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Potential use in the treatment of inflammatory disorders and obesity of selected wild edible plants from Calabria region (Southern Italy)

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ABSTRACT

The potential role of plants and their metabolites has been recently considered in the search for new well-tolerated anti-arthritic and anti-obesity drugs. This study was designed to assess the potential effectiveness of the methanolic extracts from four wild edible species from Southern Italy, *Asparagus officinalis* L., *Bellis perennis* L., *Daucus carota* L. and *Sambucus nigra* L. All these plants have a history as anti-rheumatic or anti-arthritic remedies. The chemical constituents were identified through GC–MS and HPTLC analyses and the *in vitro* antioxidant activity was determined by means of DPPH, ABTS, FRAP-Ferrozine and β-carotene bleaching tests. To assess the anti-inflammatory and anti-arthritic potentials, the capacity to inhibit nitric oxide production in murine macrophage RAW 264.7 cells and protein denaturation was measured. The anti-obesity potential was determined by evaluating the ability of the sample to inhibit pancreatic lipase, a key enzyme for dietary fats absorption. The raw extract of *D. carota* showed the best inhibitory activity on NO production (IC_{50} = 45.1 ± 1.0 μg/mL), followed by *B. perennis* and *A. officinalis* (IC_{50} equal to 193.1 ± 3.2 μg/mL and 506.3 ± 5.1 μg/mL, respectively). *D. carota* induced also inhibitory effects against the heat-induced denaturation of bovine serum albumin (IC_{50} = 878.7 ± 19.09 μg/mL) and the best lipase inhibitory potential (IC_{50} = 1.63 ± 0.07 mg/mL). Our findings suggest that this species could be a potential effective therapeutic agent to treat inflammation and arthritis, supporting the traditional popular use of this plant.

1. Introduction

Arthritis is one of the most common chronic illnesses and a leading cause of disability worldwide. It is characterized by inflammatory processes of the joints of the body. Osteoarthritis (OA) and rheumatoid arthritis (RA), a chronic autoimmune inflammatory disease, are the prevalent forms of arthritis, followed by psoriatic arthritis and related autoimmune diseases such as lupus and gout. These two main disorders are characterized by cartilage breakdown and the consequent pain and joint deformity (Laev and Salakhutdinov, 2015). The incidence of RA is rising in the last decades. The causes of this rise are unknown, but overall, researchers think it may be related to changes in environmental risk factors, among which obesity has been also considered. The results of different studies investigating this relationship are controversial. However, RA seems to be related to an altered body composition as the chronic inflammation that characterizes the disease generates metabolic alterations contributing, in combination with inactive lifestyle, to a reduced muscle mass and an increased accumulation of body fat, a condition known as rheumatoid cachexia (Crowson et al., 2013; Stavropoulos-Kalinoglou et al., 2010). Currently, available drugs for the treatment of AO and RA aim to reduce pain and inflammation and to maintain joint mobility preventing its degradation (Laev and Salakhutdinov, 2015). Even if new disease-modifying anti-rheumatic drugs (DMARDs), a group of drugs that slow or stop the immune system from destroying the joints, have been introduced for the treatment of RA, these drugs are not always effective, and non-steroidal and steroidal anti-inflammatory drugs are commonly used. The treatment of OA is also still essentially based on such older drugs, whose long-term administration may lead to side effects such as hematologic, gastrointestinal and renal complications. Therefore, the interest of the research is currently focused on complementary and alternative medicine approaches for the management of such chronic and debilitating diseases. Traditional medicines all over the world may suggest a wide range of medicinal plants and plant derived natural remedies for the treatment of these chronic disorders (Choudhary et al., 2015). The potential role of plants and their metabolites has been recently considered also in the treatment of obesity, with the aim to find new well-tolerated natural drugs. As a matter of fact, despite the huge investments for the development of effective anti-obesity agents, only a few drugs have been approved for marketing (Marrelli et al., 2016a2019).

The aim of this work was to *in vitro* evaluate the potential anti-inflammatory and anti-obesity activities of four wild edible plant

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species collected in Calabria (Southern Italy): *Asparagus officinalis* L. (common asparagus), *Bellis perennis* L. (common daisy), *Daucus carota* L. (carrot) and *Sambucus nigra* L. (elderberry). All these plants have been traditionally used to treat inflammatory disorders, as antirheumatic or antiarthritic remedies (El et al., 2008). To investigate the potential anti-arthritic activity of these raw extracts, the *in vitro* inhibitory effects on protein denaturation were assessed using bovine serum albumin (BSA) as a protein model. To the best of our knowledge, this is the first report about the *in vitro* inhibitory effects of these plant extracts on protein denaturation. Moreover, their capacity to inhibit nitric oxide (NO) production was also investigated, together with the antioxidant potential and the chemical composition of the extracts. The potential *in vitro* anti-obesity activity was assessed as well, by evaluating the ability of the sample to inhibit pancreatic lipase, a key enzyme for dietary fats absorption.

2. Materials and methods

2.1. Chemicals

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), L-glutamine, penicillin/streptomycin, phosphate buffered saline (PBS), trypan blue, 3-(4,5-dimethylthiazol-2-yl)−2,5-diphenyltetrazolium bromide (MTT), Folin-Ciocalteu reagent, aluminum chloride, ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), propyl gallate, β-carotene, linoleic acid, Tween 20, iron (II) chloride, iron (III) chloride, 3-(2-Pyridyl)−5.6-diphenyl-1.2.4-triazine-4′,4′′-disulfonic acid sodium salt (ferrozine), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS), Griess reagent, bovine serum albumin (BSA), diclofenac sodium, sodium chloride, potassium chloride, disodium hydrogen phosphate, potassium dihydrogen phosphate, Lipase Type II from porcine pancreas, 4-nitrophenyl caprylate (*p*-NPC), orlistat and reference compounds utilized in HPTLC analyses were purchased from Sigma-Aldrich S.p.A. (Milan, Italy). Normal phase glass plates 20 cm × 10 cm with glass backed layers silica gel 60 (2−10 μm; 2 mm thickness) were purchased from Merck (Darmstadt, Germany). Dipotassium peroxodisulphate was purchased from Carlo Erba Reagents. Murine macrophage cell line RAW 264.7 was purchased from ATCC no. TIB-71, UK. All solvents used were reagent grade and were purchased from VWR International s.r.l. (Milan, Italy).

