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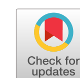
Antioxidant activity and enzymes inhibitory properties of several extracts from two Moroccan Asteraceae species



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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 ($376.11 \pm 8.22$ $\mu mol_{QE}/g_{extract}$) 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: $IC_{50} = 8.53 \pm 0.38$ 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.

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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

DPPH methanol solution (60 μM) and the mixture was incubated for 30 min at room temperature in the dark. The absorbance was recorded against a blank sample (methanol solution) at 517 nm. BHT and quercetin were used as positive controls. The antioxidant activity was evaluated by the concentration of the extract that neutralizes 50% of DPPH radicals (IC_{50} value).

2.3.2. $ABTS^{\bullet+}$ radical cation decoloration assay

The $ABTS^{\bullet+}$ stock solution (7 mM) was prepared using potassium persulfate and stored for 12–16 h in darkness at room temperature as described by Re et al. (1999). Then 10 μl of each extract, Trolox concentration (standard) or buffer (blank) were added to 190 μl $ABTS^{\bullet+}$ solution in a clear 96-well microplate, and the absorbance was measured at 734 nm, 1 min after the initial mixing. The antioxidant activity was expressed as μmol Trolox Equivalents per gram of extract ($\mu mol_{TE}/g_{extract}$) for the sample dilution that produced 20–80% inhibition of the blank absorbance.

2.3.3. Fe^{3+}/Fe^{2+} reducing power assay

The reducing power of extracts was assessed according to the method described by Yen and Chen (1995) with slight modifications. The plant extract (100 μl) was mixed with 200 mM sodium phosphate buffer pH 6.6 (250 μl) and 1% $K_3[Fe(CN)_6]$ (250 μl). After incubation for 20 min at 50 °C, 250 μl 10% TCA were added to stop the reaction. Then, the mixture was centrifuged at 650 rpm for 10 min. Finally, 100 μl of the supernatant were mixed with 100 μl of water and 20 μl of 0.1% $FeCl_3$. Ascorbic acid was used as standard and phosphate buffer as blank. The reducing activity was measured by determining the absorbance at 700 nm. The results were expressed as μmol Ascorbic Acid Equivalents per gram of extract ($\mu mol_{AAE}/g_{extract}$).

2.4. Enzyme inhibitory activities

2.4.1. AChE and BChE inhibition

Cholinesterase (ChE) inhibitory activity was measured using a 96-well microplate reader (Infinite M200, Tecan), as described by Ellman et al. (1961). Firstly, 125 μl of 3 mM DTNB were mixed with 25 μl of 15 mM ATCI or BTCI. Then, 50 μl of 100 mM phosphate buffer (pH 8.0) and 25 μl of each extract/essential oil (final concentration of 0.4 mg/ml), buffer or galanthamine at 25 μg/ml (standard inhibitor) were added. Finally, 0.28 U/ml AChE or BChE was added and the absorbance was measured at 405 nm for 5 min. A control with buffer instead of plant extract was performed and the ChE inhibitory activity was expressed as percentage inhibition.

2.4.2. Tyrosinase inhibition

The tyrosinase activity was determined spectrophotometrically according to a previously described method by Masuda et al. (2005). Briefly, 0.4 mg/ml of each sample (extracts or essential oil) or phosphate buffer as a blank were mixed with 80 μl of phosphate buffer (pH 6.8), 40 μl of tyrosinase, and 40 μl of L-DOPA. Kojic acid (200 μg/ml) was used as a positive control. The absorbance was measured at 475 nm and the tyrosinase inhibitory activity was expressed as percentage inhibition.

2.4.3. α-Glucosidase inhibition

The α-glucosidase inhibition was measured as reported by Kwon et al. (2008), with slight modifications. In a 96-well microplate, 50 μl of plant extract, essential oil (final concentration of 0.4 mg/ml) or acarbose (1 mg/ml) were mixed with 100 μl of 0.1 M phosphate buffer (pH 6.9) containing yeast α-glucosidase solution (1.0 U/ml) and then incubated at room temperature for 10 min, followed by the addition of 50 μl of 5 mM *p*-nitrophenyl-α-D-glucopyranoside solution to each well. The microplate was incubated again for 10 min and the absorbance measured with a microplate reader at 405 nm. Acarbose was used as a positive control and the α-glucosidase inhibitory activity was expressed as percentage inhibition.

