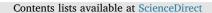
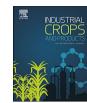
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Phenolic profile and pharmacological propensities of *Gynandriris* sisyrinchium through in vitro and in silico perspectives

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ABSTRACT

Gynandriris sisyrinchium is a traditionally used medicinal plant, yet there is a dearth of scientific information on its biological potential and polyphenolic profile. In this endeavour, we aimed to assess and compare the biological properties of different extracts (ethyl acetate, methanol, and water) of G. sisyrinchium based on enzyme inhibitory effects coupled with docking studies and antioxidant ability together with screening of bioactive compounds. Polyphenolic composition was assessed by determining total phenolic (TPC) and flavonoid (TFC) content as well as individual phenolic compounds by RP-HPLC-DAD. The antioxidant properties were determined by different assays including free radical scavenging (ABTS and DPPH), reducing power (CUPRAC and FRAP), phosphomolybdenum, and metal chelating. The enzyme inhibitory activities of the extracts were evaluated for the first time against cholinesterases, tyrosinase, α -amylase, and α -glucosidase. Docking studies were performed to elucidate the interactions of phenolic compounds with the enzymes. Results show that the methanol extract exerted better scavenging, reducing, tyrosinase, and α -glucosidase inhibitory activities while the less polar ethyl acetate extract showed better phosphomolybdenum and metal chelating activity, AChE, BChE, and α -amylase inhibition. The most abundant compounds were apigenin (4224 and 1876 µg/g extract in the methanolic and ethyl acetate extract, respectively), benzoic acid (1870 µg/g extract in the methanolic extract), pcoumaric acid (472 μ g/g extract in the methanolic extract), and quercetin (348 μ g/g extract in the methanolic extract). The present findings suggest that G. sisyrinchium can be considered as a potential source of bioactive compounds for novel phytopharmaceuticals development in the treatment and/or management of noncommunicable diseases

1. Introduction

Noncommunicable diseases (NCD's) primarily cardiovascular diseases, cancer, chronic respiratory diseases and diabetes are by far the leading cause of death in the world. The NCD's are responsible for 40 million death every year which is equivalent to 70% of all deaths globally (WHO, 2017). Many NCD's are associated with an increased oxidative stress which is caused by an imbalance between excess freeradical production and the antioxidant level in the body. These free radicals can be either oxygen derived or nitrogen derived, which occurs continuously in all cells as part of normal cellular function and is balanced by the antioxidative defense system, or through external supply in foods and/or supplements (Jadhav and Bhutani, 2002; Patel Chirag et al., 2013; Pham-Huy et al., 2008). Besides the exploration of antioxidants, research on the inhibition of key enzymes responsible for NCD's has been recently intensified.

Although the industrial revolution and the development of organic chemistry resulted in a preference for synthetic medications, their expensiveness and associated side effects on human health have renewed the interest in the exploration of natural products in drug discovery and development (Bhat, 2012). Plants have always assisted man with all his needs in terms of shelter, clothing, food, flavours and fragrances and most importantly, medicines (Gurib-Fakim, 2006). Nowadays, knowledge of ancient botanical medicinal practices and application of modern phytochemical techniques have provided excellent tools for the purification and structural elucidation of various phytocompounds, which, in turn, has given insights into their mode of action on the human body (Khan et al., 2017). In fact, 11% of the 252 drugs,

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considered as basic and essential by the World Health Organisation (WHO), are exclusively of plant origin and a number of synthetic drugs are obtained from natural precursors (Bhat, 2012).

Gynandriris sisyrinchium (L.) Parl. (syn: Moraea sisyrinchium (L.) Ker Gawl.) belongs to the family Iridaceae which comprises of about 82 genera and 1750 species (Al-Qudah et al., 2015). The genus Moraea consist of 204 accepted species, 370 synonyms, and 12 species yet to be assessed (http://www.theplantlist.org). Gynandriris sisyrinchium is a perennial herb with a corm, 15–30 cm in diameter and 10–30 cm in height, containing pale or dark bluish, violet or purple flower with usually a solitary leaf, (sometimes two) often lying-coiled on the ground. It is native to the southern parts of Europe (Spain, Portugal, Italy, Balearic Islands, Malta, and Greece) and northern of Africa (Egypt, Libya). It is also widespread in south-west Asia especially Pakistan and Himalaya. This species mostly prefers clayey loamy textured soil and is commonly found in poor or dry sandy and rocky places in garigue, rocky valleys, rocky steppe, paths, and other open grounds (Özdemir et al., 2011).

The ethnomedicinal uses of G. sisyrinchium have been reported in Pakistan (Sher et al., 2011) and Egypt (Bidak et al., 2015). For example, decoction of its corms is used twice a day as a diuretic in Khyber Pakhtunkhwa, north-west of Pakistan (Sher et al., 2015). In Kuwait, this species is seldom grazed by livestock (Mandaville, 2011). Recently, studies have probed into its pharmacological validation including antibacterial (Al-Qudah et al., 2012) and antifungal activities (Goktas et al., 2010). Furthermore, isolation and characterization of isoflavones from G. sisyrinchium revealed significant antioxidant and cytotoxic activity against human promyelocytic leukemia cells HL-60 (Al-Qudah et al., 2015). However, there is still a dearth of scientific information on other potential biological properties of this plant. For instance, the indepth antioxidant profile of G. sisyrinchium against various free radicals have not been explored so far. To the best of our knowledge, there has been no attempt to investigate into its inhibitory activity on key physiological enzymes involved in the aetiology of NCD's. In this context, the aim of the present study was to evaluate the pharmacological potential and determination of phenolic profile of G. sisyrinchium using reversed phase high-performance liquid chromatography (RP-HPLC) technique. In addition, possible interactions between major phenolics and enzyme inhibitory effects was determined in silico. Overall, we aimed to address two key research questions: (i) Are there any difference(s) in pharmacological properties of G. sisyrinchium extracts (ethyl acetate, methanol, and water) based on the different polarity of solvents used for extraction of bioactive compounds?, (ii) Are there any association between the pharmacological properties and phenolic components of G. sisyrinchium extracts?