2.2. Plant material and extraction procedure

Aerial parts from *A. officinalis* L., *B. perennis* L. and *S. nigra* L. and roots from *D. carota* L. were collected in Calabria (Italy) in April 2016 (Table 1). Voucher specimens are deposited in the Herbarium of the University of Calabria. Dried samples were extracted with methanol through maceration at room temperature (plant to solvent ratio 1:10 g/mL) and dried under vacuum. A portion of each crude extract was then suspended in methanol/water (9:1) and partitioned with *n*-hexane, in order to separate the most apolar compounds. Samples were stored at −20 °C until analyses.

2.3. Total phenolic and total flavonoid content

The total phenolic and total flavonoid content of the four raw extract were assessed by means of spectrophotometric methods. Total phenolic content was estimated using the Folin-Ciocalteau reagent as previously reported (Menichini et al., 2013), while total flavonoid content was evaluated using a colorimetric method based on the formation of a flavonoid-aluminum complex (Marrelli et al., 2016b) with absorbance measurements at 430 nm. Analyses were run in triplicate. Values were calculated from calibration curves based on the standard chlorogenic acid or quercetin (analysis of phenolics and flavonoids, respectively); final results were expressed as mg of chlorogenic acid or quercetin equivalent *per* g of dry plant material, respectively.

2.4. GC–MS analysis

The apolar volatile constituents of the *n*-hexane fractions were identified by means of gas chromatography-mass spectrometry (GC–MS). The phytochemical profile was acquired on a Hewlett-Packard 6890 gas chromatograph equipped with an SE-30 capillary column (100% dimethylpolysiloxane, 30 m length, 0.25 mm in diameter, 0.25 μm film thickness) and a selective mass detector Hewlett Packard 5973. Analyses were conducted using a programmed temperature from 60 to 280 °C (16 °C/min) with helium as carrier gas (linear velocity, 0.00167 cm/s) (Araniti et al., 2013). The comparison of GC retention factors with those of standards, and the comparison of mass spectra with those present in the Wiley 138 library allowed the identification of compounds.

2.5. HPTLC analysis

Qualitative and quantitative analyses of polar constituents of plant samples were carried out by means of High-Performance Thin Layer Chromatography (HPTLC). The utilized apparatus consisted of a Linomat 5 sample applicator connected to a TLC Visualizer (CAMAG, Muttenz, Switzerland). Normal phase glass plates 20 cm × 10 cm (silica 2−10 μm; 2 μm thickness) were used. Operating conditions were the same as previously described (Menichini et al., 2013). Plates were developed using a mixture ethyl acetate/dichloromethane/acetic acid/formic acid/water (100:25:10:10:11, v/v/v/v/v). For post-chromatographic derivatization, plates were dipped in freshly prepared NPR reagent (1 g diphenylborinic acid aminoethylester in 200 mL of ethylacetate) and anisaldehyde reagent (1.5 mL *p*-anisaldehyde, 2.5 mL H_2SO_4,1 mL AcOH in 37 mL EtOH), and heated at 100 °C for 5 min. The plates were examined under a UV light at 254 or 366 nm and under white light upper and lower (WRT) before and after derivatization.

For the qualitative analysis of phenolic compounds, the polar fractions of crude extracts (50 mg/mL in methanol) were used for TLC fingerprinting and co-chromatography with the reference compounds chlorogenic acid, caffeic acid, ferulic acid, gallic acid, cinnamic acid, *p*-coumaric acid, quercetin, catechin, rutin, luteolin, naringenin, kaempferol and naringin. For quantitative analyses, solutions at 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0 mg/mL were prepared for each marker compound. Calibration curves were prepared using absolute amount (μg/band) as

Table 1
Investigated edible plants and their total phenolic and flavonoid contents.

Botanical name	Family	Plant part	Voucher number	Yield	TP	TF
Asparagus officinalis L.	Asparagaceae	Aerial parts	CLU 26243	21.7	35.8 ± 0.1^{b}	0.97 ± 0.01^{b}
Bellis perennis L.	Asteraceae	Aerial parts	CLU 26252	11.1	16.7 ± 0.1^{c}	0.42 ± 0.01^{c}
Daucus carota L.	Apiaceae	Roots	CLU 26245	11.3	10.2 ± 0.1^{d}	0.12 ± 0.01^{d}
Sambucus nigra L.	Adoxaceae	Aerial parts	CLU 26238	19.7	42.9 ± 0.2^{a}	1.80 ± 0.01^{a}

TP,Total phenolic content; TF, total flavonoid content. Data are expressed as mean ± SD ($n = 3$). Results were expressed as mg of chlorogenic acid or quercetin equivalent *per* g of dry plant material, respectively. Letters indicate statistically significant differences at $P < 0.05$ (Bonferroni post-hoc test).

independent variable (X) and the peak area of standards as dependent variable (Y). Quantification of compounds was performed using regression equations (correlation coefficients R^2, typically > 0.98). All determinations were carried out in triplicate (three different plates).

2.6. DPPH, ABTS and FRAP-ferrozine assays

The free radical scavenging activity was assessed using a test based on the reduction of a purple methanolic solution of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). 200 μl of samples solutions at different concentrations (5–1000 μg/mL) were added to 800 μl of a 10^{-4} M methanol solution of DPPH. After 30 min in the dark, absorbances were measured at 517 nm. Ascorbic acid was used as positive control and all experiments were run in triplicate (Conforti et al., 2006).

