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A new exploration of *Dregea volubilis* flowers: Focusing on antioxidant and antidiabetic properties

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ABSTRACT

Flowers of *Dregea volubilis* (Family: Apocynaceae) are commonly consumed as seasonal vegetable in India but not yet evaluated for its health beneficiary effects. In the present study, the hydroalcoholic flower extract of *D. volubilis* (DVHA) was evaluated for antioxidant and antidiabetic activities *in vitro*. With high contents of phenolics (39.82 ± 1.22 mg GAE/g) and flavonoids (27.50 ± 0.87 mg QE/g), DVHA showed potential antioxidant activity for scavenging DPPH radical (IC_{50}, 237.86 ± 1.05 μg/mL), hydroxyl radical (IC_{50}, 170.67 ± 0.98 μg/mL), superoxide radical (IC_{50}, 219.07 ± 1.25 μg/mL), nitric oxide radical (IC_{50}, 196.38 ± 1.49 μg/mL) and ferric reducing antioxidant power (176.47 ± 3.18 μmol Fe^{2+}/g), total antioxidant capacity (39.68 ± 1.62 mg GAE/g) along with remarkable inhibitory effects on α-glucosidase (IC_{50}, 3780.09 ± 21.19 μg/mL) and α-amylase (IC_{50}, 360.68 ± 1.26 μg/mL). The characterization of the extract was evaluated by FT-IR and UHPLC analysis. The liquid chromatography study led to the identification and quantification of six compounds viz. gallic acid (412.36 ± 2.29 μg/g), ferulic acid (162.72 ± 0.89 μg/g), rutin (386.25 ± 2.00 μg/g), ellagic acid (208.8 ± 2.00 μg/g), quercetin (306.85 ± 2.24 μg/g) and cinnamic acid (213.71 ± 2.14 μg/g). The results explain the therapeutic potentialities of *D. volubilis* flowers as a potential source of natural antioxidants for use in food and pharmaceutical industries along with their possible applications to control postprandial hyperglycaemia.

1. Introduction

Non-insulin dependent diabetes mellitus, commonly called type 2 diabetes, is a chronic metabolic disease characterized by hyperglycemia which results from insufficient or inefficient insulin secretion. The increasing prevalence of diabetes mellitus has become a major health problem worldwide, reaching epidemic proportions. Controlling post-prandial hyperglycemia through the inhibition of α-amylase and α-glucosidase (carbohydrate hydrolyzing enzymes) present in the gastro-intestinal tract is one of the major management therapies. To maintain biological processes, oxidation is necessary in living organisms for the production of energy (Shukla et al., 2016). Oxygen derived free radicals and other reactive oxygen species (ROS), which are produced uninterruptedly *in vivo*, are responsible for cell death and tissue damage (Ozsoy et al., 2008). Oxygen radicals have shown to be involved in various diseases like ageing, cancer, cardiovascular diseases, diabetes etc. (Halliwell and Gutteridge, 1999). Antioxidants play an important protective role in cell injury promoted by free radical-induced oxidative stress (Kurutas, 2016). Foods containing natural antioxidants, e.g., polyphenols, which might assist to save living body systems against oxidative damage, have taken a great deal of attention to researchers (Baret et al., 2013; Dey and Lakshmanan, 2013; Hooshmand et al., 2015). Polyphenols have been reported to have antioxidant and hypoglycaemic property with ability to inhibit digestive enzymes such as α-amylase and α-glucosidase (Ang et al., 2015). α-Amylase and α-glucosidase inhibitors can retard utilization of dietary carbohydrates into absorbable monosaccharides and suppress postprandial hyperglycemia, making them applicable for treating type 2 diabetes (Wojdylo et al., 2016). Synthetic enzyme inhibitors used to control postprandial hyperglycemia are undesirable for long term usage because of gastrointestinal side effects and are costly too (Poovitha and Parani, 2016). Natural resources enriched with α-amylase and α-glucosidase inhibitors can be utilized as an effective therapy for treating postprandial hyperglycemia with minimal adverse effects.

