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Metabolite profiling and biological properties of aerial parts from *Leopoldia comosa* (L.) Parl.: Antioxidant and anti-obesity potential

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ABSTRACT

Different anti-obesity drugs have proved unsuccessful due to their adverse effects. As a consequence, there is a growing interest in herbal remedies, with the aim to find new well-tolerated effective drugs. *Leopoldia comosa* (L.) Parl. grows in Central and Southern Europe, Northern Africa and Central and South-Western Asia, and the bulbs have been commonly used for food throughout history. In this study the effectiveness of *L. comosa* (L.) Parl. leaves and inflorescences hydroalcoholic extracts and fractions was verified through the evaluation of pancreatic lipase inhibitory activity. The metabolite profiling and the antioxidant activity were also investigated. Chemical composition of *L. comosa* leaves and inflorescences was assessed by means of GC–MS and HPTLC analyses. The ethyl acetate fraction of leaves sample showed the best antioxidant activity, tested through DPPH and β-carotene bleaching test. The effects on pancreatic lipase activity were assessed through the *in vitro* evaluation of the capacity to prevent *p*-nitrophenyl caprylate hydrolysis. Interestingly, leaves and inflorescences extracts and all their fractions were effective in inhibiting pancreatic lipase. The best anti-obesity potential was demonstrated by the *n*-hexane and the ethyl acetate fractions of leaves sample, with IC_{50} values of 0.369 ± 0.020 and 0.336 ± 0.007 mg/mL. The same fractions of the inflorescences hydroalcoholic extract were also effective, with IC_{50} values equal to 0.736 ± 0.045 and 0.780 ± 0.009 mg/mL. These results suggest that investigated samples could be a source of interesting compounds able to suppress dietary fat absorption.

1. Introduction

Different drugs, such as dinitrophenol, amphetamines and sibutramine, have been utilized against obesity in the last decades. However, despite some promising results, all of them have been withdrawn because of their serious adverse effects (Marrelli et al., 2016a). For many years, orlistat ((*S*)-2-formylamino-4-methyl-pentanoic acid (*S*)-1-[[(2S,3S)-3-hexyl-4-oxo-2-oxetanyl]-methyl]-dodecyl ester) was the only anti-obesity drug approved in Europe for long-term use. This molecule is a chemically synthesized hydrogenated derivative of lipstatin, a natural product of *Streptomyces toxytricini*. Orlistat acts through the inhibition of gastric and pancreatic lipases, key enzymes for the digestion of dietary triglycerides that form unbound fatty acids from dietary triglycerides that are absorbed at the brush border of the small intestine. Although orlistat is a reversible inhibitor of these two enzymes, it is considered essentially irreversible, from a clinical point of view, because of the slow reversibility of the binding (Lucas and Kaplan-Machlis, 2001). However, also orlistat causes some side effects, such as diarrhea, fecal incontinence and dyspepsia (Marrelli et al., 2016a). From 1998, just few new drugs have been introduced, and the only two available in Europe are naltrexone sustained release (SR)/bupropion SR and liraglutide, recently approved in both USA and Europe (Krentz et al., 2016).

Recently, novel natural molecules have been studied and have been proved to play a role as key regulators in metabolic and inflammatory pathways related to obesity, such as irisin (Ferrante et al., 2016a), apelin-13 (Ferrante et al., 2016b), endomorphin-2 (Brunetti et al., 2013), chemerin (Brunetti et al., 2014a), and omentin-1 (Brunetti et al., 2014b).

Nowadays, with the aim to find well-tolerated natural effective drugs, there is a great interest towards herbal remedies (Marrelli et al., 2016a). Different classes of phytochemicals have been recently demonstrated to be effective against obesity, such as some polyphenols (Meydani and Hasan, 2010), terpenes (Birari and Bhutani, 2007; Singh et al., 2015) and phytosterols (Trigueros et al., 2013). Saponins are other interesting secondary metabolites with potential anti-obesity activity, able to play a role in body weight control by different mechanisms of action (Marrelli et al., 2016a).

Herbal products can inhibit lipid absorption, increase energy expenditure, decrease pre-adipocyte differentiation and proliferation. Other anti-obesity mechanisms are a decreased lipogenesis or an increased

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lipolysis (Hasani-Ranjbar et al., 2013). The potential health benefits of different plant extracts have been demonstrated in the last few years (Mocan et al., 2016, 2017; Savran et al., 2016) and some natural anti-obesity agents from medicinal plants have reached clinical trials (Marrelli et al., 2016a; Zengin et al., 2017). However, natural compounds have not been yet fully investigated and, potentially, new effective molecules could be discovered.

