 1.06 ^c
		Ethyl acetate	7.94 ± 0.56 ^c	3.84 ± 1.01 ^c	6.47 ± 0.76 ^c
		Methanol	45.16 ± 0.69 ^b	40.79 ± 1.98 ^b	11.03 ± 0.29 ^b
		Hot water	5.34 ± 2.02 ^c	4.54 ± 2.08 ^c	1.38 ± 0.07
	Bark	Petroleum ether	1.61 ± 0.17	0.42 ± 0.32	3.01 ± 0.67
		Ethyl acetate	54.26 ± 1.32 ^a	47.96 ± 1.83 ^a	12.78 ± 0.07 ^a
		Methanol	54.44 ± 1.15 ^a	40.40 ± 1.59 ^b	12.48 ± 0.65 ^a
		Hot water	5.98 ± 0.75	4.18 ± 0.26 ^c	1.62 ± 0.05

Values are mean of triplicate determination (n = 3) ± standard deviation.
a > b > c > d, where significance among the extracts at p < 0.05 in columns.
GAE - Gallic acid equivalents, RE - Rutin equivalents.

Table 2
Activity of *S. mundagam* leaf and bark in ABTS⁺, FRAP and phosphomolybdenum assays.

Samples	Extracts	ABTS ⁺ (μM TEAC/g extract)	FRAP (mM Fe(II)E/mg extract)	Phosphomolybdenum (g AAE/100 g extract)	
<i>S. mundagam</i>	Leaf	Petroleum ether	1592.99 ± 58.18	7.60 ± 2.97	1.85 ± 0.02
		Ethyl acetate	5582.22 ± 341.46 ^d	1022.79 ± 51.83	10.41 ± 0.86
		Methanol	24198.61 ± 803.64 ^c	3514.72 ± 3.58 ^b	34.15 ± 1.68 ^c
		Hot water	3192.73 ± 138.21	2768.28 ± 44.46 ^c	6.99 ± 0.47
	Bark	Petroleum ether	1619.99 ± 21.43	97.15 ± 10.20	18.03 ± 1.50
		Ethyl acetate	34042.30 ± 1325.01 ^b	2011.40 ± 96.61 ^d	77.17 ± 1.82 ^b
		Methanol	109686.87 ± 5771.51 ^a	6612.54 ± 32.00 ^a	268.54 ± 22.06 ^a
		Hot water	7674.71 ± 333.36 ^d	112.25 ± 18.42	38.74 ± 1.47 ^c

Values are mean of triplicate determination (n = 3) ± standard deviation.
a > b > c > d, where significance among the extracts at p < 0.05 in columns.
TEAC - trolox equivalents antioxidant capacity, Fe(II)E - Fe(II) Equivalents.
AAE - ascorbic acid equivalents.

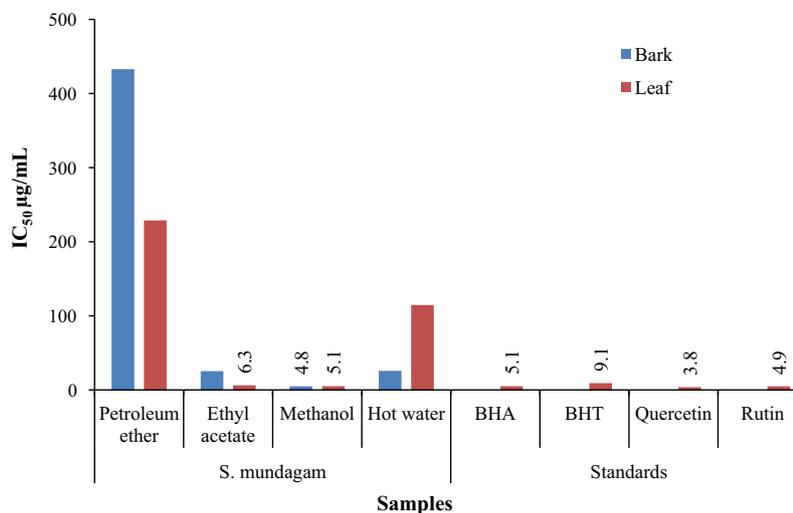


Fig. 1. DPPH radical scavenging activity of *S. mundagam* leaf and bark. IC₅₀ – Inhibitory concentration 50%.