Dregea volubilis is a large twining shrub growing in India, Sri Lanka, Myanmar, Indonesia, Thailand, and China (Anonymous, 1976). The plant, commonly known as "Jukti" in Bengali (Nandi et al., 2012),

Abbreviations: DVHA, hydroalcoholic flower extract of *D. volubilis*; GAE, gallic acid equivalent; QE, quercetin equivalent; DPPH, 1,1-diphenyl-2-picryl-hydrazyl; IC_{50}, half maximal inhibitory concentration; FT-IR, Fourier transform infrared; UHPLC, ultra high performance liquid chromatography; ROS, reactive oxygen species; PNPG, *p*-nitrophenyl-α-D-glucopyranoside; TPTZ, 2,4,6 tripyridyl-S-triazine; NBT, nitro blue tetrazolium; NED, N-(1-naphthyl)-ethylenediamine dihydrochloride; DNS, 3, 5-dinitrosalicylic acid; FRAP, ferric reducing antioxidant power; HCl, hydrochloric acid; $FeCl_3.6H_2O$, ferric chloride; $FeSO_4 \cdot 7H_2O$, ferrous sulphate; H_2O_2, hydrogen peroxide; AA, ascorbic acid; TBA, thiobarbituric acid; TCA, trichloroacetic acid; EDTA, ethylenediaminetetraacetic acid; TAC, total antioxidant capacity; TPC, total phenolic content; PBS, phosphate buffered saline.

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bears green bisexual sweet scented flowers in drooping umbel with long glabrous branches (Jadhav et al., 2012). The flowers of the plant are consumed as a seasonal vegetable in summer (Hossain et al., 2013). *D. volubilis* is traditionally used to treat inflammation, boil, abscesses, dyspepsia, piles, asthma, tumours, leucoderma, anthelmintic, paralysis, rheumatism, tonsils, neck pain etc. (Kirtikar and Basu, 1935; Chatterjee and Pakrashi, 1995; Sreeramulu et al., 2013). Volubiloside A, volubiloside B, volubiloside C, dregealol, volubilogenone, volubilol, drevogenin D, iso-drevogenin P, 17α-marsdenin, dregeanin, vicenin-2, vitexin, isovitexin, isoorientin, rutin, quercetin, luteolin, apigenin have been isolated from the flower of the plant (Sahu et al., 2002; Panda et al., 2003; Panda et al., 2006).

The present study investigated the total phenolic content, total flavonoid content, antioxidant effects, α-amylase and α-glucosidase inhibitory activities of flowers of *D. volubilis* in order to establish their medicinal and nutritional potentials.

2. Materials and methods

2.1. Chemicals

Gallic acid, ferulic acid, rutin, ellagic acid, quercetin, cinnamic acid, ascorbic acid (AA), acarbose, α-glucosidase, α-amylase, aluminium chloride, PNPG (*p*-nitrophenyl-α-D-glucopyranoside), DPPH (1,1-diphenyl-2-picryl-hydrazyl) and TPTZ (2,4,6 tripyridyl- S-triazine) were purchased from Sigma-Aldrich, St Louis, MO, USA. Folin–Ciocalteu reagent, TBA (thiobarbituric acid), 2- deoxy-D-ribose and NBT (nitro blue tetrazolium) were obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India. Sodium carbonate, phosphoric acid, acetic acid, HCl (hydrochloric acid), sulfuric acid, EDTA (ethylenediaminetetraacetic acid), sodium acetate trihydrate, $FeCl_3 \cdot 6H_2O$ (ferric chloride), $FeSO_4 \cdot 7H_2O$ (ferrous sulphate), H_2O_2 (hydrogen peroxide), sodium hydroxide, TCA (trichloroacetic acid), hydroxylamine hydrochloride, sodium phosphate, ammonium molybdate, sodium nitroprusside, NED (N-(1-naphthyl)-ethylenediamine dihydrochloride), sodium chloride, sodium dihydrogen phosphate monohydrate, disodium hydrogen phosphate dihydrate, starch, sodium potassium tartrate, DNS (3, 5-dinitrosalicylic acid) and methanol for liquid chromatography were procured from Merck Life Sciences Private Limited, Mumbai, India. The water was purified by a Milli-Q water purification system (Millipore, Bedford, MA, USA) and used for all tests. All other reagents used were of analytical grade.

2.2. Collection of plant material

Flowers of *D. volubilis* were collected in April, 2015 from Jaynagar Mazilpur, South 24 Parganas, West Bengal, India. Taxonomic identification of the plant was conducted by Dr. V.P. Prasad, Central National Herbarium, Botanical Survey of India, Botanical Garden, Howrah, West Bengal, India. A voucher specimen (voucher no DV/H/141) was deposited in Division of Microbiology, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India for future reference.