As a continuation of our ongoing studies aimed to find effective anti-obesity botanicals (Conforti et al., 2012a; Marrelli et al., 2013, 2014, 2016b, 2016c), here we report the potential anti-obesity activity of *Leopoldia comosa* (L.) Parl. aerial parts.

Leopoldia comosa (L.) Parl. (syn. *Muscari comosum* (L.) Miller, Liliaceae) grows in Central and Southern Europe, Northern Africa and Central and South-Western Asia. This species is present in the whole of Italy. The most common Italian popular name "cipollaccio" is due to similarity of the bulb shape and taste to garlic, onion and leek. The bulb of *L. comosa* (L.) Parl. has been commonly used for food throughout history and, actually, it is commonly used as food in some rural communities in the south of Campania, in Apulia, in Basilicata and in Northern Calabria, where this species is just occasionally cultivated while the harvesting of wild specimens is widespread. *L. comosa* bulbs are usually consumed fried in olive oil, occasionally mixed with cheese and eggs, boiled and served with a sweet and sour sauce, or pickled. Sometimes the bulbs, after boiling with water and vinegar, are preserved with olive oil (Casoria et al., 1999). The antioxidant activity of the bulbs of this plant has been already investigated (Pieroni et al., 2002). We also recently reported the phytochemical composition of this plant part, together with its interesting potential health benefits in the treatment of obesity (Marrelli et al., 2017).

Here we want to report the phytochemical content and the biological activity of *L comosa* aerial parts. Leaves and inflorescences were tested for their ability to inhibit pancreatic lipase *in vitro*. Lipase inhibition is one of the most important strategies used by pharmaceutical industries to decrease fat absorption after its ingestion (Marrelli et al., 2016a). The antioxidant activity and the phytochemical content of both leaves and inflorescences were investigated as well. To the best of our knowledge, this is the first study concerning the chemical composition and the biological properties of leaves and inflorescences of this plant species.

2. Materials and methods

2.1. Chemicals

Ethanol, methanol, *n*-hexane, dichloromethane and ethyl acetate of analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, *p*-nitrophenyl caprylate (NPC), orlistat, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, β-carotene, linoleic acid, propyl gallate, Tween 20, Folin-Ciocalteu reagent, aluminum chloride, chlorogenic acid, quercetin and rutin were purchased from Sigma-Aldrich S.p.a. (Milan, Italy). All other reagents, of analytical grade, were Carlo Erba products (Milan, Italy).

2.2. Plant materials: collection and extraction procedure

Leaves and inflorescences from wild *L. comosa* (L.) Parl. were collected in Calabria, Italy, in the Rende district (CS), at an altitude of 300 m, 2013 (leg. F. Conforti, det. F. Conforti) in April 2013. Fresh plant material (500 g of each sample) was extracted with 70% aqueous EtOH (3 L) through maceration (48 h × 3 times) at room temperature. The resultant total extracts were filtered and dried under reduced pressure to determine the weight and yield of extraction (Table 1). Obtained raw extracts were then suspended in methanol/water (9:1) and fractionated with *n*-hexane. Remaining solution was suspended in water and portioned with dichloromethane and ethyl acetate. Percentage yields are reported in Table 1.

Table 1
Percentage yield of *L. comosa* (L.) Parl. leaves and inflorescences hydroalcoholic extracts and fractions.

Sample	Fraction	Yield %[a]
Leaves	Raw extract	4.1
	n-Hexane	0.07
	CH_2Cl_2	0.17
	AcOEt	0.17
Inflorescences	Raw extract	5.3
	n-Hexane	0.07
	CH_2Cl_2	0.05
	AcOEt	0.15

[a] % of extraction for 500 g of dried material. Data from one representative extraction.

2.3. Total phenolic and flavonoid content estimation

The amount of total phenolics of *L. comosa* leaves and inflorescences hydroalcoholic extracts was determined by the Folin-Ciocalteau method (Araniti et al., 2014). Samples were mixed with distilled water, sodium carbonate solution and Folin-Ciocalteau reagent. After 2 h the absorbance of the blue color produced was measured at 765 nm. Chlorogenic acid was used as standard and total phenolic content was expressed in mg *per* g of crude extract.

Flavonoid content was instead determined using a method based on the formation of a flavonoid-aluminum complex (Marrelli et al., 2015). Each sample (2 mg/mL in EtOH 80%) was mixed with 2% $AlCl_3$ in EtOH and absorbance was measured at 430 nm after 15 min. Results were expressed as quercetin equivalents in mg *per* g of crude extract.

Both quantifications were conducted in triplicate.