2.4.4. α-Amylase inhibition

The α-amylase inhibition assay was carried out according to the procedure described by Kwon et al. (2008) with slight modifications. A volume of 500 μl of each extract or essential oil (final concentration of 0.4 mg/ml) was added to 500 μl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) and porcine pancreatic α-amylase (0.5 mg/ml). The mixture was incubated at 25 °C for 10 min. Then, 500 μl of a 1% starch solution in phosphate buffer was added to each tube. After incubation at 25 °C for 10 min the reaction was stopped with 1.0 ml of dinitrosalicylic acid color reagent. Finally, the test tubes were incubated in a boiling water bath for 5 min, cooled to room temperature and then diluted with distilled water. Acarbose at 1 mg/ml was used as reference and α-amylase inhibitory activity was expressed as percentage inhibition.

2.5. Statistical analysis

All the experiments were carried out in triplicate and were repeated three times. The data were described as the mean ± standard error of mean and expressed by one-way analysis of variance (ANOVA), followed by Duncan's New analysis to identify significant differences between means using Multiple Range Test ($p < 0.05$). The correlations were calculated using Pearson's test. All statistical analysis were determined using the SPSS statistical package for Windows (release 18.0; SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Extraction yield and total phenolic contents

Extraction efficiency depends on different conditions; e.g., chemical nature of phytochemicals, the extraction method, the solvent used, as well as on the presence of interfering substances (Stalikas, 2007). In this study, the extraction yields varied with the plant species, the solvent and the method used (Table 1). The highest extraction yields were obtained for the infusion in both plants (29.32 and 32.70% for *B. imbricatum* and *C. arabicus*, respectively), followed by the maceration with MeOH in the case of *B. imbricatum* (20.98%) and UAE with MeOH/W in the case of *C. arabicus* (24.70%).

Total phenolic contents (Table 2) ranged from 178.81 ± 8.95 to 337.47 ± 6.67 $\mu mol_{GAE}/g_{extract}$ in *C. arabicus*, and from 742.36 ± 5.95 to 1611.13 ± 14.23 $\mu mol_{GAE}/g_{extract}$ in *B. imbricatum*. The highest amounts of phenolics were obtained using maceration with MeOH as a solvent for *C. arabicus* (7.97 mg_{GAE}/g_{DW}), and also maceration but with MeOH/W for *B. imbricatum* (47.96 mg_{GAE}/g_{DW}). These values were higher than those found in MeOH/water (80/20) extract from *B. imbricatum* (6.93 mg_{GAE}/g_{DW}) and in extracts from other Asteraceae species from Morocco, e.g., MeOH extracts from *Artemisia campestris* L. (2.43 mg_{GAE}/g_{DW}) and *Artemisia herba-alba* Asso (5.61 mg_{GAE}/g_{DW}) (Tlili et al., 2013), and MeOH/water (80/20) extracts from *Conyza Canadensis* (L.) Cronquist (2.54 mg_{GAE}/g_{DW}), *Pulicaria dysenterica* (L.) Gaertn. (7.81 mg_{GAE}/g_{DW}) and *Senecio anteuphorbium* (L.) Sch.Bip. (3.99 mg_{GAE}/g_{DW}) (El Guiche et al., 2015). However, only the TPC of the MeOH/W extract of *B. imbricatum* was higher than those found in *Anvillea radiata* Coss. & Durieu (11.54 mg_{GAE}/g_{DW}), *A. herba-alba*

Table 1
Extraction yields (%) of extracts from *C. arabicus* and *B. imbricatum* obtained by using different extraction methods and solvents.

Extraction method	*C. arabicus*	*B. imbricatum*
Maceration MeOH	13.89	20.98
Maceration MeOH/W	14.80	17.50
UAE MeOH	15.92	15.24
UAE MeOH/W	24.70	17.15
Infusion	32.70	29.32

MeOH: methanol; MeOH/W: methanol/water; UAE: ultrasound assisted extraction.

Table 2
Total phenolic and flavonoid contents of different extracts from *C. arabicus* and *B. imbricatum*.

