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Research paper

In-vitro antioxidant and antidiabetic potentials of *Dianthus basuticus* Burtt Davy whole plant extracts

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ABSTRACT

Dianthus basuticus is a popular South African medicinal plant used in the management of diabetes mellitus. This study evaluated the antioxidant and antidiabetic potential of *D. basuticus* using an *in-vitro* model. The antioxidant activity was determined using iron chelation, 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl and superoxide anion radical scavenging abilities of the aqueous, ethanol and hydro-ethanol extracts of *D. basuticus* while the antidiabetic potential was assessed by evaluating the inhibitory effects of the extracts on the activities of α-amylase, α-glucosidase, maltase and sucrase. The aqueous extract displayed significantly higher ($p < 0.05$) DPPH (2.56 μg/mL) and superoxide radical (7.22 μg/mL) scavenging abilities while ethanol (10.56 μg/mL) and hydro-ethanol (6.95 μg/mL) extracts exhibited strongest hydroxyl radical scavenging and iron chelation activities respectively. The ethanol extract displayed significantly higher ($p < 0.05$) inhibition of α-amylase (34.02 μg/mL) while aqueous extract exhibited strongest inhibition of α-glucosidase (6.59 μg/mL), maltase (31.21 μg/mL) and sucrase (20.98 μg/mL). Hydro-ethanol and aqueous extract inhibited α-amylase and α-glucosidase in a mixed non-competitive and pure non-competitive manner respectively while the aqueous extract competitively inhibited both maltase and sucrase activities. It can be concluded that *D. basuticus* extracts possessed antioxidant and antidiabetic activities, and one of its mechanism of antidiabetic action is through the inhibition of diabetes-related enzymes.

1. Introduction

Diabetes mellitus is a pathological condition characterized by hyperglycaemia due to partial or total loss of insulin secretion (International Diabetes Federation, 2013). It is a metabolic disorder as it disturbs carbohydrate, fat and protein metabolism, leading to several complications such as nephropathy, neuropathy and retinopathy (Altan, 2003). Current statistics suggest that about 382 million people live with diabetes worldwide and this number is estimated to increase to 552 million by 2035 (International Diabetes Federation, 2014). Hyperglycemia-induced metabolic dysfunction may be caused by reactive oxygen species (ROS) produced in the mitochondrial electron transport chain (Brownlee, 2005). ROS such as the superoxide anion radical (O_2^-) and hydroxyl radicals (OH^-) are physiological metabolites formed as a result of respiration in aerobic organisms but their excessive levels have been linked to the onset of diseases such as cancer, stroke and diabetes (Niedowicz and Daleke, 2005).

Therefore, the search for the discovery of antioxidant and antidiabetic agents from plant sources is an important strategy required to combat the widespread nature of this condition. This is because the present synthetic drugs have many drawbacks ranging from limited efficacy and several side effects such as hypoglycaemia, weight gain and chronic tissue damage (Michael et al., 2005). In South Africa one of the plants used in the management of diabetes is *Dianthus basuticus. D. basuticus* Burtt Davy is known as Hlokoa-la-tsela and Lesotho Dianthus, in Sesotho and English respectively. It is widely distributed in many regions of South Africa especially the Free State province and Lesotho (Foden and Potter, 2005). Amongst the Basotho people, the plant is used as an immune modulator, for flatulence and to increase the fertility of bulls. It is also widely used in the management of chest pains, mumps and sugar-related disorders such as diabetes mellitus (Moteetee and Van Wyk, 2011).

The antimicrobial and cytotoxic potential of *D. basuticus* was previously reported in the author's laboratory (Lamula and Ashafa, 2014). The authors have also reported the safety of this plant in an

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animal model (Ashafa and Kazeem, 2015). Despite the ongoing usage of this plant in the treatment of diabetes mellitus, there is a paucity of information on its antioxidant and antidiabetic potential. This study was therefore carried out to evaluate the antioxidant and antidiabetic potentials of different extracts of *D. basuticus* using an *in-vitro* model.

2. Materials and methods

2.1. Plant material

The plant material (whole plant, *i.e.* aerial parts and the roots) were collected in January 2013 from a multiple population in the field around Qwaqwa within the Golden Gate Mountains (28° 28″ 111′ S and 28° 48″ 314′ E; altitude 11950 m). The species' abundance was taken into consideration and collections were made in such a way that the existence of the species was not threatened. Proper identification and authentication were done at the Bews Herbariun of the University of Kwazulu Natal, Pietermaritzburg Campus by Dr C.J. Potgieter. An herbarium voucher with reference number (LamMed/01/2013/Qhb) was already deposited at the UFS-Qwaqwa campus herbarium.