2.4. HPTLC analysis

For the analysis of polar compounds, the AcOEt fractions of *L. comosa* extracts were investigated by means of High Performance Thin Layer Chromatography (HPTLC). One microliter of the two samples (50 mg/mL in methanol) was applied in triplicate on silica gel 60 glass plates 20 cm × 10 cm (VWR International s.r.l., Milan, Italy) using Linomat 5 automated TLC applicator (CAMAG, Muttenz, Switzerland) according to the same operating conditions previously described (Menichini et al., 2013). The presence of different flavonoids, such as quercetin, quercitrin, catechin, kaempferol, naringin and rutin was assessed using the corresponding standard at a concentration of 3 mg/mL. Plates were developed using the mobile phase AcOEt/CH_2Cl_2/CH_3COOH/HCOOH/H_2O (100:25:10:10:11; v/v/v/v/v) and successively dried at room temperature and at 100 °C for 10 min. TLC plates were then observed under white light and at 254 and 366 nm before and after derivatization with NPR (1 g diphenylborinic acid aminoethylester in 200 mL of ethyl acetate) and anhysaldehyde (1.5 mL *p*-anisaldehyde, 2.5 mL H_2SO_4,1 mL AcOH in 37 mL EtOH). HPTLC analyses allowed a good separation and visualization of the chemical constituents. Band stability was checked inspecting the resolved peaks at intervals of 12, 24 and 48 h, and repeatability was determined by running three analyses. RF values for main selected compounds varied less than 0.02%. The effects of small changes in the mobile phase composition and mobile phase volume were reduced by the direct comparison.

For rutin quantitative analysis a calibration curve ($R^2 = 0.9842$) was prepared using different concentrations of the standard compound (from 0.5 to 10.0 mg/mL) that were spotted on HPTLC plates to give different amounts from 0.5 to 10 μg/band.

2.5. GC–MS analysis

Chemical composition of the *n*-hexane and dichloromethane fractions of leaves and inflorescences hydroalcoholic extracts was

investigated by gas chromatography–mass spectrometry (GC–MS). 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) directly coupled to a selective mass detector (model 5973, Hewlett Packard) was used. Electron impact ionization was carried out in Electron Impact mode (EI, 70 eV). Analyses were realized using a programmed temperature from 60 to 280 °C (rate 16° min^{-1}) using helium as carrier gas. Injector and detector were set at temperatures of 250° and 280 °C, respectively (Araniti et al., 2013). Identification of molecules was based on the comparison of the GC retention factors with those of standards and the comparison of the mass spectra with those present in the Wiley 138 library data of the GC–MS system. In order to highlight the metabolomic differences between leaves and inflorescences an internal standard (fenchone at the concentration of 500 ppm) was added to the extracts. Repeatability was determined by running three analyses.

2.6. Evaluation of antioxidant activity

The antioxidant activity of *L. comosa* leaves and inflorescences extracts and fractions was evaluated through the DPPH and the β-carotene bleaching test. The free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was utilized for the determination of the radical scavenging potency of analyzed samples, as previously described (Conforti et al., 2012b). Test samples solutions at different concentrations (5–1000 μg/mL) were added to a 10^{-4} M methanol solution of DPPH. Absorbance was measured at 517 nm after 30 min in the dark using a Perkin Elmer Lambda 40 UV/VIS spectrophotometer. Experiments were run in triplicate and ascorbic acid was used as positive control.

The β-carotene/linoleic acid system was instead used to assess the ability of the samples to inhibit lipid peroxidation (Menichini et al., 2013). A chloroformic 0.5 mg/mL β-Carotene solution (1 mL) was added to linoleic acid (0.02 mL) and 100% Tween 20 (0.2 mL). An emulsion was prepared after evaporation of chloroform and dilution with 100 mL of water. Each sample (0.2 mL) was then added to 5 mL of emulsion. Different concentrations of each sample were tested (100, 50, 25, 10, 5, 1, 0.5 and 0.025 μg/mL). Obtained solutions were placed in a water bath at 45 °C and absorbance was measured at 470 nm at initial time, 30 and 60 min. Experiments were run in triplicate and propyl gallate was used as positive control. The antioxidant activity was measured in terms of successful prevention of β-carotene bleaching.

2.7. Measurement of pancreatic lipase activity

To assess the potential anti-obesity effects of *L. comosa* L. extracts and fractions, porcine pancreatic lipase (type II) activity was measured using *p*-nitrophenyl caprylate (NPC) as a substrate (Marrelli et al., 2013). Samples were incubated at 37 °C for 25 min with the enzyme solution (1 mg/mL in water), 5 mM NPC solution and Tris-HCl buffer (pH = 8.5). Absorbance was then measured at 412 nm. Experiments were run in triplicate and orlistat (final concentration 20 μg/mL) was used as positive control.