2.3. Preparation of plant extract

Flowers of *D. volubilis* were shade dried at room temperature and the dried plant material was powdered using mechanical grinder. 100 g of powdered material was extracted with 1000 mL of a hydroalcoholic solvent mixture prepared by mixing 70 volume methanol and 30 volume water using soxhlet apparatus. The crude extract was filtered through Whatman filter paper No. 1 and concentrated by rotary evaporation under vacuum and then the material was evaporated to dryness with a percentage yield of 12.6% (*w*/w). Extract (DVHA) was stored in the dark at a temperature of 10 °C for future use.

2.4. Total phenolic content

The content of soluble phenolics in DVHA was determined using Folin–Ciocalteu assay (Vongsak et al., 2013) with slight modifications and the volumes of the reagents added were scaled down to fit the microtiter plate volume. Folin–Ciocalteu reagent was prepared with ten-fold of dilution in water. Briefly, 40 μL of DVHA (1000 μg/mL) was mixed with 100 μL of Folin–Ciocalteu reagent in microtiter plate and 160 μL sodium carbonate (7.5%, *w*/*v*) was added to the mixture. It was allowed to left for 30 min at room temperature with occasional shaking. The absorbance was read at 765 nm using a microplate reader (Multiskan GO Microplate Spectrophotometer, Thermo Fisher Scientific, USA) against reagent blank containing water in place of sample. Gallic acid was used as standard to estimate total phenol content (TPC) and a calibration curve (absorbance vs. μg/mL) was constructed using different concentrations of gallic acid (20–100 μg/mL). The values for TPC are presented in mg gallic acid equivalent (GAE)/g dried extract.

2.5. Total flavonoids content

The aluminium chloride colorimetric method (Pothitirat et al., 2009) was adapted for the determination of total flavonoids with minor changes. An aliquot of 120 μL of DVHA (1000 μg/mL) was mixed with an equal volume of a solution of 2% (*w*/*v*) aluminium chloride solution in a microtiter plate. The absorbance of the mixture was measured at 415 nm after 10 min of incubation against a blank without aluminium chloride solution using a microplate reader (Multiskan GO Microplate Spectrophotometer, Thermo Fisher Scientific, USA). Result was expressed as mg of quercetin equivalent (QE)/g of dried extract with a calibration curve of quercetin (20–100 μg/mL).

2.6. Fourier transform infrared spectroscopy analysis

Fourier transform infrared (FTIR) spectra of DVHA were recorded in ATR mode using Nicolet iS10 FT-IR Spectrometer (Thermo Fisher Scientific, USA) with a total of 30 scans at a resolution of 4 cm^{-1} in the wave number range between 4000 cm^{-1} and 525 cm^{-1} (Tarantilis et al., 2008). A background spectrum was obtained by collecting the spectrum of the clean ATR crystal immediately before acquiring the spectrum of sample. DVHA was then placed on the ATR accessory and pressed for collecting the FTIR spectrum of the sample. The study was used to acquire information on the nature of the functional groups and chemical bonds present in phytochemicals of DVHA by studying their peak values (cm^{-1}). The spectral acquisitions were done using OMNIC software supplied from the manufacturer of the spectrometer. The analysis represented qualitative information regarding the types of phytoconstituents present in the flowers of *D. volubilis*.

2.7. Identification and quantification of phenolic compounds by UHPLC

The phenolic compounds present in DVHA were identified and quantified by ultra high performance liquid chromatography using a UHPLC+ focused system consisting of a Dionex Ultimate 3000 Pump, a Dionex Ultimate 3000 auto sampler column compartment and a Dionex Ultimate 3000 variable wavelength detector (Karaman et al., 2010). A mixed standard stock solution (1 mg/mL) containing gallic acid, ferulic acid, rutin, ellagic acid, quercetin and cinnamic acid was prepared in HPLC grade methanol and subsequently different concentrations (6, 10, 20, 30, 40 and 50 μg/mL) of standard solutions were prepared by diluting the mixed standard stock solution for calibration curves in order to quantify the phenolic compounds present in DVHA. All solutions were filtered through a 0.45 μm membrane filter. Separations of phenolic compounds were performed using a C18 column (250 mm × 4.6 mm i.d.) with a particle size of 5 μm, Hypersil GOLD (Thermo Fisher Scientific, U.S.A.) and column oven temperature was maintained at 25 °C. The chromatographic separation was carried out