3.9. Acute toxicity

The acute oral toxicity study showed the safety of the extract up to a maximum dose of 2000 mg/kg body weight. The extract administration neither caused any significant change in the behaviours nor the death of animals. 1/10th and 1/20th of the maximum dose was fixed for further analyses. The animals were divided into 4 groups of six animals each, Group I treated with standard glibenclamide, group II and III with 100 and 200 mg extract/kg respectively and group IV kept untreated.

3.10. Oral glucose tolerance test (OGTT)

The rats treated with SMBM showed significant reduction of blood glucose levels after an initial rise in the 30th min (Fig. 5). The higher dose (200 mg/kg) could lower down the glucose level by 59.70% which indicates the ability of the extract to provide tolerance to the glucose loaded rats. The extract could reduce 177 mg/dL of blood glucose at 180th min. The 100 mg/kg dose was also able to bring down the glucose level in blood to near normal after 180 min.

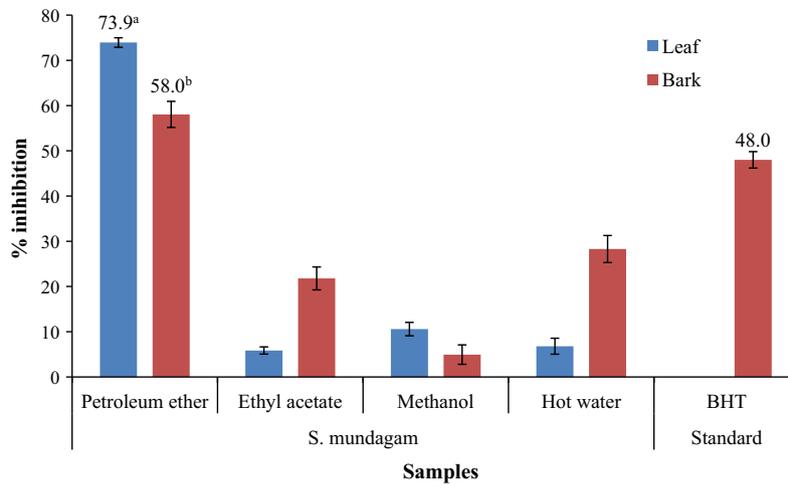


Fig. 2. Metal chelating activity of *S. mundagam* leaf and bark. Values are mean of triplicate determination (n = 3) ± standard deviation. a > b, where significance among the extracts at p < 0.05.

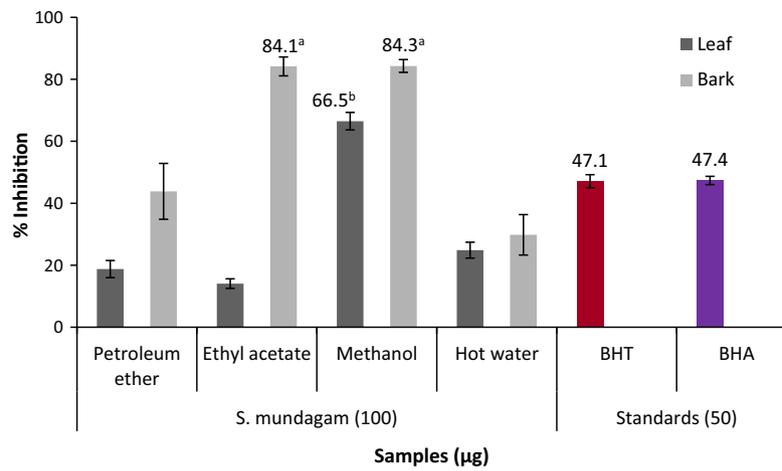


Fig. 3. Superoxide radical scavenging activity of *S. mundagam* leaf and bark. Values are mean of triplicate determination (n = 3) ± standard deviation. a > b, where significance among the extracts at p < 0.05.