2.8. Statistical analysis

All measurements were carried out in triplicate and data were expressed as mean value ± S.E.M. Raw data were fitted through non-linear regression in order to calculate the IC_{50} values (concentration providing 50% inhibition). Graphs were built using GraphPad Prism Software (version 6, San Diego, CA, USA).

Differences between IC_{50} values were evaluated through univariate analysis. Data were first checked for normality (D'Agostino-Pearson test) and tested for homogeneity of variances (Levene's test). Successively, statistical significances among samples were assessed through one-way analysis of variance (ANOVA) using SigmaStat Software (Jantel scientific software, San Rafael, CA, USA). Significant differences among means were analyzed using Tukey's *post hoc* test with $P \leq 0.05$.

To highlight metabolic differences between *Leopoldia* leaves and inflorescences GC–MS data were analyzed using the software Metaboanalyst 3.0 (Xia et al., 2015). Data, expressed as metabolite concentrations, were checked for integrity and missing values were replaced with a small positive value. Data were successively normalized by the pre-added internal standard (fenchone at the concentration of 500 ppm), transformed through "Log normalization" and scaled through Pareto-Scaling. Data were then classified through Principal Component Analysis (PCA) and metabolite variations were clusterized and presented through a heatmap (Araniti et al., 2017). Statistically significant differences between organs (leaves and inflorescences) were showed through a volcano plot considering significantly different compounds with a fold change ≥1.5 and a *P* value ≤ 0.05. The addition of the internal standard fenchone was carried out in order to both normalize the data, before the statistical analysis, and to do a relative quantification of the metabolite identified.

3. Results

3.1. Extraction procedure

Fresh leaves and inflorescences wild from *L. comosa* (L.) Parl. were extracted with 70% aqueous EtOH through maceration procedure, and both raw extracts were then partitioned between *n*-hexane, dichloromethane and ethyl acetate. Flowers crude extract showed a higher extraction yield than leaves sample (5.3% and 4.1%, respectively, Table 1). Yields of the different fractions obtained from the two extracts ranged from 0.05 to 0.17%.

3.2. Total phenolics and flavonoids content

Total phenolics and total flavoinoids contents were assessed by means of two spectrophotometric methods. Leaves and inflorescences raw extracts showed an amount of phenolic compounds equal to 50.50 ± 1.53 and 47.67 ± 2.62 mg/g of extracts, respectively, while a flavonoid content of 4.59 ± 0.15 and 5.61 ± 0.07 mg/g was instead observed (Fig. 1).

3.3. HPTLC analysis

The identification of polar compounds was conducted by means of High Performance Thin Layer Chromatography (HPTLC). Analyses aimed to assess the presence of different flavonoids, such as quercetin,

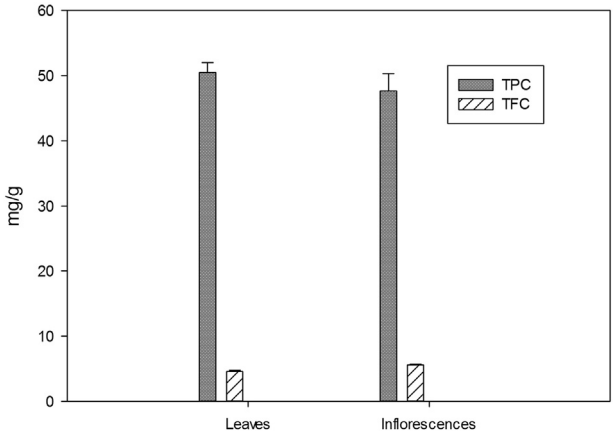

Fig. 1. Total phenolic content (TPC) and total flavonoid content (TFC) of *L. comosa* (L.) Parl. leaves and inflorescences hydroalcoholic extracts. Data are expressed as means ± ES (n = 3) in mg/g of extract.

quercitrin, catechin, kaempferol, naringin and rutin in the polar fractions (AcOet samples) of the two hydroalcoholic extracts. The flavonoid glycoside rutin was identified in both leaves and inflorescences samples, as illustrated in Fig. 2, which reports the chromatographic profiles of AcOEt fractions from *L. comosa* extracts and the standard (Rf = 0.19).

Quantitative analyses were conducted in order to compare the rutin content of the two AcOEt fractions. Leaves of *L. comosa* showed a highest content, with an amount of 0.118 ± 0.005 mg/g of crude extracts. A value equal to 0.076 ± 0.001 mg/g was obtained for the inflorescences.

3.4. GC–MS analysis

The GC–MS analysis carried out on *L. comosa* leaves and inflorescences *n*-hexane fractions allowed the identification of 21 metabolites. In particular, 18 fatty acids and 3 phytosterols were identified (Table 2). Palmitic acid was the most abundant compound, followed by stearic acid and myristic acid. Nine compounds were instead identified in the two dichloromethane fractions (Table 3). Different phenolic acids, such as cinnamic acid, *p*-hydroxycinnamic acid, *p*-coumaric acid and ferulic acid were found in these two last samples.