2.2. Extract preparation

20 g each of the dried powdered material was extracted in 200 mL distilled water, ethanol or hydro-ethanol (50:50), with constant shaking on Labcon platform shaker (Laboratory Consumables, PTY, Durban, South Africa) for 24 h. The extracts were centrifuged (Hermle Laboratory Centrifuge, Lasec, South Africa) and later filtered using Whatman No. 1 filter paper. The ethanol extract was concentrated to dryness under vacuum using a rotary evaporator (Cole–Parmer, South Africa), while the aqueous extract was freeze-dried in a lyophilizer (Virtis BenchTop, SP Scientific Series, USA). The hydro-ethanol extract was initially concentrated using a rotary evaporator and later freeze-dried in the lyophilizer. Extracts were dissolved in dimethysulphoxide (DMSO) to give stock solutions of 1.0 mg/mL and different concentrations (3.13, 6.25, 12.5, 25, 50 and 100 μg/mL) of the extracts were prepared using a serial dilution method with distilled water. All extracts were stored at 4 °C prior to analysis.

2.3. Chemicals and reagents

Porcine pancreatic α-amylase, rat intestinal α-glucosidase, 1,1-diphenyl-2-picrylhydrazyl, gallic acid, acarbose and *para*-nitrophenyl–glucopyranoside were products of Sigma–Adrich Co., St. Louis, USA while starch soluble (extra pure) was obtained from J. T. Baker Inc., Phillipsburg, USA. Other chemicals and reagents were of analytical grade and the water used was glass-distilled.

2.4. Antioxidant activities

2.4.1. DPPH free radical scavenging ability

The free radical scavenging ability of the extracts against DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical was evaluated by a modified method of Saha et al. (2008). Different concentrations (3.13–100 μg/mL) of the extracts (150 μL) were mixed with 150 μL of 0.4 mmol/L methanolic solution containing DPPH radicals. The mixture was left in the dark for 30 min and the absorbance was measured at 516 nm. The DPPH free radical scavenging ability of each extract was subsequently calculated with respect to the reference (which contains all the reagents without the test sample). DPPH free radical scavenging ability of a standard antioxidant was also tested by replacing the extract with gallic acid (3.13–100 μg/mL)

2.4.2. Hydroxyl radical scavenging ability

The ability of the plant extracts to prevent Fe^{2+}/H_2O_2 induced decomposition of deoxyribose was carried out using the modified method described by Oboh and Rocha (2006). Briefly, 40 μL freshly prepared extracts (3.13–100 μg/mL) (Section 2.2) was added to a reaction mixture containing 20 μL 20 mM deoxyribose, 80 μL 0.1 M phosphate buffer, 10 μL 20 mM hydrogen peroxide and 10 μL 500 μM $FeSO_4$, and the volume was made up to 200 μL with distilled water. The reaction mixture was incubated at 37 °C for 30 min, and the reaction was stopped by the addition of 50 μL of 2.8% TCA (trichloroacetic acid), this was followed by the addition of 50 μL of 0.6% TBA solution. The mixtures were subsequently incubated for 20 min and the absorbance was measured at 532 nm in a microplate reader (Model 680, BIO-RAD). Hydroxyl radical scavenging ability of a standard antioxidant was also tested by replacing the extract with gallic acid (3.13–100 μg/mL)

2.4.3. Iron chelation assay

The chelation of ferrous ions by the plant extracts was determined by the modified method of Dorman et al. (2003). Briefly described, 200 μL of 0.2 mM $FeCl_2$ was added to 40 μL aliquots of extracts (3.13–100 μg/mL) (as prepared in Section 2.2). The reaction was initiated by the addition of 5 mM ferrozine (80 μL), the mixture was vigorously shaken and left to stand at room temperature for 10 min. The absorbance was then measured at 562 nm in a microplate reader (Model 680, BIO-RAD, USA). Iron chelating potential of a standard antioxidant was also tested by replacing the extract with gallic acid (3.13–100 μg/mL).

2.4.4. Superoxide anion radical scavenging ability

Measurement of superoxide anion scavenging activity of the various extracts was based on the method described by Liu et al. (1997). Superoxide radicals were generated in 50 μL of Tris-HCl buffer (16 mM, pH 8.0) containing 50 μL of NBT (50 mM) solution, 50 μL NADH (78 mM) solution and different concentrations (3.13–100 μg/mL) of *D. basuticus* extracts (100 μL). The reaction started by adding 1 mL of phenazine methosulphate (PMS) solution (10 mM) to the mixture. The reaction mixture was incubated at 25 °C for 5 min, and the absorbance measured at 560 nm. Superoxide anion radical scavenging ability of a standard antioxidant was also tested by replacing the extract with gallic acid (3.13–100 μg/mL).

2.5. Antidiabetic potentials

2.5.1. α-Amylase inhibitory assay

This assay was carried out using a modified procedure of McCue and Shetty (2004). A total of 250 μL of each extract (3.13–100 μg/mL) (Section 2.2) was placed in a test tube and 250 μL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution was added. This solution was pre-incubated at 25 °C for 10 min, after which 250 μL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at timed intervals and then incubated at 25 °C for 10 min. The reaction was terminated by adding 500 μL of dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5 min and cooled to room temperature. The reaction mixture was diluted with 5 mL distilled water and the absorbance was measured at 540 nm using a spectrophotometer (Biowave II, Biochrom, UK). The control was prepared using the same procedure replacing the extract with distilled water while activity of the standard was tested by replacing the extract with acarbose (3.13–100 μg/mL). The α-amylase inhibitory activity was calculated as percentage inhibition, thus;