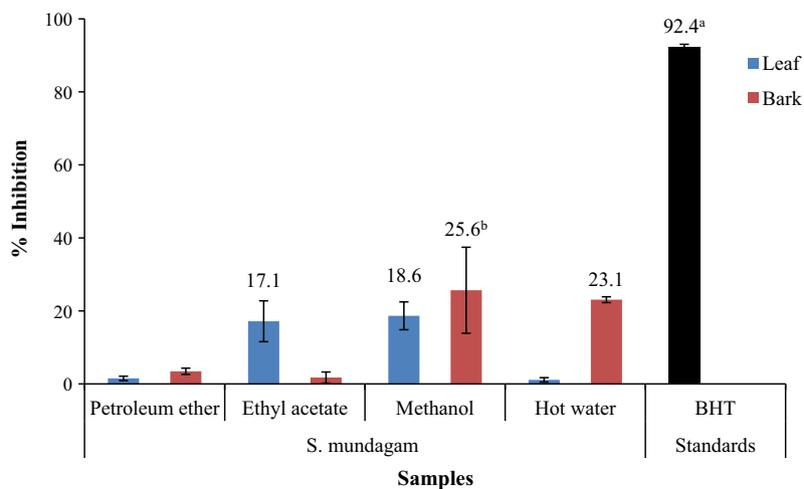


Fig. 4. Nitric oxide radical scavenging activity of *S. mundagam* leaf and bark. Values are mean of triplicate determination (n = 3) ± standard deviation. a > b, where significance among the extracts at p < 0.05.

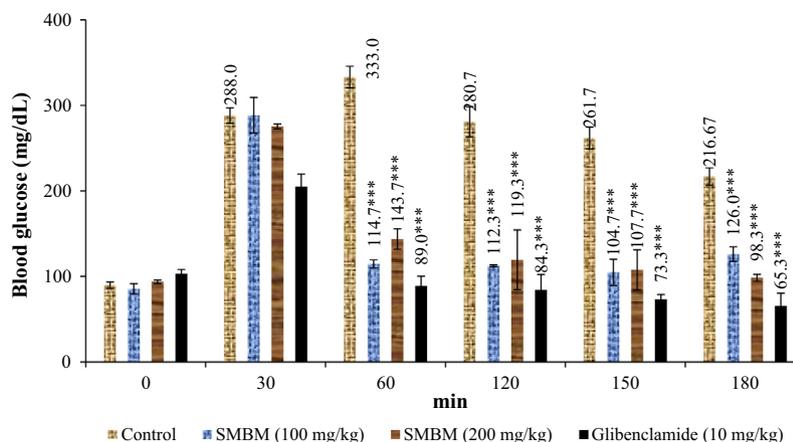


Fig. 5. Oral glucose tolerance of *S. mundagam* bark methanol extract. The data represents mean \pm SEM (n = 6). Significantly different at *** $p < 0.001$, when compared to control. SMBM- *S. mundagam* bark methanol extract.

3.11. Anti-hyperglycemic activity

SMBM showed a good ability in reducing the glucose level by 38.16%. When compared to control rats, hypoglycemic effect of the extract was much higher. There was no significant reduction ($p < 0.05$) observed in control rats as the blood glucose was brought down by only 0.35% (Fig. 6).

4. Discussion

The present study highlights the antioxidant and hypoglycemic activity of *Syzygium mundagam*. Excessive production of free radicals may lead to oxidative stress which may lead to subsequent mechanisms in pancreatic beta-cell death and the development of insulin-dependent diabetes mellitus-induced by STZ.²⁸ Hence the antioxidant property shown by the plant extracts would support hypoglycemic and antihyperglycemic property. The attempt has been made to determine the ability of the plant to assess the action over the acute diabetic condition. The results from the investigation could also encourage carrying out a detailed study using chronic diabetic models in rat.