3.5. Principal Component Analysis (PCA)

The multivariate data analysis of the raw data, carried out through Principal Component Analysis (PCA), pointed out that the metabolic profile of leaves and inflorescences was clearly separated, confirming that the metabolic composition is extremely variable among different plant organs (Fig. 3-A). Leaves and inflorescences separation was achieved using the principal components PC1, which explained a variance of 96.3%, and the PC2 component, which explained the lowest variance (1.8%) in the subspace perpendicular to PC1 (Fig. 3-A). The PCA loading plot reported in Fig. 3 highlighted that PC1 was mainly dominated by *p*-coumaric acid, syringic acid, mevalonic acid lactone, behenic acid and linolenic acid, whereas the PC2 by 7-hydroxy-3-(1-1-dimethyl prop-2-nyl) coumarin, cinnamic acid, eicosanoic acid and margaric acid (Fig. 3-B).

PCA and heatmap visualization of metabolomic data showed distinct segregation between *L. comosa* leaves and inflorescences (Fig. 3).

Finally, data where analyzed through the volcano plot analysis considering statistically different compounds characterized by a fold change of 1.5 and a *P* value ≤ 0.05. The univariate analysis pointed out that 28 out of the 31 metabolites identified were significantly different among plant organs (Fig. 4 and Table 4). In particular, cinnamic acid, myristic acid and palmitic acid were the only compounds characterized by no statistical differences between leaves and inflorescences organs (Table 4).

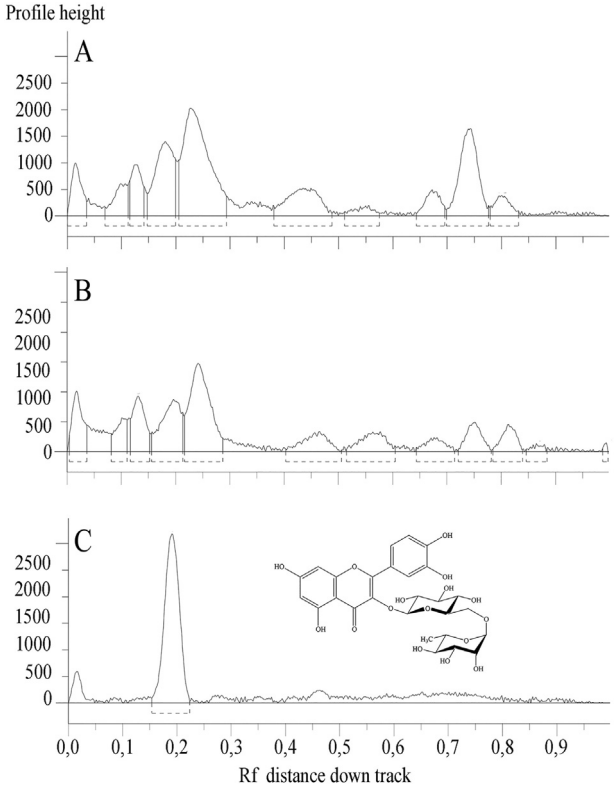

Fig. 2. HPTLC chromatographic profile of AcOEt fractions from *L. comosa* extracts. Mobile phase: AcOEt/CH_2Cl_2/CH_3COOH/HCOOH/H_2O (100:25:10:10:11; v/v/v/v/v). A, AcOet fraction of leaves extract. B, AcOet fraction of inflorescences extract. C, rutin (Rf = 0.19).

Table 2
Fatty acids and phytosterols from the *n*-hexane fractions of *L. comosa* (L.) Parl. leaves and inflorescences extracts.

Fatty acids[a]	RT[b]	mg/g of extract[c]	
		Leaves	Inflorescences
Butanedioic acid	9.878	–	Tr[d]
Caprylic acid	10.129	0.3	–
Azelaaldehydic acid	13.850	0.6	–
Undecanoic acid	14.713	0.1	–
Lauric acid	15.084	0.5	–
Myristic acid	16.845	1.5	1.2
Oleic acid	17.302	–	0.1
Pentadecanoic acid	17.656	0.5	0.3
Tetradecanoic acid	17.702	0.2	–
Palmitic acid	18.588	19.8	15.5
Margaric acid	18.919	0.5	0.9
10,13-Octadecadienoic acid	19.445	0.2	–
Linoleic acid	19.719	0.3	1.1
Stearic acid	19.897	2.3	7.9
Linolenic acid	19.954	0.5	–
Eicosanoic acid	20.994	0.4	1.2
Behenic acid	22.263	0.9	–
Lignocerid acid	23.743	0.2	0.6
Phytosterols[(a)]			
3α, 5-cyclo-ergosta-7,22-dien-6-one	28.224	–	0.3
Stigmast-7-en-3-ol, (3β, 5α)-	34.699	0.3	–
Stigmasta-3,5-dien-7-one	35.860	–	0.3

[a] Compounds listed in order of elution from SE30 MS column.
[b] Retention time (as minutes).
[c] Relative quantification based on the internal standard at known concentration added to the extract during the analysis.
[d] Compositional values less than 0.1 mg/g are denoted as traces. Data are expressed as mean (n = 3).

