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Original Article

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

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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 μM 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.

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

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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 tulsi.[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′aninobis(3-ethylbenzothiozoline-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 contents[17] 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′-azinobis (3-ethylebenzothiozoline-6-sulphonic acid) ($ABTS^{\cdot+}$)

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 $DPPH^{\cdot}$ method

The DPPH radical scavenging activity of extracts was determined by the method of Blois.[21] More significantly the IC_{50} 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 (O_2^-) 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 performed[25] according to the OECD (Organization for Economic Co-operation and Development)

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