Flavonoids and phenolics acids are the most important groups of secondary metabolites and bioactive compounds in plants and good sources of natural antioxidants in human diets,²⁹ therefore the quantification of these metabolites was done to confirm the presence of antioxidant activity in this plant. Methanol was found highly efficient in extracting phenolic compounds from bark than

leaves. This indicates the physiological process of the plant where the secondary metabolite like phenolics might have produced higher in bark than leaves. The tannin and flavonoid content was also higher in bark than leaf, indicating that bark could be the best antioxidant agent. Similar results were observed in a closely related species of *S. mundagam*.¹¹

ABTS is one of the simplest radicals that can be used to estimate even a small amount of antioxidant molecule. The activity can be confirmed by the reduction in the colour of reaction mixture from dark green to a colourless solution. The bark methanol extract has shown the best ability to scavenge ABTS radical produced when reacted with potassium persulphate and depicts the presence of phytochemicals that are very strong scavengers equivalent to trolox. The DPPH radical scavenging activity was determined by the decrease in absorbance caused by the extracts. The lower IC_{50} depicts the higher activity. It was noted that the activity of methanol extract of leaf and bark was close to the activity shown by standard antioxidants. It has been found that antioxidant molecules such as ascorbic acid, tocopherol, flavonoids and tannins reduce and decolorize DPPH due to their hydrogen donating ability.³⁰ This could be predicted that the phenolic molecule in the extract could have high hydrogen donating ability. Several studies reported that higher phenolic compounds in plant extracts are capable of forming metal complexes and stabilizing transition metal ions, making them unable to participate in metal-catalysed initiation and hydroperoxide decomposition reactions.³¹ As observed in previous assays the methanol extract of bark was

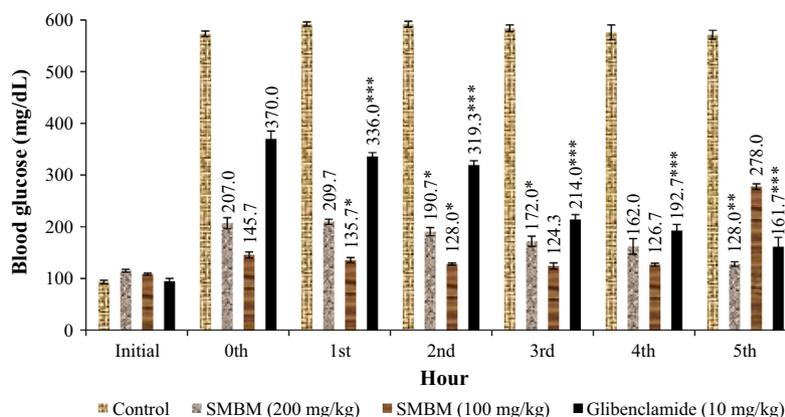


Fig. 6. Antihyperglycemic activity of *S. mundagam* bark methanol extract. The data represents mean \pm SEM (n = 6). Significantly different at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, when compared to control. SMBM- *S. mundagam* bark methanol extract.

found to be efficient reducing agent indicating the presence of higher phenolics that are capable of forming stable complexes. However, petroleum ether extract was found to be the active extract which could chelate the metal ion.

It has been observed that superoxide radicals may give rise to other reactive radicals like hydrogen peroxides, hydroxyl radicals and singlet oxygen that may lead to lipid peroxidation, DNA damage, damage to proteins and lipids.³² Flavanoids could reduce the levels of these radicals and prevent the damage caused to biomolecules.³³ The ability of antioxidants can be detected by the decrease in the absorbance of reaction mixture where the phytochemicals in the extract are able to inhibit the blue NBT formation.³⁴ The inhibiting property of methanol extract (84.30%) is an indication of its ability to protect damage of cellular components. The phosphomolybdenum activity shown by the methanol extract (268.54 g AAE/100 g extract) was in agreement with the study by Chandran et al.¹¹ in *S. calophyllifolium*. Nitric oxide radical may cause various disease conditions carcinomas and inflammation, juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis.³⁵ The scavenging property shown by the extract could reduce such risk factors. The antioxidant factor in the extract could compete with the oxygen molecules thereby blocking the nitric oxide radical formation. The radical scavenging activity of methanol extract clearly shows that it contains higher concentration of phytochemicals that can act as antioxidants in a synergetic manner.