The ABTS assay was performed in Spectrophotometer Jasco V-530 according the method described by Venditti et al. (2013) with slight modifications. ABTS radical was generated by mixing 5 mL of a 2 mM ABTS solution with 100 μL of 7 mM K_2S_2O8 and incubating in the dark for 24 h at room temperature. Before usage, the ABTS radical solution was diluted (1 – 25 mL methanol) to obtain an Abs value of 0.7 at 734 nm. Methanolic extracts were solubilized in water and tested in a concentration range of 25–100 μg/mL and Trolox (Tr) was tested in a concentration range of 5–50 μM. 100 μL of each samples (or Trolox) were added to 900 μL of the diluted ABTS radical solution and the Abs at 734 nm was recorded after 1 min. The anti-radical capacity of the samples was calculated by comparing ABTS radical solution decolorization with that of Trolox. All the experiments were run in triplicate and the results are expressed as IC_{50} (μg/mL).

The FRAP-Ferrozine assay was performed in the microplate reader Victor TM X3 PerkinElmer according to the method described by Venditti et al. (2013). A calibration curve was created for the Fe^{2+}/ferrozine complex using 100 μL of $FeCl^2$ increasing concentrations (from 10 to 80 μM) and 100 μL of Ferrozine (2.5 mM in distilled water) in a total volume of 1 mL. A blank was prepared with ferrozine only. Methanolic extracts were tested in a concentration range of 25–100 μg/mL and Trolox from 10 to 80 μM. 100 μL of extract (or Trolox) were added to 200 μL of a previously prepared mixture containing $FeCl_3$ (1 mM) and Ferrozine (5 mM) and the volume was brought to 1 mL with distilled water. The Abs was read using the microplate reader at 570 nm after 5 min of incubation at room temperature. The Abs of the $FeCl_3$/ferrozine mixture was subtracted from that obtained with extracts or Trolox. The amount of Fe^{2+} produced by the extracts at the different concentrations was calculated from the calibration curve. Results are expressed in FRAP value, which represents the μg of sample necessary to obtain 100 μM of Fe^{2+}.

2.7. β-carotene bleaching-linoleic acid assay

The antioxidant activity was determined using the β-carotene bleaching test as previously reported (Conforti et al., 2012). Briefly, 1 mL of a β-carotene solution (0.5 mg/mL in $CHCl_3$) was added to 0.02 mL of linoleic acid and 0.2 mL of 100% Tween 20. An emulsion was prepared by evaporation of chloroform and dilution with 100 mL of water. 0.2 mL of different samples solutions (1–100 μg/mL) were added to 5 mL of the prepared emulsion that was placed in a water bath at 45 °C; absorbances were measured at 470 nm at initial time and after 30 and 60 min. The antioxidant activity was measured in terms of successful prevention of β-carotene bleaching. The experiments were run in triplicate and propyl gallate was used as positive control.

2.8. Inhibition of nitric oxide production

The *in vitro* anti-inflammatory potential of edible plant species was tested by verifying their ability to inhibit nitric oxide (NO) production in lipopolysaccharide-stimulated murine macrophage RAW 264.7 cells. Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 1% L-glutamine, 1% antibiotic solution (penicillin/streptomycin) 10% fetal bovine serum (FBS), and under 5% CO_2 at 37 °C. Cells were removed from culture flask by scraping and cells counts and viability were performed using a standard trypan blue cell counting technique. Cells were then sub-cultured onto 96 well culture plates (1×10^5 cells/well). The nest day cells were incubated with different samples (concentrations ranging from 25 to 1000 μg/mL) in the presence of 1 μg/mL LPS for further 24 h. The presence of nitrite, a stable oxidized product of NO, was determined in cell culture media by means of the Griess reagent (1% sulfanamide and 0.1% N-(1-naphtyl) ethylenediamine dihydrochloride in 2.5% H_3PO_4) (Delfine et al., 2017). Briefly, the same volumes (100 μL) of cell culture supernatant and Griess reagent were combined in 96-well plates and absorbance was measured at 550 nm using a microplate reader (GDV DV 990 B/V, Roma, Italy).

The 3-(4,5-dimethylthiazol-2-yl)–2,5-diphenyltetrazolium bromide (MTT) assay (Marrelli et al., 2016c) was used to verify the absence of cytotoxic effects. Cells were incubated with 0.5% w/v MTT in phosphate buffered saline (100 μL/well) and after 4 h of incubation dimethyl sulfoxide (100 μL/well) was added to dissolve the formazan crystals. Absorbance values were measured at 550 nm.

2.9. Anti-arthritic potential

The anti-arthritic potential of investigated wild edible plants was estimated by means of the *in vitro* protein denaturation assay. The test was performed using the method described by Palit et al. (2018) with some modifications. 0.10 mL of each sample (concentrations ranging from 1000 to 50 μg/ml in water) were added to 2.40 mL of 3.5% bovine serum albumin (BSA). Water (0.10 mL) was used in untreated control group, and diclofenac sodium (250 μg/mL) was used as positive control. Product control groups were prepared without bovine serum albumin. pH was adjusted at 6.3 using 1 N HCl and samples were then incubated at 37 °C for 20 min and then heated at 72 °C for 5 min. After cooling, 2.5 mL of phosphate buffered saline (pH 6.3) were added to each sample. Buffer was prepared by dissolving 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4 and 0.24 g of KH_2PO_4 in 800 mL of distilled water; pH was adjusted at 6.3 using 1 N HCl and the final volume was brought to 1000 mL with distilled water. The turbidity of obtained solutions was measured spectrophotometrically at 660 nm. The control represents 100% of protein denaturation. The percentage of protein denaturation inhibition was calculated as shown in the following equation:

Percentage of inhibition = [1 – (Abs test solution – Abs product control) / Abs untreated control] × 100

2.10. Pancreatic lipase inhibition

The anti-obesity potential was verified by evaluating the inhibition of pancreatic lipase using a method previously described (Marrelli et al., 2018). Test samples (100 μL) at different concentrations (ranging from 0.125 to 5 mg/mL) were added to 100 μL of type II crude porcine pancreatic lipase solution (1 mg/mL in water), 100 μL of 5 mM 4-nitrophenyl caprylate (NPC) solution in dimethyl sulfoxide and 4 mL of Tris–HCl buffer (pH= 8.5). This mixture was incubated at 37 °C for 25 min and absorbance was measured at 412 nm. Experiments were run in triplicate and orlistat (final concentration 20 μg/mL) was used as positive control.