	TPC ($\mu mol_{GAE}/g_{extract}$)		TFC ($\mu mol_{QE}/g_{extract}$)	
	C. arabicus	*B. imbricatum*	*C. arabicus*	*B. imbricatum*
Maceration MeOH	337.47 ± 6.67 a	985.63 ± 13.09 b	157.64 ± 3.85 a	268.53 ± 7.57 b
Maceration MeOH/W	283.04 ± 6.29 b	1611.13 ± 14.23 a	36.88 ± 3.63 c	376.11 ± 8.22 a
UAE MeOH	310.06 ± 3.09 ab	742.36 ± 5.95 d	137.20 ± 1.78 b	211.81 ± 3.44 d
UAE MeOH/W	180.52 ± 2.09 c	1005.44 ± 11.71 b	20.92 ± 1.21 d	243.03 ± 6.76 c
Infusion	178.81 ± 8.95 c	854.66 ± 21.23 c	21.06 ± 2.15 d	170.09 ± 7.50 e

Values are expressed as mean ± SE (n = 3). In the same column, values marked with different letters indicate significant differences ($p < 0.05$). MeOH: methanol; MeOH/W: methanol/water; UAE: ultrasound assisted extraction; TPC: total phenolic content; TFC: total flavonoid content.

(19.86 mg_{GAE}/g_{DW}) and *Inula viscosa* (L.) Aiton (25.26 mg_{GAE}/g_{DW}) (El Guiche et al., 2015). On the other hand, the results indicated that maceration was more effective for the extraction of phenolic compounds from both plants, which is in agreement with preceding research in other species (Naima et al., 2015; Cujić et al., 2016; Rocha et al., 2017). Previous investigations also showed that MeOH is one of the most suitable solvents for the extraction of phenolic compounds (Galvez et al., 2005; Nacif de Abreu and Mazzafera, 2005). TFCs varied widely among both plants and extraction assays. As observed for the TPC, the greatest TFC were obtained using maceration with MeOH for *C. arabicus* (6.62 mg_{QE}/g_{DW}) and MeOH/W for *B. imbricatum* (19.89 mg_{QE}/g_{DW}). This highest TFC value obtained for *B. imbricatum* was similar to that found by El Guiche et al. (2015) in MeOH/W of the same species (23.14 mg_{QE}/g_{DW}), but much lower than those observed in MeOH/W extracts from other Asteraceae species. In previous studies with *Centaurea* species, an important genus within the Asteraceae family, it was found that MeOH extracts showed much higher TFC values than aqueous extracts (Aktumsek et al., 2013; Zengin et al., 2018).

3.2. UHPLC–MS analysis

The UHPLC–MS analysis confirmed the presence of a great diversity of flavonoids and phenolic acids in the extracts (Table 3). Fifteen compounds were identified in the extracts of both plants including mainly phenolic acids and flavonoids. Isochlorogenic acid was the major phenolic compound identified in the majority of the extracts from both Asteraceae species, followed by ferulic acid in *B. imbricatum* extracts and by caffeic acid in *C. arabicus* extracts. The same compounds were detected in all extracts of each species with the exception of gallic and benzoic acids which were found, although in lower percentages (0.01–0.2% to the total phenolic compounds identified), only in *B. imbricatum* extracts. On the other hand, cinnamic acid was found only in *C. arabicus* extracts (0.02–4.45%).

Isochlorogenic acid has been cited in previous studies as a strong antioxidant compound and inhibitor of the studied enzymes (Tundis et al., 2010; Erdogan-Orhan et al., 2011). Other phenolic acids (caffeic, vanillic, protocatechuic, ferulic, gallic, *p*-hydroxybenzoic and benzoic acids) also identified in the extracts are involved in the inhibition of enzymes and recognized as having strong antioxidant effect (Hitayezu et al., 2015; Liu et al., 2016). Among the compounds identified, different flavones were detected, including apigenin, luteolin and diosmetin, which have shown their efficacy against different enzymes like cholinesterases, α-glucosidase and α-amylase (Tadera et al., 2006).

3.3. Antioxidant activity

The different extracts of both plants have been studied for their antioxidant capacity using three complementary tests: DPPH and ABTS radical scavenging capacity, and FRAP.