Oxidative stress is believed to increase the severity of diabetes and its complications. Several studies have also indicated the role of free radicals in the pathogenesis of diabetes.³⁶ Hence drug with antioxidant activity can have an effective role in treatment of diabetes. Our study has shown antioxidant property of *S. mundagam* with the best being the methanol extract (SMBM) was checked for antihyperglycemic activity. The acute toxicity study shows safety level of the extract even at the dose of 2000 mg/kg and thus can be administered as drug without any adverse effects. The extract could reduce the blood glucose level in both glucose loaded rats and STZ induced diabetic rats. A similar study was done with bark methanol extract of *S. calophyllifolium* in rats and proved to be a good hypoglycemic agent.¹¹ The extract could have enhanced the ability of tissues to uptake the glucose by decreasing the insulin resistance. As discussed earlier, another mechanism could be that the extract might have reduced the free radical generation under diabetic condition and resulting hypoglycaemia. This study also signifies the antidiabetic compounds present in methanol extract which needs to be characterized.

5. Conclusion

In summary, this study represents the first report on the antioxidant and antihyperglycemic activity of *S. mundagam*. The results clearly depict the role of antioxidants in lowering blood glucose level in both glucose loaded and STZ induced diabetic rats. The extractability of methanol and higher concentration of bioactive molecules might have generated the higher antioxidant activity that in-turn induced the hypoglycaemia in rats. Hence the study signifies the need of much detailed study on antioxidant compounds responsible for the antihyperglycemic activity to validate the extract as a competent therapeutic agent for the treatment of diabetes.

Acknowledgement

The authors are thankful to Department of Science and Technology, Govt. of India and INSPIRE programme (DST/INSPIRE Fellow-

ship 2010/IF10618) for providing financial support for carrying out the work.

