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Antioxidant and antiglycemic potentials of a standardized extract of *Syzygium malaccense*

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ABSTRACT

The present study was designed to identify the ability of the leaf extract from *Syzygium malaccense* (L.) Merr. & L.M. Perry to scavenge DPPH, ABTS and NO radicals; to inhibit the carbohydrate-hydrolyzing enzymes α-glucosidase and α-amylase; and, eventually, to identify and quantify its bioactive compound(s). The *S. malaccense* leaf extract was a far better scavenger of DPPH and ABTS than of nitric oxide. It also inhibited α-glucosidase more significantly than the positive control, acarbose, but was a poor α-amylase inhibitor. Myricitrin was identified by using high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LCMS) techniques as the major bioactive compound present in the extract. The percent yield of myricetin derivatives in the extract was determined to be 3.3 ± 0.05%. The presence of the potent antioxidant and antihyperglycemic agent myricitrin in the *S. malaccense* leaf extract indicates the potential use of the extract in the management of diabetes mellitus and its related complications.

1. Introduction

Increased blood glucose levels are believed to accelerate the generation of free radicals (Maritim, Sanders, & Watkins, 2003) via various mechanisms (Baynes & Thorpe, 1999). Imbalance in radical-generating and endogenous free-radical-scavenging defense systems leads to oxidative stress and results in oxidative damage and tissue injury, the hallmark of diabetes and its related complications. The interconnection between free radicals and oxidative stress in the pathogenesis of diabetes and its co-morbidities is well established (Baynes & Thorpe, 1999; Maritim et al., 2003). Therefore, natural antioxidants originating from various plants and their derivatives became a wise option for the management of oxidative stress-induced diabetes (Bajaj & Khan, 2012; Pereira, Valentao, Pereira, & Andrade, 2009).

Syzygium malaccense (L.) Merr. & L.M. Perry falls under the family of Myrtaceae. It is locally known as the 'Malay apple' and was originally found in Malaysia and India. Various parts of the plant have been applied in traditional medicine. Interestingly, its bark extract has been shown to effectively serve as a hypoglycemic agent that improved the fasting blood-sugar level and the liver-glycogen depletion and reduced diabetes-induced hyperlipidemia in diabetic rats (Bairy, Sharma, & Shalini, 2005). The plant extract of *S. malaccense* is believed to be able to prevent the development of diabetes-induced cataractogenesis based on its strong inhibitory effect towards aldose reductase (Guzman & Guerrero, 2005). The above findings clearly suggest the potential use of *S. malaccense* in the management of diabetes mellitus.

The present study is designed to evaluate the antioxidant and antiglycemic characteristics of the leaf extract of *S. malaccense*. In addition, the bioactive compounds potentially responsible for its antioxidant and anti-hyperglycemic activities will be identified through a bioassay-guided fractionation technique. The findings from this study are expected to provide the probable underlying principles of the potential use of *S. malaccense* as an anti-hyperglycemic agent and its ability to manage oxidative stress-induced diabetes mellitus.

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2. Materials and methods

2.1. Analytical reagents and chemicals

Quercetin dihydrate was the product of Calbiochem (Darmstadt, Germany). Acetonitrile (HPLC grade), formic acid and dimethyl sulfoxide (DMSO) were obtained from Friendemann Schmidt (Germany). Acarbose and myricitrin (purity > 99.0%) were purchased from Sigma Chemical Co., Ltd. (St. Louis, MO). All other reagents used were of analytical grade. All of the experiments were performed at 28 °C unless otherwise stated.

2.2. Collection of plant and preparation of S. malaccense leaf extract

The leaves of *S. malaccense* were collected from a plantation in Johor Bahru, Malaysia and authenticated (sample number: PID220712-15) by the Herbarium at the Forest Research Institute of Malaysia (FRIM). The leaves were washed with distilled water, left to dry in a circulating oven at 40 °C and powderized. Ethanol was added to the powderized leaves in a 1:20 ratio (w/v), extraction was performed at 37 °C for 24 h on an orbital shaker at 200 rpm and the final suspension mixture was filtered using Whatman® grade 114 filter papers. The ethanol filtrate obtained was concentrated using a rotary evaporator. The dried residue was recovered, weighed and reconstituted with absolute ethanol to a concentration of 50 mg/ml and stored at 4 °C for not more than two weeks, prior to the evaluation of its total phenolic content, antioxidant and antiglycemic activities.

