Food and Chemical Toxicology 113 (2018) 179–186

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Biological, chemical and *in silico* fingerprints of *Dianthus calocephalus* Boiss.: A novel source for rutin

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A R T I C L E I N F O

Keywords:
α-amylase
Cholinesterases
Tyrosinase
Phenolics
Molecular docking and dynamics
Natural agents

A B S T R A C T

Extracts (methanol, ethyl acetate, and water) from *Dianthus calocephalus* Boiss. prepared by different extraction techniques (maceration, Soxhlet, and ultrasonication) were studied for possible inhibitory action against key enzymes (α-amylase, α-glucosidase, acetyl cholinesterase, butyryl cholinesterase, and tyrosinase). Antioxidant potential was established using a battery of assays and phenolic compounds profiled by RP-HPLC. Binding pose of tyrosinase with rutin was studied by means of molecular docking. Methanol extracts showed the highest phenolic (39.35–40.25 mgGAE/g) content and rich in rutin (61.38–72.07 mg/g extract). Ethyl acetate extracts of *D. calocephalus* were potent inhibitors of acetyl (1.45–1.48 mgGALAE/g) and butyryl (2.44–2.74 mgGALAE/g) cholinesterases. Docking studies showed that rutin interacts with the side chains of the key amino acid residues and to the copper atom found at the active site of tyrosinase. Methanol extracts showed highest antioxidant capacity. *D. calocephalus* showed interesting biological properties that could be further studied to manage diabetes, neurodegenerative diseases, Alzheimer's disease, and hyperpigmentation.

1. Introduction

Historically, one of the major hallmark of progress in the pharmaceutical industry was the discovery of salicin from the plant *Salix alba*, which lead to the development of salicylic acid; the active molecule of aspirin (Dutra et al., 2016). Since, then there has been a renewed interest in the potential of traditionally used medicinal plants as a source of new pharmacophores. More than 50 000 plants species have been reported to be used in traditional medicine worldwide, providing the basis for modern drug development (Wangchuk et al., 2017). Plants provide an abundance of natural bioactive compounds which have many proved health-promoting actions (Złotek et al., 2016). Indeed, a growing number of scientific studies aim towards the identification of natural phenolic compounds which possess wide spectrum of pharmacological activities (Omar et al., 2017). Besides, a vast repertoire of biologically active chemical constituents have been isolated from plants, consequently patented and exploited by the pharmaceutical industry (Balbani et al., 2009).

Turkey is among the countries which possess the richest plant biodiversity (11 014 identified taxa, of which 33% is endemic) owing to its geographical location, geomorphologic structure, and the diverse climate types (Güler et al., 2015). The use of plants for curative purposes is of high interest among the Turkish population. A recent mapping study performed in Turkey, revealed that 60.5% of people refer to traditional complementary medicine, including medicinal plants, for the treatment and/or management of human ailments (Şimşek et al., 2017). The daunting challenges regarding healthcare faced by the global community along with the lack of scientific information on the implication of medicinal plants for the management of global pandemics, are major concerns (Cesur et al., 2017). Scientific investigations are of utmost importance for providing baseline data which might serve in the development of new lead drugs to manage global health problems.

The genus *Dianthus* is in the family Caryophyllaceae in the major group of the angiosperms. Several members from this genus have been documented to be used traditionally in the management of various diseases. For instance, whole plant of *Dianthus anatolicus* Boiss is used as an antipyretic in intermittent fever and general tonic. Flower buds of *Dianthus caryophyllus* L. has been reported to be used in the treatment/management of gum infections, gastro-intestinal disorder, wounds,

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https://doi.org/10.1016/j.fct.2018.01.049
Received 23 October 2017; Received in revised form 26 January 2018; Accepted 27 January 2018
Available online 31 January 2018
0278-6915/

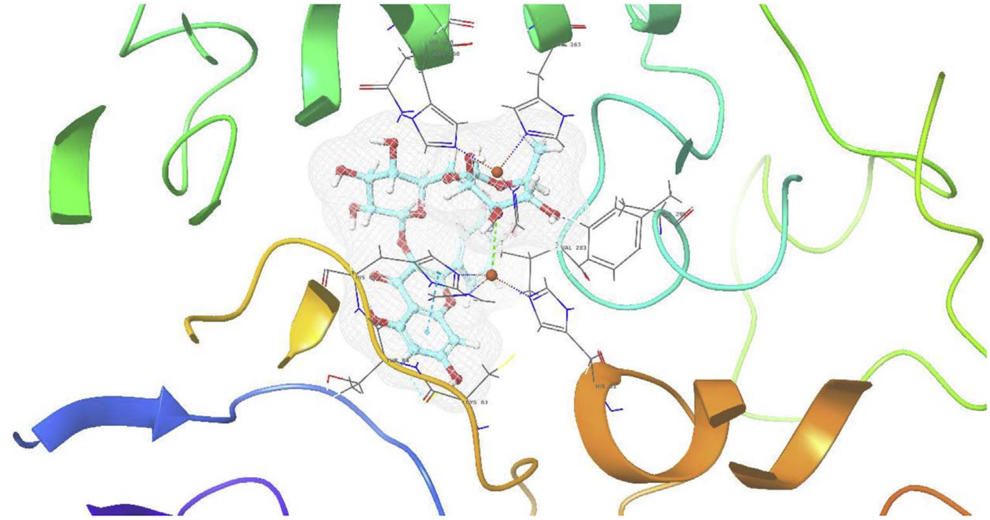

Fig. 1. Rutin docked into the enzymatic pocket of tyrosinase. The best ranking pose was obtained by Gold 6.0 with chemscore scoring function. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Total phenolic and flavonoid contents of *D. calocephalus* extracts.