Table 3
Chemical composition and relative quantification of dichloromethane fractions from *L. comosa* (L.) Parl. leaves and inflorescences extracts.

Compound[a]	RT[b]	mg/g of extract[c]	
		Leaves	Inflorescences
Mevalonic acid lactone	12.312	3.7	–
Cinnamic acid	13.850	Tr[d]	tr
Benzoicacid, 4-hydroxy-, methyl ester	14.187	2.2	tr
Siringic aldehyde	16.090	–	tr
p-Hydroxycinnamic acid	16.867	0.9	tr
p-Coumaric acid	17.228	–	3.3
Syringic acid	17.428	–	1.9
Ferulic acid	17.845	–	tr
7-Hydroxy-3-(1,1-dimethylprop-2-enyl)coumarin	20.942	0.2	–

[a] Compounds listed in order of elution from SE30 MS column.
[b] Retention time (as minutes).
[c] Relative quantification based on the internal standard at known concentration added to the extract during the analysis.
[d] Compositional values less than 0.1 mg/g are denoted as traces. Data are expressed as mean (n = 3).

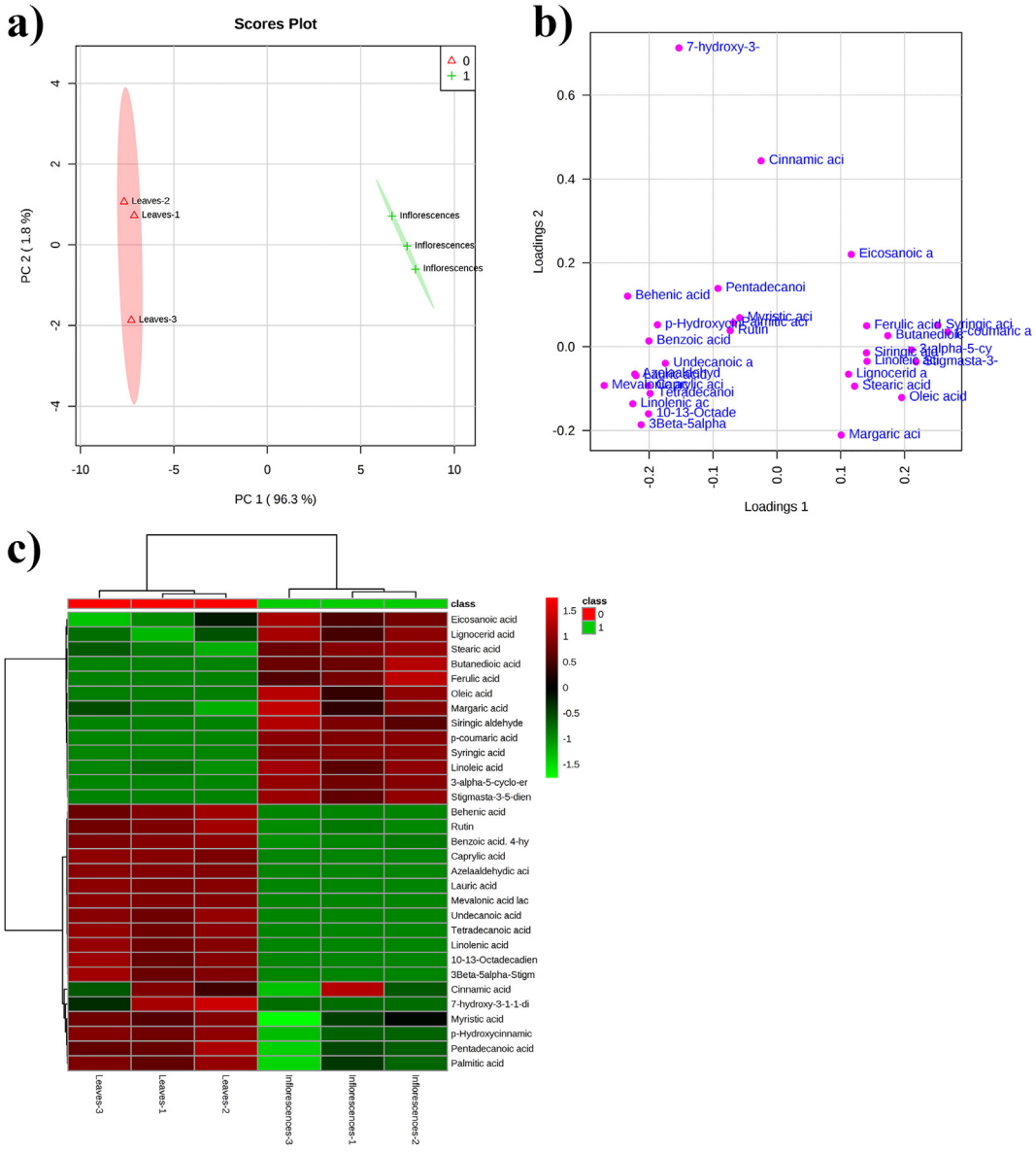

Fig. 3. PCA analysis carried on the metabolite identified and quantified in *L. comosa* leaves and inflorescences. Principal Component analysis model scores A) and loading plot B) of metabolite profile of leaves (Leaves_1 – Leaves_3, replicates of leaves samples) and inflorescences (Inflorescences_1 – Inflorescences_3, replicates of Inflorescences samples). Both score and loading plots were generated using the first two PCs, PC1 *vs* PC2, with the explained variances shown in brackets; C) overlay heat map of metabolite profiles identified in *L. comosa* organs (leaves and inflorescences). Each square represents the metabolite concentration expressed as false-color scale. Red or green regions indicate increase or decrease metabolite content, respectively.

