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Antioxidant activity and enzymes inhibitory properties of several extracts from two Moroccan Asteraceae species

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ABSTRACT

The present work reports investigation on phenolic compounds, antioxidant activity and enzyme inhibitory activities (acetylcholinesterase, butyrylcholinesterase, tyrosinase, α-amylase and α-glucosidase) of different extracts from two Moroccan Asteraceae species; *Bubonium imbricatum* Cav. and *Cladanthus arabicus* (L.) Cass. *B. imbricatum* extracts contained the highest amounts of phenolics and flavonoids, and also exhibited higher antioxidant activity. In this species, the highest total phenolic (1611.13 ± 14.23 $\mu mol_{GAE}/g_{extract}$) and flavonoid (376.11 ± 8.22 $\mu mol_{QE}/g_{extract}$) contents were observed in aqueous-methanol extract obtained by maceration. Further, UHPLC–MS analysis of *C. arabicus* and *B. imbricatum* extracts revealed the presence of several flavonoids (diosmetin, luteolin, apigenin 7-glucoside, and apigenin) and phenolic acids (benzoic, protocatechuic, *p*-coumaric, gallic, vanillic, caffeic, ferulic and isochlorogenic acids). The antioxidant activity of the extracts was dependent of the extraction process and solvent used. Aqueous-methanol extract of *B. imbricatum* prepared by maceration showed the highest activity with DPPH, ABTS and FRAP tests (respectively: IC_{50} = 8.53 ± 0.38 μg/ml, 3461.8 ± 9.38 $\mu mol_{TE}/g_{extract}$ and 3281.6 ± 47.43 $\mu mol_{AAE}/g_{extract}$). The results indicated that most of the tested extracts or essential oils exhibited activity towards the tested enzymes. Overall, the results obtained in this work indicated the two Moroccan species studied, particularly *B. imbricatum*, as valuable sources of natural agents beneficial for human health.

1. Introduction

In the last years, the use of medicinal plants as a source of bioactive compounds, particularly of natural antioxidants, has increased substantially worldwide (Jdey et al., 2017; Muddathir et al., 2017). Among plant bioactive compounds, phenolics are probably the most important since they can act on specific molecular targets and play an important role in human health (Naczk and Shahidi, 2004). Many studies demonstrated that phenolic compounds can reduce the risk of various chronic diseases as Alzheimer's disease (AD) and diabetes mellitus type 2, which is mainly related with their strong antioxidant properties (Maritim et al., 2003; Wojtunik-Kulesza et al., 2016).

Although AD has not been fully clarified, the valid hypothesis being accepted is a lack of acetylcholine levels in the hippocampus and cortex of the brain (Nordberg, 2006). Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are key enzymes that play important roles in cholinergic transmission by hydrolyzing the neurotransmitter acetylcholine (Millard and Broomfield, 1995) and, therefore, the use of inhibitors of these enzymes is considered an effective therapy for AD (Tewari et al., 2018). On the other hand, the inhibition of α-amylase and α-glucosidase retards the degradation of starch and consequently, reduces the postprandial blood glucose levels in diabetic patients (Balogun and Ashafa, 2017). Tyrosinase is the enzyme that catalyzes the production of melanin, a pigment that helps to prevent UV damage to the skin, hair and eyes, but in excess is associated with hyperpigmentation and neurodegenerative disorders such as Parkinson's disease. This enzyme is also responsible for browning in fruits and vegetables (Kim and Uyama, 2005). Tyrosinase inhibitors have been used for

Abbreviations: DPPH, 2,2-Diphenyl-1-picrylhydrazyl; ABTS, 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid radical cation; FRAP, Ferric reducing ability power; BHT, Butylated hydroxytoluene; AChE, Acetylcholinesterase; AD, Alzheimer's disease; ATCI, Acetylthiocholine iodide; ChE, Cholinesterase; BChE, Butyrylcholinesterase; BTCI, Butyrylthiocholine; DTNB, 5,5′-Dithiobis(2-nitrobenzoic acid); F–C reagent, Folin–Ciocalteu reagent; GAE, Gallic acid equivalents; UHPLC–MS, Ultra-high performance liquid chromatography-mass spectrometer; TCA, Trichloroacetic acid; TE, Trolox equivalents; TEAC, Trolox equivalent antioxidant capacity; Trolox, 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; UAE, Ultrasound-assisted extraction; MeOH/W, Methanol/Water; MeOH, Methanol; TFC, Total flavonoid content; TPC, Total phenolic content.