2.11. Statistical analysis

Experiments were run in triplicate, except for test involving cell cultures, for which four replicates were performed. Data were

expressed as means ± S.E.M. D'Agostino-Pearson's K2 test was used for assessing normality of data and Levene's test for homogeneity of variances. Raw data were then fitted through nonlinear regression in order to deduce the IC_{50} parameter (Graph-Pad Prism Software, San Diego, CA, USA). Statistical differences between the control and treated groups were tested by one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. Statistical significance of differences among treated group means were estimated by one-way ANOVA followed by Bonferroni post-hoc test ($P \leq 0.05$) (SigmaStat Software, Jantel Scientific Software, SanRafael, CA).

3. Results and discussion

3.1. Phytochemical constituents

All samples were extracted with methanol through maceration procedure. *A. officinalis* and *S. nigra* crude extracts showed higher extraction yields (21.7% and 19.7%, respectively) compared to *B. perennis* and *D. carota* (yield about 11.0%, Table 1). The *n*-hexane fractions were analyzed by means of GC–MS, and identified fatty acids, terpenes and phytosterols are reported in Table 2. Fatty acids were the most abundant identified compounds. Myristic acid (8.8%) was found to be the major constituents of *A. officinalis*, while the apolar fractions of *B. perennis* and *S. nigra* were mainly characterized by the presence of α-linolenic acid (13.6% and 2.6%, respectively) and linoleic acid (5.1% and 16.1%). Myristic acid was also found to be abundant in *S. nigra* (9.6%). Two terpenes were identified: the diterpene neophytadiene, present in all the samples except for *D. carota*, and the terpenoid ketone citronellyl acetone. *A. officinalis* was the sample with the highest number of phytosterols, being β-sitosterol (6.7%) the most abundant one. Total phenolic and total flavonoid content of investigated raw extracts was also assessed. The amounts were expressed as chlorogenic acid and quercetin equivalents *per* g of dry material. The highest amount of phenolic compounds was detected in *S. nigra* methanolic extract (42.9 ± 0.2 mg/g, Table 1). This sample showed also the highest content of flavonoids (1.80 ± 0.01 mg/g). The total phenolic and total flavonoid contents of the raw extract of *A. officinalis* were 35.8 ± 0.1 mg/g and 0.97 ± 0.01 mg/g, respectively. Lower levels were detected in *B. perennis* and *D. carota*.

The polar residue of the methanolic extracts after fractionation with *n*-hexane were analyzed by HPTLC, which allowed to tentatively identify the presence of chlorogenic acid and rutin in more than one extract (Fig. 1). Chlorogenic acid has been detected in the polar fractions of *B. perennis* and *S. nigra* raw extracts, as indicated by the typical blue spots (Fig. 1(b); Tracks: 3–5; Rf = 0.33). The flavonoid glycoside rutin is recognizable in this latter sample and in *A. officinalis* (tracks 2,3,13; Rf = 0.18) as a yellow spot after derivatization with NP reagent. Chromatographic profiles of investigated samples and utilized standards are reported in Fig. 2. Quantitative analyses were performed using regression equations. As evidenced in Fig. 3, *S. nigra* showed the greatest amount of chlorogenic acid (51.86 ± 2.17 mg/g of fraction). 33.28 ± 0.60 mg/g of this phenolic compound were detected in *B. perennis*. The amount of rutin identified in *S. nigra* and *A. officinalis* was 36.78 and 37.56 mg/g, respectively. Our results are in agreement with those of Lee and Finn (2007), who reported chlorogenic acid and rutin as the major polyphenolic compounds in *S. nigra*. Rutin is a well-known phytochemical compound of *A. officinalis* (Lee et al., 2010), and the presence of chlorogenic acid in *B. perennis* is also documented (Scognamiglio et al., 2012).

3.2. Antioxidant activity

The radical scavenging activity of the raw methanolic extracts was first determined by means of the DPPH and ABTS colorimetric assays. *S. nigra* raw extract emerged as the most effective sample from both the assays, with IC_{50} values of 43.65 ± 0.48 μg/mL and 38.48 ± 1.00 μg/mL for DPPH and ABTS assays, respectively (Table 3). IC_{50} values of 168.4 ± 4.10 μg/mL (DPPH assay) and 74.69 ± 8.70 μg/mL (ABTS assay) were observed for *B. perennis*, while lower radical scavenging potential was observed for the other two species. The antioxidant activity of tested samples was verified also by means of the β-carotene bleaching method.

Table 2
Fatty acids, terpenes and sterols composition of the *n*-hexane fractions of analyzed plants.

Fatty acids[a]	RT[b]	RAP[c]			
		A. officinalis L.	*B. perennis* L.	*D. carota* L.	*S. nigra* L.
Pentadecanoic acid	17.599	Tr[d]	tr	0.5	–
Palmitic acid	18.125	1.6	1.5	tr	tr
14-Methylpentadecanoic acid	18.131	–	–	0.8	–
Myristic acid	18.388	8.8	1.0	–	9.6
8,11-Octadecadienoic acid	19.417	–	–	1.0	tr
α-linolenic acid	19.451	–	13.6	–	2.6
Linoleic acid	19.656	–	5.1	1.0	16.1
7,10,13-Hexadecatrienoic acid	19.788	–	–	–	1.0
Stearic acid	19.862	0.8	1.5	–	–
Terpenes[a]					
Neophytadiene	17.450	0.6	8.9	–	6.8
Citronellyl acetone	17.827	–	–	–	tr
Phytosterols[a]					
Campesterol	31.167	1.2	–	–	–
β-Sitosterol	32.489	6.7	–	–	–
Stigmasta-7,22-dien-3-ol	33.253	–	6.9	–	–
Stigmast-5-en, 3-ol	33.339	–	–	–	tr
Tremulone	36.934	tr	–	–	–
Others[a]					
2-Phytene	17.490	–	0.9	–	–
Phytone	17.513	0.3	–	–	–
1-Octadecene	19.325	–	–	–	tr
9,17-Octadecadienal	19.679	1.4	–	–	tr
Cyclotetracosane	20.079	–	–	–	1.1
Tigogenin	31.801	1.9	–	–	–

[a] Compounds listed in order of elution from SE30 MS column. [b] Retention time (as minutes). [c] Relative area percentage (peak area relative to total peak area%). [d] Compositional values less than 0.1% are denoted as traces.