The results of the antioxidant activity are summarized in Table 4. The results of DPPH test, expressed as IC_{50} values, varied between 8.53 ± 0.38 and 49.75 ± 0.93 μg/ml for *B. imbricatum*, and between 33.11 ± 1.25 and 173.83 ± 0.27 μg/ml for *C. arabicus*. *B. imbricatum* extracts showed high antioxidant activity with IC_{50} values lower than that obtained by the synthetic antioxidant BHT (BHT, IC_{50} = 14.46 ± 0.25 μg/ml), for most of the extracts (Table 4). *C. arabicus* extracts showed also considerable antioxidant activity especially the extract obtained by maceration with MeOH (IC_{50} = 33.11 ± 1.25 μg/ml). Overall, MeOH extracts from Turkish Asteraceae species reached 50% DPPH inhibition at higher concentrations (0.20–0.50 mg/ml) than that used in the present work. In addition, Tlili et al. (2013) observed IC_{50} values of 0.73 ± 0.10 and 0.33 ± 0.10 mg/ml, respectively for *A. campestris* and *A. herba-alba*. In a previous study with *B. imbricatum* using DPPH assay, El Guiche et al. (2015) found an inhibition of 89.82% using a high concentration of sample (1 mg/ml). Therefore, the results obtained in the present work showed considerably higher antioxidant activity.

The results of ABTS assay also showed higher antioxidant activity. *B. imbricatum* extracts obtained by maceration with MeOH/W showed the highest activity (3461.8 ± 9.38 $\mu mol_{TE}/g_{extract}$), whereas the

Table 3
Analysis of phenolic compounds found in several extracts from *C. arabicus* and *B. imbricatum*.

	BZA	4-HZA	CA	V	PCA	*p*-CA	VA	GA	CFA	FA	A	L	D	ICA	A7G
B. imbricatum															
Maceration MeOH	0.10	NF	NF	0.2	0.05	0.26	2.61	0.01	0.05	77.30	1.55	0.12	18.03	NF	0.04
Maceration MeOH/W	0.04	NF	NF	0.1	1.64	0.12	1.16	0.20	5.36	4.25	0.02	0.73	0.05	86.30	0.36
UAE MeOH	NF	0.05	NF	0.01	0.23	2.25	0.40	0.03	0.08	44.48	2.07	7.44	20.50	NF	21.51
UAE MeOH/W	1.20	NF	NF	0.02	5.21	0.10	4.81	0.07	9.44	11.52	0.09	1.28	0.03	63.30	1.40
Infusion	NF	NF	NF	0.33	0.80	1.07	3.50	0.01	3.44	23.10	2.20	6.30	12.60	32.10	11.20
C. arabicus															
Maceration MeOH	NF	0.01	1.20	0.30	11.90	0.44	0.88	NF	30.95	1.43	1.48	5.52	1.45	43.21	1.01
Maceration MeOH/W	NF	4.65	0.02	0.06	0.04	0.04	0.50	NF	2.10	1.25	0.01	0.20	0.15	90.48	0.03
UAE MeOH	NF	0.02	0.98	0.50	7.41	2.84	1.98	NF	29.4	3.47	2.98	4.31	2.78	37.98	4.10
UAE MeOH/W	NF	4.21	0.12	0.04	2.15	1.64	2.20	NF	6.30	4.25	0.41	1.17	0.20	75.30	1.20
Infusion	NF	1.10	4.45	0.20	5.20	0.98	5.17	NF	12.18	4.95	0.04	4.14	7.10	44.30	8.54

NF: Not found. The values are expressed as mean percentage (%) of total polyphenols.
BZA: Benzoic acid; 4-HZA: 4-Hydroxybenzoic acid; CA: Cinnamic acid; ICA: Isochlorogenic acid; PCA: Protocatechuic acid; *p*-CA: *p*-Coumaric acid; VA: Vanillic acid; GA: Gallic acid; CFA: Caffeic acid; FA: Ferulic acid; A: Apigenin; L: Luteolin; D: Diosmetin; V: Vanillin; A7G: Apigenin 7-glucoside; MeOH: methanol; MeOH/W: methanol/water; UAE: ultrasound assisted extraction.