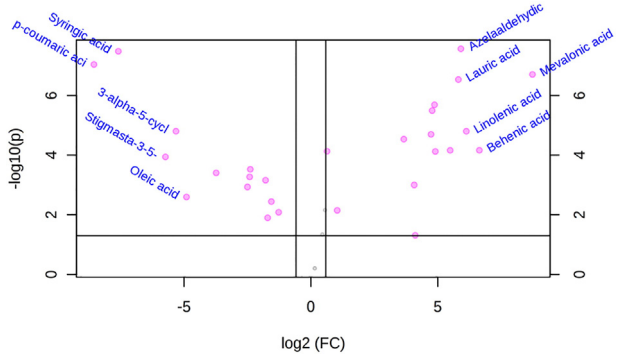

Fig. 4. Volcano Plot carried on the metabolite identified and quantified in *L. comosa* leaves and inflorescences. Statistically significant features, with a fold change ≥1.5 and a *P* value ≤ 0.05, are reported in pink color (n = 3).

3.6. Antioxidant activity

Radical scavenging activity of hydroalcoholic extracts and their fraction was assessed through the DPPH test. Testing different concentration of samples, IC_{50} values equal to 154.8 ± 10.6 and 316.6 ± 11.69 μg/mL were obtained for leaves and inflorescences raw extracts, respectively (Table 5). The best activity was detected for the two polar fractions, particularly the ethyl acetate fraction of leaves sample (IC_{50} = 86.09 ± 1.98 μg/mL, Tukey's test).

This same sample showed also the strongest capacity to protect linoleic acid from peroxidation, as assessed by the β-carotene bleaching test (Table 5). After 30 min of incubation, an IC_{50} value of 23.73 ± 1.11 μg/mL was observed. The antioxidant activity, however, decreased after 60 min. Also inflorescences AcOEt fraction induced a good inhibitory activity, with an IC_{50} value equal to 53.35 ± 2.92 μg/mL.

Table 4
Volcano plot data regarding the metabolites statically different between *L. comosa* leaves and inflorescences organs (data from Fig. 4).