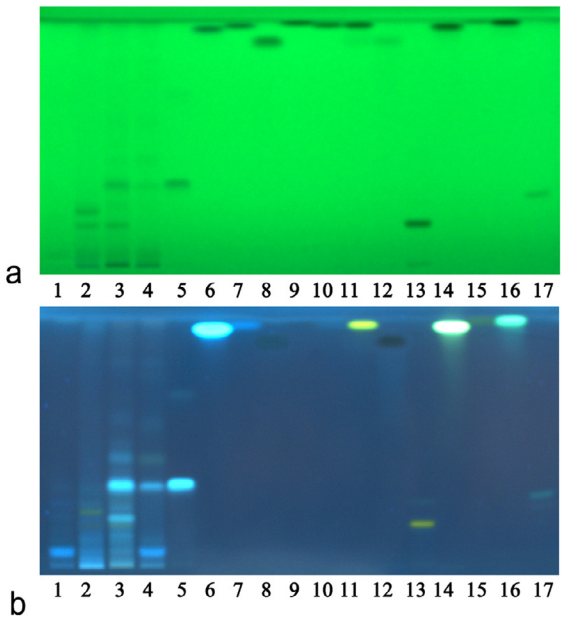

Fig. 1. HPTLC analysis of the polar fractions of investigated plants. Mobile phase: ethyl acetate/dichloromethane/acetic acid/formic acid/water (100:25:10:10:11, v/v/v/v/v). a Visualization: 254 nm. b visualisation: 366 nm, Derivatisation: NPR. Tracks: 1, *D. carota* L.; 2, *A. officinalis* L.; 3, *S. nigra* L.; 4, *B. perennis* L.; 5, chlorogenic acid; 6, caffeic acid; 7, ferulic acid; 8, gallic acid; 9, cinnamic acid; 10, *p*-coumaric acid; 11, quercetin; 12, catechin; 13, rutin; 14, luteolin; 15, naringenin; 16, kaempferol; 17, naringin.

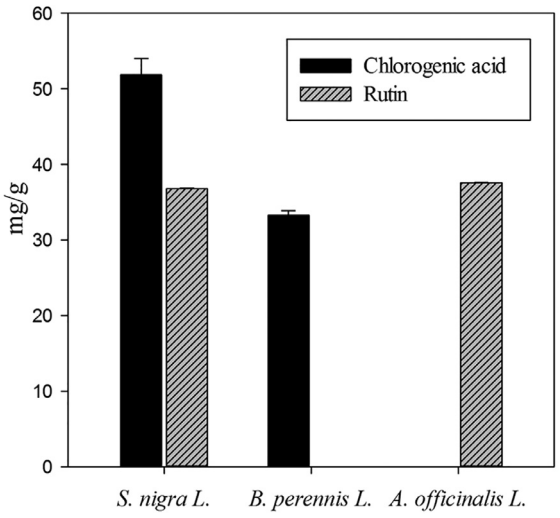

Fig. 3. Quantitative analysis of phenolic compounds. Data are expressed as mean ± SD (n = =3).

S. nigra showed the best antioxidant activity also in this assay, with an IC_{50} value equal to 5.07 ± 0.04 μg/mL after 30 min of incubation and to 6.22 ± 0.19 μg/mL after 60 min (Table 3). *A. officinalis* showed also a good biological activity (IC_{50} values equal to 13.48 ± 0.30 and 16.29 ± 0.46 μg/mL after 30 and 60 min, respectively). The capacity of tested samples to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) was evaluated through the FRAP-Ferrozine assay and it emerged that *S. nigra* was the most effective sample with a FRAP value of 162.18 ± 0.27 (Table 3). The best antioxidant and antiradical capacity registered for *S. nigra* compared to the other investigated plants is consistent with the found total phenolic and flavonoid contents which are well known for their antioxidant properties (Mandrone et al., 2012) and have already shown linearity with biological activities (Chiocchio et al., 2018).

Dawidowicz et al. (2006) also assessed the antioxidant properties of alcoholic extracts from *S. nigra*, reporting a good biological activity for leaves, berries and flowers, while the antioxidant activity of *A.*