Table 4
Antioxidant capacity of *C. arabicus* and *B. imbricatum* extracts determined by DPPH, ABTS and FRAP tests.

	DPPH (IC_{50} µg/ml)		ABTS ($\mu mol_{TE}/g_{extract}$)		FRAP ($\mu mol_{AAE}/g_{extract}$)	
	C. arabicus	*B. imbricatum*	*C. arabicus*	*B. imbricatum*	*C. arabicus*	*B. imbricatum*
Maceration MeOH	33.11 ± 1.25 c	8.98 ± 0.51 b	419.61 ± 19.6 a	1707.51 ± 21.08 b	1084.82 ± 26.5 a	1845.33 ± 8.63 b
Maceration MeOH/W	55.84 ± 0.69 e	8.53 ± 0.38 b	235.37 ± 3.58 b	3461.8 ± 9.38 a	608.04 ± 8.63 b	3281.60 ± 47.43 a
UAE MeOH	41.62 ± 0.61 d	9.10 ± 0.10 b	425.75 ± 11.70 a	1314.03 ± 35.91 c	1086.42 ± 6.58 a	1715.83 ± 38.75 b
UAE MeOH/W	95.41 ± 0.58 f	49.75 ± 0.93 d	175.74 ± 5.41 c	1379.20 ± 39.77 c	258.22 ± 6.75 c	896.60 ± 62.18 d
Infusion	173.83 ± 0.27 g	11.11 ± 0.27 b	153.15 ± 6.30 c	1665.38 ± 9.37 b	170.07 ± 14.50 d	1346.24 ± 40.34 c
Quercetin	1.51 ± 0.02 a					
BHT	14.46 ± 0.25 c					

Values are expressed as mean ± SE (n = 3). In the same column, values marked with different letters indicate significant differences ($p < 0.05$). MeOH: methanol; MeOH/W: methanol/water; UAE: ultrasound assisted extraction; DPPH: 2-diphenyl-1-picrylhydrazyl; ABTS: 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid radical cation; FRAP: ferric reducing ability power.

lowest activity was recorded in the MeOH UAE (1314.03 ± 35.91 $\mu mol_{TE}/g_{extract}$). These results were much higher than those recently found (175.96–391.43 $\mu mol_{TE}/g_{extract}$) in methanol and water extracts from two *Centaurea* spp. (Zengin et al., 2018). The extracts obtained from *C. arabicus* were less active; the highest activity did not exceed 425.75 ± 11.7 $\mu mol_{TE}/g_{extract}$ and was observed in the extract obtained by UAE with MeOH. However, in general, the *C. arabicus* values were higher than those of other Asteraceae species (Tlili et al., 2013). Only the lowest antioxidant activity observed in the infusion of *C. arabicus* (12.53 mg_{TE}/g_{DW}) was an intermediate value between the ABTS results of MeOH extracts from *A. campestris* (16.10 mg_{TE}/g_{DW}) and *A. herba-alba* (10.16 mg_{TE}/g_{DW}) (Tlili et al., 2013).

In the case of *B. imbricatum*, the highest FRAP was observed in the extract obtained by maceration with MeOH/W (3281.6 ± 47.43 $\mu mol_{AAE}/g_{extract}$). In *C. arabicus*, the best results were obtained using pure MeOH as solvent both with maceration (1084.82 ± 26.5 $\mu mol_{AAE}/g_{extract}$) or UAE (1086.42 ± 6.58 $\mu mol_{AAE}/g_{extract}$).

As shown in Table 5 the results presented a strong linear correlation between antioxidant activity and TPC or TFC. These data are in agreement with the literature since various works confirmed that correlation (Stefanović et al., 2015; Muddathir et al., 2017). Hence, the highest activity of all extracts could be attributed to the higher TPC and TFC, as already demonstrated by many researches (Wang et al., 2006; Ismail et al., 2010; Vijayalaxmi et al., 2015) including with Asteraceae species (Zengin et al., 2018). Phenolic acids particularly, isochlorogenic, ferulic and caffeic acids, that are the major compounds found in the extracts, certainly contribute to the strong antioxidant activity as previously reported by other authors (Hitayezu et al., 2015; Shahidi and Ambigaipalan, 2015).