References

- Knentz AJ, Natras M. Diabetic ketoacidosis, non ketotic hyperosmolar coma and lactic acidosis. In: Pickup JC, Williams G, eds. *Handbook of Diabetes*. 2nd ed. Blackwell Science; 1991:479–494.
- Lin Y, Sun Z. Current views on type 2 diabetes. *J Endocrinol*. 2010;204:1–11.
- Wolff SP, Dean RT. Glucose autooxidation and protein modification. The potential role of 'autoxidative glycosylation' in diabetes. *Biochem J*. 1987;245:243–250.
- Jiang ZY, Woollard AC, Wolff SP. Hydrogen peroxide production during experimental protein glycation. *FEBS Lett*. 1990;268:69–71.
- Halliwell B, Gutteridge JMC. Role of free radicals and catalytic metal ions in human disease: an overview. *Meth Enzymol*. 1990;186:1–85.
- Hogg N, Kalyanaraman B, Joseph J, Struck A, Parthasarathy S. Inhibition of low-density lipoprotein oxidation by nitric oxide. Potential role in atherogenesis. *FEBS Lett*. 1993;334:170–174.
- Kawamura M, Heinecke JW, Chait A. Pathophysiological concentrations of glucose promote oxidative modification of low density lipoprotein by a superoxide dependent pathway. *J Clin Invest*. 1994;94:771–778.
- Tsai EC, Hirsch IB, Brunzell JD, Chait A. Reduced plasma peroxyl radical trapping capacity and increased susceptibility of LDL to oxidation in poorly controlled IDDM. *Diabetes*. 1994;43:1010–1014.
- Prince PSM, Menon VP, Pari L. Hypoglycemic activity of *Syzygium cumini* seeds: effects on lipid peroxidation in alloxan diabetic rats. *J Ethnopharmacol*. 1998;61:1–7.
- Gurusamy K, Kokilavani R, Ananta Teepa KS. Effect of *Syzygium calophyllifolium* Walp. seed extract on transaminases and phosphatases in alloxan induced diabetic rats. *Ancient Sci Life*. 2007;26:28–33.
- Chandran R, Sathyanarayanan S, Rajan M, Kasipandi M, Parimelazhagan T. Antioxidant, hypoglycemic and anti-hyperglycemic properties of *Syzygium calophyllifolium*. *Bangladesh J Pharmacol*. 2015;10:672–680.
- Chandran R, Parimelazhagan T, Shanmugam S, Thankarajan S. Antidiabetic activity of *Syzygium calophyllifolium* in Streptozotocin-Nicotinamide induced Type-2 diabetic rats. *Biomed Pharmacother*. 2016;82:547–554.
- Oudhia P. Drawbacks of indigenous medicinal orchids and ferns and *Syzygium mundagam* (Bourd.) Chithra based herbal formulations. Pankaj Oudhia's expert comments on world patents on herbal medicines. Audio bank on biodiversity and traditional healing. <<http://www.pankajoudhia.com>>; 2012.
- Narayanan MKR, Anilkumar N, Balakrishnan V, Sivadasan M, Ahmed AH, Alatar AA. Wild edible plants used by the Kattunaikka, Paniya and Kuruma tribes of Wayanad District, Kerala, India. *J Med Plants Res*. 2011;5:3520–3529.
- Siddhuraju P, Becker K. Studies on antioxidant activities of Mucuna seed (*Mucuna pruriens* var. utilis) extracts and certain non-protein amino/imino acids through *in vitro* models. *J Sci Food Agri*. 2003;83:1517–1524.
- Siddhuraju R, Manian S. The antioxidant activity and free radical scavenging capacity of dietary phenolic extracts from horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) seed. *Food Chem*. 2007;105:950–958.
- Zhishen J, Mengecheng T, Jianming W. The determination of flavonoid contents on mulberry and their scavenging effects on superoxide radical. *Food Chem*. 1999;64:555–559.
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *J Free Rad Biol Med*. 1999;26:1231–1237.
- Pulido R, Bravo L, Sauro-Calixto F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *J Agri Food Chem*. 2000;48:3396–3402.
- Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitative of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem*. 1999;269:337–341.
- Blois MS. Antioxidants determination by the use of a stable free radical. *Nature*. 1958;4617:1199–1200.
- Dinis TCP, Madeira VMC, Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch Biochem Biophys*. 1994;315:161–169.
- Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal Biochem*. 1971;44:276–277.
- Sreejayan N, Rao MNA. Nitric oxide scavenging by curcuminoids. *J Pharm Pharmacol*. 1997;49:105–107.
- Ecobichon DJ. *The Basis of Toxicology Testing*. New York: CRC Press; 1997. 43–86.
- Jalil AMM, Ismail A, Chong PP, Hamid M, Kamaruddin SHS. Effects of cocoa extract on glucometabolism, oxidative stress, and antioxidant enzymes in obese-diabetic (Ob-db) rats. *J Agri Food Chem*. 2008;56:7877–7884.
- Masiello P, Broca C, Gross R, et al. Development of a new model of type 2 diabetes in adult rats administered with streptozotocin and nicotinamide. *Diabetes*. 1998;47:224–229.
- Koo KB, Suh HJ, Ra KS, Choi JW. Protective effect of cyclo (his-pro) on streptozotocin-induced cytotoxicity and apoptosis *in vitro*. *J Microbiol Biotechnol*. 2011;21:218–227.

29. Kim D, Jeond S, Lee C. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chem.* 2003;81:321–326.
30. Kumaran A, Karunakaran RJ. In vitro antioxidant activities of methanol extracts of five *Phyllanthus* species from India. *Lebensm-Wiss Technol.* 2007;40:344–352.
31. Bourgou S, Ksouri R, Bellila A, Skandrani I, Falleh H, Marzouk B. Phenolic composition and biological activities of Tunisian *Nigella sativa* L. shoots and roots. *Compte Rendu de Biologies.* 2008;331:48–55.
32. Wickens AP. Aging and the free radical theory. *Respi Physiol.* 2001;128:379–391.
33. Pietta PG. Flavonoids as antioxidants. *J Nat Prod.* 2000;63:1035.
34. Parejo I, Viladomat F, Bastida J, et al.. Comparison between the radical scavenging activity and antioxidant activity of six distilled and non-distilled Mediterranean herbs and aromatic plants. *J Agri Food Chem.* 2002;50:6882.
35. Huie RE, Padmaja S. The reaction of no with superoxide. *Free Radic Res Commun.* 1993;18:195–199.
36. Rahimi R, Shekoufeh N, Bagher L, Mohammad AA. A review on the role of antioxidants in the management of diabetes and its complications. *Biomed Pharmacother.* 2005;59:365–373.