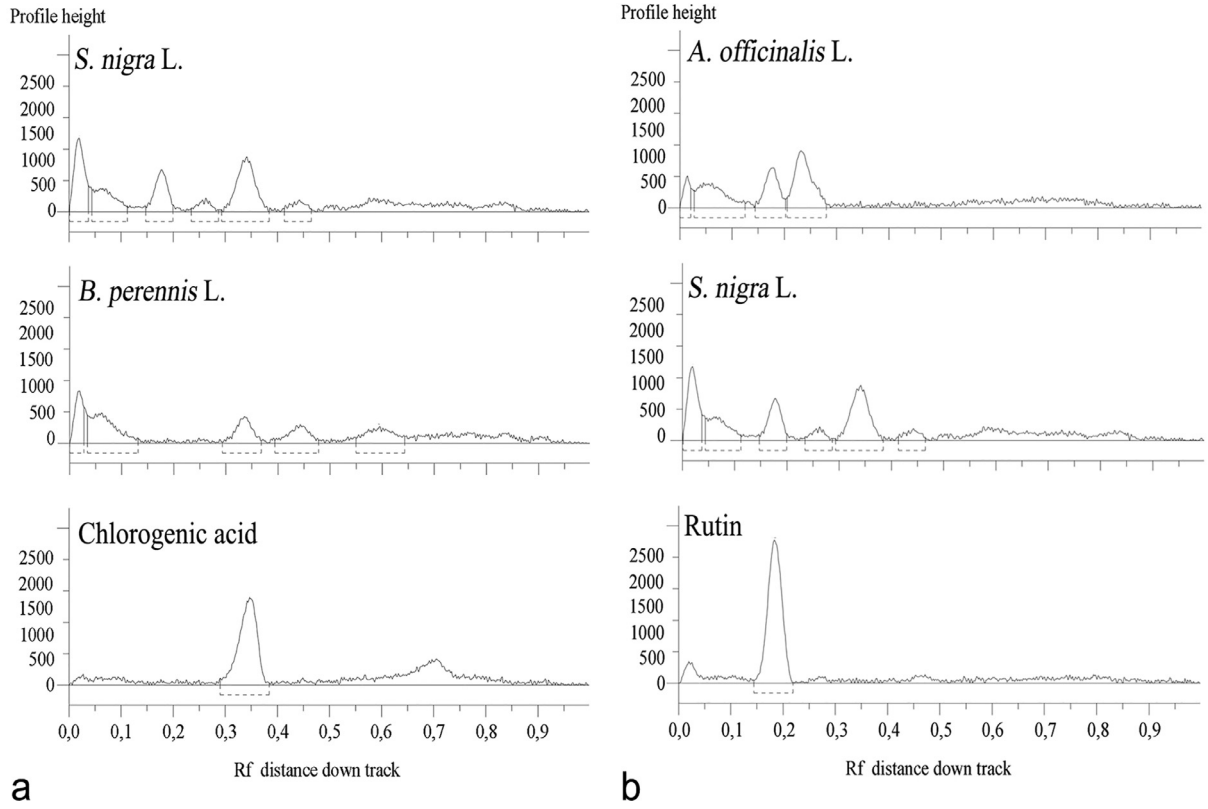

Fig. 2. HPTLC chromatograms of analyzed samples and standards. (a) Detection of chlorogenic acid (Rf = 0.33). (b) Detection of rutin (Rf = 0.18).

Table 3
Anti-inflammatory, anti-arthritic, anti-obesity and antioxidant activities of investigated wild edible plants.

Sample	IC_{50} (μg/mL)							
	NO inhibition	BSA denaturation inhibition	Lipase inhibition (mg/mL)	DPPH test	β-carotene bleaching test		ABTS	FRAP_FZ
					30 min	60 min		FRAP value
A. officinalis L.	506.3 ± 5.1 ^{c}	> 1000	> 5	351.6 ± 4.37 ^{d}	13.48 ± 0.30 ^{c}	16.29 ± 0.46 ^{c}	107.66 ± 12.20 ^{b}	323.99 ± 15.05 ^{c}
B. perennis L.	193.1 ± 3.2 ^{b}	> 1000	> 5	168.4 ± 4.10 ^{c}	78.45 ± 0.77 ^{d}	85.28 ± 1.22 ^{e}	74.69 ± 8.70 ^{ab}	557.89 ± 2.55 ^{d}
D. carota L.	45.1 ± 1.0 ^{a}	878.7 ± 19.09	1.63 ± 0.07	513.7 ± 4.49 ^{e}	> 100	> 100	212.62 ± 33.50 ^{c}	649.62 ± 22.14 ^{e}
S. nigra L.	> 1000	> 1000	> 5	43.65 ± 0.48 ^{b}	5.07 ± 0.04 ^{b}	6.22 ± 0.19 ^{b}	38.48 ± 1.00 ^{ab}	162.18 ± 0.27 ^{b}
Indomethacin*	58.0 ± 0.9 ^{a}	–	–	–	–	–	–	–
Diclofenac*	–	15.73 ± 0.16	–	–	–	–	–	–
Lipase *	–	–	0.018 ± 0.001	–	–	–	–	–
Ascorbic acid*	–	–	–	2.00 ± 0.01 ^{a}	–	–	–	–
Propyl gallate*	–	–	–	–	1.00 ± 0.02 ^{a}	1.00 ± 0.02 ^{a}	–	–
Trolox	–	–	–	–	–	–	4.13 ± 0.20 ^{a}	40.87 ± 1.27 ^{a}

Data are expressed as mean ± SEM. FRAP value represents the μg of sample necessary to obtains 100 μM of Fe^{2+}. Different letters along columns or between columns (β-carotene) indicate statistically significant differences at $P < 0.05$ (Bonferroni post-hoc test). For protein denaturation assay and lipase inhibition assay the statistical difference between *D. carota* extract and the positive controls was determined by *t*-test ($P < 0.001$). * Positive controls.

officinalis has been already investigated by Hafizur et al. (2012) and Sun et al. (2007). Wang et al. (2011) reported that the aqueous extract induced 11.54 ± 0.25% of inhibition at 0.5 mg/mL.