The antioxidant activity could also be due to the presence of flavonoids such as apigenin, diosmetin and luteolin, known for their strong activity (Cheng et al., 2014; Telange et al., 2017), that can be attributed to their chemical structure, particularly to the substitution pattern of free hydroxyl groups on the flavonoid skeleton (Fraga et al., 2010).

3.4. Enzyme inhibitory activity

B. imbricatum and *C. arabicus* extracts and essential oils were also tested for their inhibitory activities against AChE, BChE, tyrosinase, α-glucosidase and α-amylase. To the best of our knowledge, there is no report about enzyme inhibitory activities of extracts and essential oils from both plant species. As seen in Table 6, all the extracts and essential oils have inhibitory abilities on AChE, BChE and tyrosinase, and the extracts have also inhibitory capacity against α-glucosidase. None of the extracts or essential oils at the tested concentration demonstrated capacity to inhibit α-amylase. *C. arabicus* essential oil showed the highest inhibitory capacity against AChE, BChE and tyrosinase (67.52%, 58.53% and 63.64%, respectively). The inhibitory potential of *B. imbricatum* essential oil was lower (37.14%, 41.56% and 54.89%, respectively).

A straight relationship between the AChE and BChE inhibitory capacity of monoterpenoids, the main compounds of essential oils, was noted and already reported in several studies (Orhan et al., 2008; Zarrad et al., 2015). In addition, many investigations proved that a bicyclic monoterpene hydrocarbon containing an allylic methyl group was a strong inhibitor of AChE and BChE enzymes. Other studies have also proved the strong inhibition of monoterpenes hydrocarbons (Perry et al., 2002), the main compounds of many essential oils. In accordance with the obtained results, the predominance of sabinene, α-pinene and β-pinene in *C. arabicus* essential oil (Aghraz et al., 2017) may be responsible for its inhibitory activity.

The results showed that the extract obtained by maceration with MeOH exhibit the highest inhibition percentage on AChE and BChE for the two species (Table 6), 62.94% and 43.88%, respectively for *B. imbricatum* and 640% and 58.10%, respectively for *C. arabicus*. The extracts obtained by the infusion of both species showed the lowest BChE inhibitory activity, with an inhibition lower than 20%. Overall, the AChE and BChE inhibition percentages obtained were higher than those observed for similar concentration of MeOH and water extracts from other Asteraceae species (0–24.54% for AChE and 0–45.50% for BChE)

Table 5
Pearson's correlation coefficients between assays.[a]

	Bioactive compounds	Antioxidant activity			Enzyme inhibitory activity			
		DPPH	ABTS	FRAP	AChE	BChE	TYR	α-Gluc
C. arabicus	TPC	−0.860**	0.889**	0.945**	0.428	0.855**	0.818**	0.785**
	TFC	−0.723**	0.972**	0.946**	0.301	0.931**	0.923**	0.468
B. imbricatum	TPC	−0.064	0.950**	0.808**	−0.346	0.069	0.663**	0.849**
	TFC	−0.082	0.858**	0.831**	−0.130	0.409	0.409	0.786**

TPC: total phenolic content; TFC: total flavonoid content; DPPH: 2-diphenyl-1-picrylhydrazyl; ABTS: 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid radical cation; FRAP: ferric reducing ability power; AChE: acetylcholinesterase; BChE: butyrylcholinesterase; TYR: tyrosinase; α-Gluc: glucosidase.

[a] Data represents Pearson correlation coefficient R.

** Indicates $p < 0.01$.

Table 6
Enzyme inhibitory activity (AChE, BChE, tyrosinase and α-glucosidase) of extracts (0.4 mg/ml) from *C. arabicus* and *B. imbricatum*.