Extraction methods	Solvents	Total phenolic content (mg GAE/g)	Total flavonoid content (mg RE/g)
Maceration	*EA*	33.08 ± 0.10[a]	19.67 ± 0.18[b]
	MeOH	39.35 ± 0.44[b]	42.32 ± 0.19[b]
	Water	26.47 ± 0.42[c]	4.39 ± 0.21[b]
Soxhlet	*EA*	27.95 ± 0.45[b]	11.99 ± 0.27[c]
	MeOH	40.04 ± 0.59[a]	42.26 ± 0.25[b]
	Water	33.27 ± 0.89[b]	24.57 ± 0.15[a]
Ultrasonication	*EA*	24.18 ± 0.19[c]	22.12 ± 0.14[a]
	MeOH	40.25 ± 0.34[a]	43.14 ± 0.21[a]
	Water	37.55 ± 0.62[a]	3.96 ± 0.17[c]

GAE, gallic acid equivalents; RE, rutin equivalents; TE, trolox equivalents. * Values expressed are means ± S.D. of three parallel measurements. Data marked with different letters within the same column indicate statistically significant differences in same solvents for each extraction methods ($p < 0.05$).

throat infection, cardiotonic, diaphoretic, alexiteric, and used as vermifuge. Interestingly a glycosylated flavonol Kaempferide triglycoside has been isolated from *D. caryophyllus* that exhibit inhibitory properties against human colon cancer cell line induced to over express estrogen receptor (Martineti et al., 2010). Another potent plant is *Dianthus chinensis* L., is a well-known for ornamental flowering plants, whereby the whole plant is used traditionally in the treatment of menostasis, gonorrhea, diuretic, emmenagogue, and coughs (Chandra and Rawat, 2015).

In the present study, *Dianthus calocephalus* Boiss., a poorly studied medicinal plant from the *Dianthus* genus was investigated for its potential application for the treatment and/or management of diabetes, neurodegenerative diseases, and skin hyperpigmentation disorders via inhibition of key enzymes involved in such pathologies. Different extraction solvents and techniques were used to study its biological potential against key enzymes relevant to these pathologies. The extracts were also evaluated for possible antioxidant potential using a plethora of assays. The phenolic composition was established using HPLC and *in silico* docking studies used to study molecular interaction of abundant compounds against the target enzymes.

2. Material and methods

2.1. Plant material and preparation of extracts

Aerial parts of *Dianthus calocephalus* were collected from Ali Mount (Kayseri/Turkey) (in summer 2015) and air dried at the room temperature. The collected plant was authenticated by Dr. Murad Aydın Sanda, botanist, from Selcuk University. The aerial parts were finely grounded and extracted by soxhlet extraction, maceration, and ultrasonication using three solvents, namely ethyl acetate, methanol, and water (Uysal et al., 2017).

2.2. Profile of bioactive compounds

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

The phytochemical composition of the methanol extracts was established using RP- HPLC-DAD (Shimadzu Scientific Instruments, Kyoto, Japan). Separation procedure was achieved at 30 °C on Eclipse XDB C-18 reversed-phase column (250 mm × 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. The amount of each phenolic compounds was expressed as mg per gram of extract using external calibration curves, which were obtained for each phenolic standard at corresponding absorption maxima for specific phenolic classes (i.e. phenolic acids, flavonoids). Chromatographic conditions employed in the present study were previously described by Mocan et al. (2016).

2.3. Determination of antioxidant and enzyme inhibitory effects

The metal chelating, phosphomolybdenum, FRAP, CUPRAC, ABTS, and DPPH activities of *D. calocephalus* extracts were assessed following the methods described by Grochowski et al. (2017). The antioxidant activities were reported as trolox equivalents, whereas EDTA was used for metal chelating assay. The possible inhibitory effects of *D. calocephalus* extracts against cholinesterases (by Ellman's method), tyrosinase, α-amylase and α-glucosidase were evaluated using standard *in vitro* bioassays (Grochowski et al., 2017).

2.4. Molecular modeling

2.4.1. Enzyme preparation

The crystal structure of the tyrosinase was downloaded in PDB format from the Protein Data Bank (2Y9X) (Ismaya et al., 2011). The enzyme was co-crystallized with its inhibitor tropolone. The raw structures were prepared for computational calculations using PrepWizard module of Maestro 2017 (Maestro, 2017a). The crystal