3.3. Anti-inflammatory and anti-arthritic potentials

The potential role of edible plants in the treatment of inflammatory disorders was investigated trough the evaluation of their ability to inhibit the LPS-induced production of NO in murine macrophage RAW 264.7 cell line. Nitrite, a stable oxidized product of NO, was used as an indicator of NO production, and its presence in cell culture medium was verified by means of the Griess reagent (1% sulfanamide and 0.1% N-(1-naphtyl) ethylenediamine dihydrochloride in 2.5% H_3PO_4). Cells were cultured with different concentrations (25–1000 μg/ml) of the raw extracts in the presence of LPS (final concentration 1 μg/ml) for 24 h. Moreover, the capacity to protect bovine serum albumin from denaturation was used to estimate the potential anti-arthritic activity. The denaturation of tissue proteins is one of causes of inflammatory and arthritic, and this damage is also related to the production of auto-antigens that occurs in some arthritic diseases. Therefore, agents able to prevent protein denaturation could be useful for the development of new anti-inflammatory drugs (Chandra et al., 2012). *B. perennis, D. carota* and *S. nigra* samples induced a dose-dependent inhibition of NO production (Fig. 4(a) and (b)), without affecting cell viability in performed MTT test. The raw extract of *D. carota* induced 92.7 ± 3.0% of inhibition at 1000 μg/mL, and it was still effective even at the lowest concentration tested (32.9 ± 0.6% at 25 μg/mL, Fig. 4(a)). *B. perennis* raw extracts, able to cause 71.7 ± 2.3% inhibition at 1000 μg/mL, also showed a significant activity at the lowest concentrations compared to the control ($P < 0.001$, Dunnett's multiple comparison test). *A. officinalis* strongly affected NO production at the highest concentration (95.3 ± 1.2%) but did not show a significant activity at 100 μg/mL. The crude extract of *D. carota* showed the best inhibitory activity on NO production, with an IC_{50} value of 45.1 ± 1.0 μg/mL (Table 3). A lower but still interesting effectiveness was observed for *B. perennis* and *A. officinalis* crude extracts (IC_{50} equal to 193.1 ± 3.2 μg/mL and 506.3 ± 5.1 μg/mL, respectively). A study by Metzger and Barnes (2009) reported that polyacetylenes from *D. carota* extract were effective in inhibiting nitric oxide production in RAW 264.7 cells. An inhibitory activity on NO production has been documented for *A. officinalis* from China (Bor et al., 2006). This sample, causing less than 40% inhibition at the concentration 200 μg/mL, was less effective compared to our data. The methanolic extract of wild *B. perennis* flowers from Turkey also showed anti-inflammatory activity on RAW 264.7 macrophages (Karakas et al., 2017).

The *in vitro* inhibitory effects of investigated raw extracts on protein denaturation are shown in Fig. 4(c) and (d). *A. officinalis* and *D. carota* induced a dose-dependent inhibition of albumin denaturation. At the highest concentration tested, *A. officinalis* raw extract caused 48.26 ± 0.11% inhibition (Fig. 4(c)). An inhibition equal to 51.53 ± 0.77% was detected for *D. carota* sample, for which an IC_{50} value equal to 878.7 ± 19.09 μg/mL was calculated (Fig. 4(d), Table 3). Vasudevan et al. (2006) investigated the potential anti-inflammatory properties of the seeds extract from *D. carota*, demonstrating that it was effective in inhibiting carrageenan, histamine and serotonin-induced paw edema and formaldehyde-induced arthritis in rats. No previous studies investigated the *in vitro* potential inhibitory activity of *D. carota* extracts on protein denaturation in BSA model to the best of our knowledge.

3.4. Anti-obesity potential

The ability of samples to inhibit pancreatic lipase was assessed by means of a spectrophotometric method based on the hydrolysis of 4-nitrophenylcaprylate (*p*-NPC), which releases the yellow chromogen *p*-nitrophenol. At the highest concentration tested, 5 mg/mL, all the samples were significantly effective compared to the control ($P < 0.001$, Dunnett's multiple comparison test). The raw extract of *D. carota* induced 93.66 ± 2.84% inhibition of the enzyme, while for the other sample percentages of inhibition lower than 50% were detected (Fig. 5(a)). *D. carota* raw extract showed the best biological activity, with an IC_{50} value equal to 1.63 ± 0.07 mg/mL (Fig. 5(b), Table 3). The effectiveness of our sample was more interesting than that reported for the hydroalcoholic extract of the same species from Slovenia, as reported by Slanc et al. (2009). These authors assessed the lipase inhibitory potential using *p*-nitrophenylpalmitate and 5-bromo-4-chloro-3-indoxylpalmitate as substrates and *D. carota* showed inhibition of pancreatic lipase below 40%. Interestingly, the *D. carota* pomace obtained from juice extraction was also assessed for its hypolipidemic potential on albino rats (Afify et al., 2013). The dietary supplementation of the powder obtained from carrot pomace was able to significantly decrease total lipid, total cholesterol and triglycerides. Body weight gain was also significantly affected.

4. Conclusion

Medicinal and culinary plants traditionally used in local popular medicine represent a great resource for the search of new effective drugs, as an alternative to synthetic drugs commonly utilized. In this context, the results of the present work demonstrate the *in vitro* potential effectiveness of *A. officinalis, B. perennis, D. carota* and *S. nigra*, plants traditionally used as anti-rheumatic or anti-arthritic remedies.