	AChE (%)		BChE (%)		Tyrosinase (%)		α-Glucosidase (%)	
	C. arabicus	*B. imbricatum*	*C. arabicus*	*B. imbricatum*	*C. arabicus*	*B. imbricatum*	*C. arabicus*	*B. imbricatum*
Maceration MeOH	64.00 ± 2.29 a	62.94 ± 2.11 a	58.10 ± 2.39 a	43.88 ± 0.70 a	24.74 ± 1.48 c	51.26 ± 1.53 ab	29.55 ± 1.61 b	26.52 ± 2.66 ab
Maceration MeOH/W	45.58 ± 2.32 bc	54.91 ± 2.48 ab	45.63 ± 1.02 c	25.65 ± 2.01 b	30.68 ± 1.15 b	37.01 ± 2.07 c	38.92 ± 1.96 a	29.60 ± 2.70 a
UAE MeOH	54.55 ± 4.02 b	42.59 ± 2.06 c	56.17 ± 1.66 ab	42.5 ± 1.54 a	23.38 ± 0.58 c	47.55 ± 1.12 b	23.04 ± 1.61 bc	21.59 ± 2.33 b
UAE MeOH/W	51.82 ± 2.44 bc	41.19 ± 2.07 c	51.90 ± 1.09 b	24.28 ± 0.95 b	25.43 ± 0.69 c	34.11 ± 1.10 c	20.16 ± 2.56 c	15.17 ± 0.87 c
Infusion	46.62 ± 2.25 bc	52.53 ± 0.69 b	15.18 ± 0.70 d	13.67 ± 0.88 c	28.73 ± 1.34 b	36.51 ± 2.01 c	23.79 ± 2.40 bc	8.60 ± 1.01 d
Essential oil	67.52 ± 0.58 a	37.14 ± 0.62 c	58.53 ± 2.33 a	41.56 ± 1.71 a	63.64 ± 0.89 a	54.89 ± 0.77 a	NA	NA
Standard	73.54 ± 0.81		86.10 ± 0.69		80.85 ± 0.77		81.12 ± 0.25	

Values are expressed as mean ± SE (n = 3). In the same column, values marked with different letters indicate significant difference ($p < 0.05$). MeOH: methanol; MeOH/W: methanol/water; UAE: ultrasound assisted extraction; AChE: acetylcholinesterase; BChE: butyrylcholinesterase.

(Aktumsek et al., 2013). In the case of tyrosinase inhibitory assay, different responses were observed. The best results for *B. imbricatum* were observed with extracts obtained by methanolic maceration (51.26%) and UAE (47.55%), and for *C. arabicus* in extracts obtained by maceration with MeOH/W (30.68%) and infusion (28.73%). Extracts obtained by maceration with MeOH/W showed significantly higher inhibitory activity of α-glucosidase, 38.00 and 30.00% for *C. arabicus* and *B. imbricatum*, respectively. Based on these findings, the highest effects were noted against AChE and BChE. Similar to the previously observed by other authors (Zengin et al., 2018), no significant correlation (Table 5) was observed between TPC and TFC and the inhibitory effects against AChE and BChE, indicating that the recorded activity is related with non-phenolic compounds. Previous studies conducted by Szwajgier (2015) and Ekin et al. (2016) showed a high activity of different flavonoids and phenolic compounds (nordihydroguaiaretic acid, rosmarinic acid, caffeic acid, gallic acid, cyanidin, delphinidin, kaempferol, myricetin, phloridzin, pelargonidin or quercetin) tested singly, but a lower activity when those compounds were simultaneously tested suggesting an antagonistic interaction between phenolic acids and flavonoids.

In spite of the significant number of phenolics and flavonoids in the extracts, no inhibition of α-amylase was observed. An explanation for this is the possible interaction between phenolic compounds and non-phenolic phytochemicals present in the extracts as observed in previous studies (Ranilla et al., 2010; Shai et al., 2010; Ademiluyi et al., 2015). It is also noteworthy that all the extracts significantly inhibited α-glucosidase more than α-amylase which agrees with earlier findings (Adefegha et al., 2014; Custódio et al., 2015; Zengin et al., 2018).

4. Conclusion

This study investigated the phytochemical properties, antioxidant activity, and enzyme inhibitory activities of *B. imbricatum* and *C. arabicus* extracts obtained using different extraction methods. The results indicate that the extracts of both species are rich in a great diversity of phenolic compounds, particularly isochlorogenic acid, and also exhibit a high antioxidant activity and a moderate inhibitory capacity against the enzymes AChE, BChE, tyrosinase and α-glucosidase. Results suggest that the extracts from these two endemic and underused species from Morocco can have a positive effect on Alzheimer's disease, diabetes mellitus type 2, and skin diseases although more preclinical and clinical studies along with phytochemical studies are required.

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