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medical and food applications (Seo et al., 2003). The inhibition of tyrosinase could be useful for the treatment of skin cancer and other dermatological disorders associated with hyperpigmentation of melanin as reported by several studies (Wang et al., 2010).

Many plants of the Asteraceae family are well known for their medicinal properties and several studies showed that some of them, e.g., *Achillea*, *Artemisia* and *Matricaria* species, contain high amounts of phenolics and consequently strong antioxidant activity (Polatoğlu et al., 2013; Tlili et al., 2013; Metrouh-Amir et al., 2015). However, there are some less known species as is the case of *Cladanthus arabicus* (L.) Cass. and *Bubonium imbricatum* Cav., two medicinal plants endemic from Morocco. Previous studies reported that *C. arabicus* is used for its anti-icteric properties, antifeedant activity and as an ornamental plant (Bellakhdar, 1997). El Hanbali et al. (2005) studied the chemical composition of *C. arabicus* essential oil and its antibacterial activity. Moreover, Aghraz et al. (2017) reported the chemical composition, *in vitro* antioxidant, antimicrobial and insecticidal activities of the essential oil. On the other hand, a previous work reports the chemical composition, antioxidant, antimicrobial and insecticidal activities of *B. imbricatum* essential oil (Aghraz et al., 2016), that also showed a strong activity against the agricultural pathogenic fungi *Penicillium digitatum*, *Penicillium expansum* and *Botrytis cinerea* (Alilou et al., 2008). The current work aimed to investigate the phenolic contents, antioxidant activity and enzyme inhibitory capacity against acetylcholinesterase, butyrylcholinesterase, tyrosinase, α-amylase and α-glucosidase of extracts from *B. imbricatum* and *C. arabicus*. The enzymes inhibitory potential of the essential oils is also reported for the first time.

2. Materials and methods

2.1. Standards and reagents

Folin–Ciocalteu's phenol reagent (F–C reagent), gallic acid, sodium carbonate (Na_2CO_3) and iron (III) chloride ($FeCl_3$) were purchased from VWR (Leuven, Belgium). Trichloroacetic acid (TCA) was obtained from Panreac (Barcelona, Spain). 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) tablets, potassium persulfate, acetylthiocholine iodide (ATCI), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), acetylcholinesterase (AChE) (Electric-eel, EC 3.1.1.7, Type VI-S), butyrylcholinesterase (BChE) (horse-serum, EC 3.1.1.8), butyrylthiocholine chloride (BTCI), galanthamine hydrobromide, kojic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), mushroom tyrosinase (EC 1.14.18.1), 3,4-dihydroxy-L-phenylalanine (L-DOPA), porcine pancreatic α-amylase (EC 3.2.1.1), yeast α-glucosidase (EC 3.2.1.20), *p*-nitrophenyl-α-D-glucopyranoside, starch, 3,5-dinitrosalicylic acid and acarbose were purchased from Sigma–Aldrich (Steinheim, Germany). Potassium ferricyanide ($K_3[Fe(CN)_6]$) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Acros Organics (Geel, Germany). Methanol, acetonitrile and acetone were purchased from Biosolve (Valkenswaard, The Netherlands). Phenolic standards: gallic acid, quercetin, 4-hydroxybenzoic acid, protocatechuic acid, vanillic acid, caffeic acid, syringic acid, ferulic acid, vanillin, hydroxityrosol, luteolin, apigenin-7-glucoside, diosmetin, *p*-coumaric acid, tyrosol and apigenin were purchased from Sigma-Aldrich (Steinheim, Germany). Acetic and formic acids were purchased from VWR International (Roden, The Netherlands).