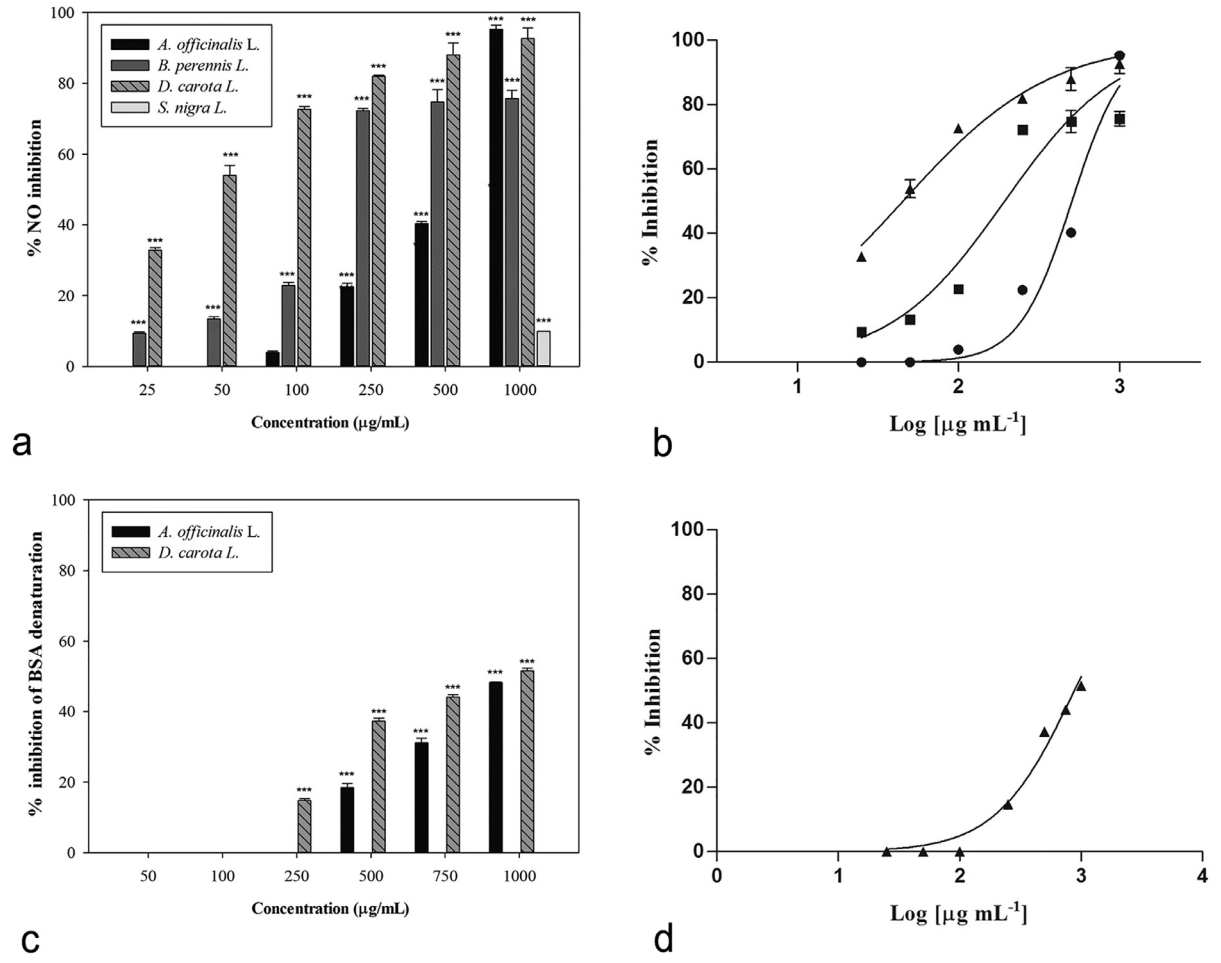

Fig. 4. Anti-inflammatory and anti-arthritic potential of investigated raw extracts. (a) Inhibition of NO production. Data were expressed as means ± S.E.M. (n = 4). Mean values of samples showing significant difference from the control were denoted with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ in one-way ANOVA followed by Dunnett's test. (b) Non-linear regression curves: • A. officinalis L., □ B. perennis L., ▲ D. carota L. (c) Inhibition of BSA denaturation. Data were expressed as means ± S.E.M. (n = 3). (d) Non-linear regression anaysis of D. carota L. extract.

D. carota, particularly, has been observed to exert significant anti-arthritic effect *in vitro* experimental studies, being effective in inhibiting protein denaturation. This property has not been reported for this plant so far to the best of our knowledge. In conclusion, together with the evaluation of the antioxidant and anti-obesity potential of investigated raw extracts, this study provides a scientific support to the

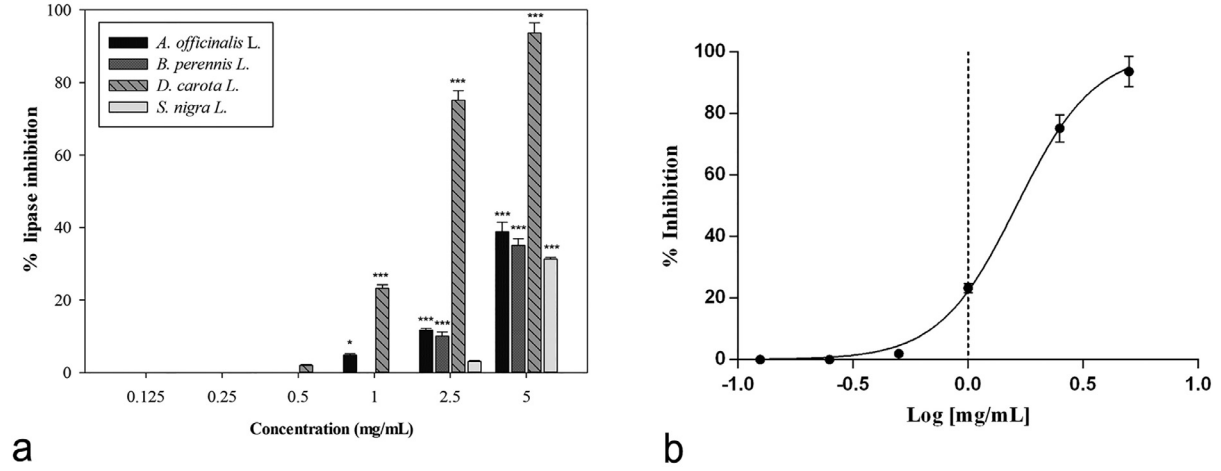

Fig. 5. Lipase inhibitory potential of investigated raw extracts. (a) Data were expressed as means ± S.E.M. (n = 3). Mean values of samples showing significant difference from the control were denoted with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ in one-way ANOVA followed by Dunnett's test. (b) Non-linear regression analysis of *D. carota* L. lipase inhibitory activity.

traditional popular use of this plants as antirheumatic or antiarthritic remedies. Further studies are required for the isolation and chemical characterization of bioactive constituents and a deeper understanding of their mechanisms of action, with the aim to find new potential agents with anti-inflammatory and anti-obesity potential.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.sajb.2019.11.029.

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