2. Materials and methods

2.1. Plant material and extracts preparation

Aerial parts of *Gynandriris sisyrinchium* were collected from Pozantı (Adana/Turkey) (at the flowering season in 2015 summer) and air dried at room temperature. Taxonomic identification was confirmed by the senior taxonomist Dr. Murad Aydın Sanda, from the Department of Biology, Selcuk University, Turkey.

The dried plant samples were ground to a fine powder (about 0.2 mm) using a laboratory mill. The powdered plant material (2 g) of each sample was extracted with 20 mL of organic solvents (methanol or ethyl acetate), for 45 min in a sonication bath at 30 °C. The extracts were filtered through Whatman No.1 filter paper and concentrated under vacuum at 40 °C. For the water extracts, plant samples (5 g) were boiled (100 °C) with 100 mL of distilled water for 20 min followed by freeze-drying (–80 °C, 48 h). All the extracts were stored at + 4 °C until further analysis (Uysal et al., 2018; Zengin et al., 2018a,b).

2.2. Determination of polyphenolic composition

The total phenolic and flavonoid contents were determined using the Folin-Ciocalteu and $AlCl_3$ assays, respectively (Slinkard and Singleton, 1977; Zengin et al., 2016b). Results were expressed as gallic acid (mg GAEs/g extract) and rutin equivalents (mg REs/g extract) for respective assays.

The phenolic profile of the studied extracts were determined using RP- HPLC (Shimadzu Scientific Instruments, Kyoto, Japan). Separation procedure was achieved at 30 °C on Eclipse XDB C-18 reversed-phase column (250 mm \times 4.6 mm length, 5 µm particle size, Agilent, Santa Clara, CA, USA) under optimized experimental conditions. Identification and quantitative analysis were performed by comparison with standards. All analytical standards (gallic acid, protocatechuic acid, (+)-catechin, *p*- hydroxybenzoic acid, chlorogenic acid, caffeic acid, epicatechin, syringic acid, vanilin, *p*- coumaric acid, ferulic acid, sinapic acid, benzoic acid, *o*-coumaric acid, rutin, hesperidin, rosmarinic acid, eriodictyol, cinnamic acid, quercetin, luteolin, kaempferol and apigenin) (all of them were HPLC grade)) were purchased from Sigma Aldrich (Darmstadt, Germany). Chromatographic conditions employed in the present study was followed using previously described method by Mocan et al. (2016).

2.3. Evaluation of pharmacological properties

The metal chelating, phosphomolybdenum, FRAP, CUPRAC, ABTS, and DPPH activities of the tested extracts were assessed following the methods described by Grochowski et al. (2017). The possible inhibitory effects of these extracts against cholinesterases (by Ellman's method), tyrosinase, α -amylase and α -glucosidase were evaluated using standard *in vitro* bio-assays as described by Grochowski et al. (2017). The experimental procedures were briefly explained below.

2.4. DPPH scavenging activity

After combining 1.0 mL of extract solution with 4 mL of DPPH (0.267 mM), samples were incubated for 30 min at room temperature in darkness. Afterwards, absorbance of samples were measured at 517 nm. Results were calculated as milligrams of Trolox equivalents per gram of dry extract (TE/g).

2.4.1. ABTS radical cation scavenging activity

Formation of $ABTS^+$ radical cation is an effect of incubation in darkness in room temperature mixture of 7 mM ABTS with 2.45 mM potassium persulfate. Prepared solution was diluted with methanol until its absorbance reached 0.700 \pm 0.02 at 734 nm. 1 mL of extract solutions were combined with previously prepared 2 mL of ABTS⁺ solution and after 30 min of incubation, absorbance at 734 nm was measured. Results were expressed as milligrams of trolox equivalents per gram of dry extract (mg TE/g extract).

2.4.2. Phosphomolybdenum method

Tested extract solutions (0.3 mL) were added to reagent mixture, containing 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. After 90 min of incubation at 95 °C, the absorbance of the mixture was read at 695 nm against blank sample (0.3 mL methanol with 3 mL reagent mixture). Millimoles of trolox per gram of dry extract were the measurement unit (mmol TE/g extract).

2.4.3. Cupric ion reducing (CUPRAC) method

Extract solutions (0.5 mL) were added to reaction mixture [CuCl2 (1 mL, 10 mM), neocuproine (1 mL, 7.5 mM), NH4Ac buffer (1 mL, 1 M, pH 7.0)] and the absorbance was recorded at 450 nm after 30 min of incubation at room temperature. Similarly, a blank sample (prepared in the same manner but without the extract) was prepared and analysed according to this procedure. Milligrams of Trolox equivalents per gram

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