2.2. Plant materials

The aerial parts of *B. imbricatum* and *C. arabicus* were collected during the flowering period (in April and June, respectively) from Essaouira (South West Morocco, latitude: 31.51, longitude: −9.76) and Ourika (region of Marrakesh, latitude: 31, longitude: 7). The plant material was identified according to the flora of Morocco. Voucher specimens (Buimb 023 and CLA 023) were deposited at the Herbarium of the Laboratory of Biotechnology, Protection and Valorization of Plant Resources (Cadi Ayyad University). Plant material was dried at room temperature, powdered to achieve a mean particle size less than 2 mm and kept in the dark until future use.

2.2.1. Extraction

The essential oils were obtained by hydrodistillation using a Clevenger-type apparatus (Aghraz et al., 2016, 2017). Methanol extracts were prepared by maceration and ultrasound assisted extraction (UAE). The plant material (5 g) was extracted with 50 ml of methanol (MeOH) or an aqueous/methanol mixture (50/50), at room temperature (2 × 24 h) under shaking, in the case of maceration, or in an Elmasonic S 100 (H) (37 kHz) ultrasound bath (Elma Hans Schmidbauer GmbH & Co. KG, Singen, Germany) for 30 min at 50 °C. To prepare the infusion, the plant material was added to boiling distilled water, maintained for 5 min and then left to stand at room temperature for 10 min. The MeOH was eliminated under vacuum (40 °C) with a rotary evaporator and the infusions were lyophilized. The yield of each extract was calculated based on the dry weight of the plant. The obtained extracts were stored at −4 °C in darkness until their use.

2.2.2. Determination of total phenolic (TPC) and flavonoid contents (TFC)

TPCs were determined using the Folin–Ciocalteu assay as described by Ainsworth and Gillespie (2007). A standard curve was evaluated using gallic acid concentrations ranging from 4 μM to 0.5 mM. Firstly, 200 μl of 10% (v/v) F–C reagent was mixed with 100 μl of each extract in phosphate buffer (75 mM, pH 7.0). The reaction was incubated at room temperature for 2 h after the addition of 800 μl of 700 mM Na_2CO_3. Gallic acid and phosphate buffer were used, respectively, as a positive and negative control. The absorbance was determined at 765 nm. TPCs were expressed as μmol gallic acid equivalents per gram of dry weight of extract ($\mu mol_{GAE}/g_{extract}$).

TFCs were assessed by the aluminum chloride colorimetric method (Woisky and Salatino, 1998). Briefly, 0.5 ml of sample was added to 0.5 ml of a 2% $AlCl_3$ ethanolic solution. Then, the absorbance was measured at 420 nm after 1 h of incubation at room temperature. TFCs were expressed as μmol quercetin equivalents per gram of dry weight of extract ($\mu mol_{QE}/g_{extract}$).

2.2.3. UHPLC–MS analysis

The extracts were analyzed by ultra-high-performance liquid chromatography coupled to mass spectrometry (UHPLC–MS) for the determination of single polyphenols. UHPLC–MS analyses were carried out according to the method already reported by Di Stefano et al. (2017). A Dionex Ultimate 3000 System, equipped with an autosampler controlled by the Chromeleon 7.2 software (Thermo Fisher Scientific, Bremen, DE and Dionex Softron GmbH, Germering, DE), and with an UHPLC column (Phenomenex Lun C18 50 × 1 mm, 2.5 μm), was employed. Compound elution was achieved in gradient mode, with a mobile phase composed of water containing 0.1% acetic acid (v/v) pH 3.2, and acetonitrile. The detection of all compounds was performed by ultrahigh performance liquid chromatography (UHPLC system) coupled with heated electrospray ionization (HESI) quadrupole Orbitrap mass spectrometry (Q Exactive; Thermo Scientific, Germany) in negative ion mode. The identification of compounds was based on the retention times and exact mass in comparison with those of pure standards. The quantification procedure was performed by comparing the areas of the peaks, providing the proportion of each phenolic compound present in extracts obtained with different extraction methods.

2.3. Evaluation of the antioxidant activity

2.3.1. DPPH radical scavenging assay

The extracts capacity to reduce the radical DPPH was assessed using the method described by Masuda et al. (1999) with slight modifications. 50 μl of each extract at different concentrations were added to 2 ml of