2.3. Antioxidant assays

2.3.1. Determination of total phenolic content (TPC)

The TPC was analyzed according to the method reported by Oki et al. (2002) with slight modifications, using 96-well microtiter plates. The *S. malaccense* leaf extract (50 μl) was incubated with 50 μl of 10% (v/v) Folin-Ciocalteu phenol reagent for three minutes in the dark at room temperature. This was followed by the addition of 100 μl of a 10% (w/v) sodium carbonate (Na_2CO_3) solution and one hour of incubation before the absorbance was measured spectrophotometrically at 750 nm on a Benchmark™ Plus microplate spectrophotometer (Bio-Rad, USA). The mean TPC value was expressed as the amount of the phenolic content (mg gallic acid equivalents, GAE) in one gram of extract.

2.3.2. DPPH radical-scavenging assay

A DPPH assay was performed according to the method described by Gerhauser et al. (2003) with some modifications, using 96-well microtiter plates. The extract (5 μl) and quercetin (positive control) of various concentrations were mixed with 195 μl of a 100 μM ethanolic solution of the DPPH reagent. The absorbance was read after incubation in the dark for 20 min at 515 nm.

2.3.3. ABTS radical-scavenging assay

An ABTS assay was performed according to the method described by Kanagasabapathy, Malek, Kuppusamy, and Vikineswary (2011) in 96-well microtiter plates with slight modifications. The samples (10 μl) were mixed with 90 μl of ABTS. + reagent. The absorbance was measured after four minutes at 734 nm.

2.3.4. Nitric oxide (NO) radical-scavenging assay

An NO assay was performed according to the method described by Ebrahimzadeh, Nabavi, Nabavi, Bahramian, and Bekhradnia (2010) with some modifications, using 96-well microtiter plates. Ten microliters of the samples were mixed with 90 μl of the SNP reagent (final concentration of SNP: 10 mM), prepared freshly in a phosphate buffered solution (pH 7.4). The mixture was incubated for 2.5 h in the presence of light. Then, 100 μl of pre-mixed Griess-Ilosvay's nitrite reagent was added to the wells, which were incubated for 30 min in the dark, and the absorbance was measured at 540 nm.

2.3.5. Determination of IC_{50} values for free-radical-scavenging assays

The free radical (DPPH, ABTS and NO)-scavenging activities of the extract and quercetin were calculated according to the following formula:

$$\text{Percentage of radical quenched}(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100,$$

where A = absorbance.

The control was prepared by replacing the sample volume with the respective diluents (ethanol for the extract; DMSO for quercetin). The absorbances of the samples at various concentrations without a reagent (substituted with water) were excluded to eliminate the sample color and background interferences.

The scavenging activity of the extract was expressed in IC_{50} value, the concentration of the sample required to scavenge 50% of the radicals.

2.4. α-amylase and α-glucosidase enzyme-inhibition assays

The diluted extracts and acarbose (positive control) at various concentrations in the reaction buffer were subjected to α-amylase and α-glucosidase enzyme-inhibition assays as described by Manaharan, Appleton, Ming, and Palanisamy (2012).

The α-amylase enzyme inhibition assay: Eighty microliters of samples were incubated with 40 μl of the amylase enzyme for 10 min at room temperature. This was followed by the addition of 40 μl of starch solution and incubation at 37 °C for 10 min. Finally, 80 μl of 3,5,-dinitrosalicylic acid solution was added and the mixture was incubated for 10 min at 95 °C to detect the presence of reducing sugar. Absorbance was read at 540 nm.

The α-glucosidase inhibition assay: Twenty microliters of fresh DL-dithiothreitol solution (1 mM) was added to 20 μl of samples. This was followed by the addition of 20 μl of 6 mM 4-nitrophenyl-α-D-glucopyranoside solution and 20 μl of α-glucosidase enzyme (0.4 U/ml in 0.1 M sodium phosphate buffer, pH 6.8 supplemented with 0.2% BSA) to the mixture. The reaction mixture was incubated at 37 °C for 15 min. Finally, the reaction was stopped with 80 μl of 0.2 M sodium carbonate solution and absorbance was read at 400 nm.

The percentage of inhibition of the samples was calculated according to the following formula:

$$\text{Percentage of inhibition}(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100,$$

where A = absorbance.

The control was prepared without the sample by replacing the sample volume with buffer. The absorbance of the samples and their contributions in the reaction at various concentrations without the enzyme (substituted with buffer) were excluded to eliminate the sample color and background interferences.

The inhibitory activity of the extract was computed from the plot of the percentage of inhibition against the concentration of the samples. The IC_{50} values were expressed as the concentration of the samples required to inhibit 50% of the enzymatic activity.

2.5. Bioassay-guided fractionation of the S. malaccense leaf extract by HPLC

The leaf extract of *S. malaccense* (10 mg/ml in ethanol) filtered using syringe filter (0.20 μm) was injected into an Agilent 1200