Compounds	FC	log_2(FC)	*P* value	-Log_{10}(*P*)
Azelaaldehydic acid	60.333	59.149	2.69E-04	75.709
Syringic acid	0.005172	−75.949	3.26E-04	74.871
p-Coumaric acid	0.002655	−85.571	9.03E-04	70.445
Mevalonic acid lactone	426.67	8.737	1.95E-03	67.099
Lauric acid	56.333	58.159	2.90E-03	65.383
Caprylic acid	29.333	48.745	2.04E-02	56.904
Benzoic acid. 4-hydroxy-. methyl ester	27.5	47.814	3.19E-02	54.961
3-α-5-Cyclo-ergosta-7-22-dien-6-one	0.025	−53.219	1.57E-01	48.035
Linolenic acid	70	61.293	1.58E-01	48.023
Tetradecanoic acid	26.667	4.737	2.00E-01	46.987
Undecanoic acid	12.667	3.663	2.90E-01	45.376
Behenic acid	100	66.439	6.86E-01	41.634
3β-5α-Stigmast-7-en-3-ol	45	54.919	6.89E-01	41.619
Rutin	1.553	0,63508	7.39E-01	41.311
10-13-Octadecadienoic acid	30	49.069	7.52E-01	4.124
Stigmasta-3-5-dien-7-one	0.01875	−5.737	0.000115	39.406
Linoleic acid	0.1907	−23.906	0.000299	35.241
Butanedioic acid	0.075	−3.737	0.000394	34.041
Siringic aldehyde	0.1875	−2.415	0.000538	32.694
Stearic acid	0.28936	−17.891	0.000697	3.157
p-Hydroxycinnamic acid	16.842	4.074	0.000998	30.009
Ferulic acid	0.17647	−25.025	0.001166	29.333
Oleic acid	0.033333	−49.069	0.002518	2.599
Lignocerid acid	0.33913	−15.601	0.003587	24.453
Pentadecanoic acid	20.513	10.365	0.007084	21.497
Margaric acid	0.41389	−12.727	0.008218	20.852
Eicosanoic acid	0.30652	−17.059	0.012591	18.999
7-Hydroxy-3-(1-1-dimethyl prop-2-nyl) coumarin	17.333	41.155	0.048782	13.117

Statistically significant differences between samples were observed, as biological activity of leaves fractions was significantly higher than the corresponding one of inflorescences.

3.7. In vitro inhibition of pancreatic lipase

A very interesting inhibitory activity on pancreatic lipase was assessed for all tested samples (Table 6, Fig. 5). Leaves hydroalcoholic extract (IC_{50} = 3.819 ± 0.119 mg/mL) was more active than inflorescence sample (IC_{50} = 6.561 ± 0.167 mg/mL), as evidenced by statistical analysis. The best anti-obesity potential was demonstrated by the *n*-hexane and the AcOet fractions of both samples, and particularly, also in this case, leaves samples, with IC_{50} values of 0.369 ± 0.020 and 0.336 ± 0.007 mg/mL. The same fractions of the inflorescences hydroalcoholic extract were also effective, even if the activity was lesser than that of the previous ones, with IC_{50} values equal to 0.736 ± 0.045 and 0.780 ± 0.009 mg/mL for the *n*-hexane and the ethyl acetate samples, respectively (Table 6). A lower but still interesting activity was demonstrated by the two dichloromethane fractions. Any significant difference was observed between the lipase inhibitory activity of these two samples, with IC_{50} values of 1.409 ± 0.033 and 1.570 ± 0.027 mg/mL for leaves and inflorescences, respectively.

Table 5
Antioxidant activity of *L. comosa* (L.) Parl. leaves and inflorescences extracts and fractions.

Sample	Fraction	IC_{50} (μg/mL)		
		DPPH test	β-Carotene bleaching test	
			30 min	60 min
Leaves	Raw extract	154.8 ± 10.6^{c}	44.46 ± 2.64^{c}	>100
	n-Hexane	>1000	>100	>100
	CH_2Cl_2	>1000	>100	>100
	AcOEt	86.09 ± 1.98^{b}	23.73 ± 1.11^{b}	84.08 ± 3.60^{d}
Inflorescences	Raw extract	316.6 ± 11.69^{d}	84.42 ± 2.42^{d}	>100
	n-Hexane	>1000	>100	>100
	CH_2Cl_2	472.1 ± 24.67^{e}	>100	>100
	AcOEt	102.4 ± 4.91^{c}	53.35 ± 2.92^{c}	>100
Ascorbic acid*		2.00 ± 0.01^{a}	–	–
Propyl gallate*		–	1.00 ± 0.02^{a}	1.00 ± 0.02^{a}

Data are expressed as mean ± SE (n = 3). Different letters along column (DPPH test), or between columns (β-carotene bleaching test) indicate statistically significant differences at $p < 0.05$ (Tukey's test).
* Positive controls.

4. Discussion

Obesity is a serious health problem as this condition is associated with an increased risk of several diseases, including type II diabetes, cancer and cardiovascular events. Different drugs have already proved unsuccessful because of their serious adverse effects. Even Orlistat, naltrexone sustained release (SR)/bupropion SR and liraglutide, the only anti-obesity medications currently approved in Europe, cause some adverse effects. For this reason, there is a growing interest in herbal remedies, aiming to find well-tolerated natural effective drugs (Marrelli et al., 2016a). Moreover, herbal products have been proved to be useful in the treatment of clinical symptoms related to obesity,

Table 6
Inhibition of pancreatic lipase induced by *L. comosa* (L.) Parl. leaves and inflorescences extracts and fractions.

Sample	Fraction	IC_{50} (mg/mL)
Leaves	Raw extract	3.819 ± 0.119^{e}
	n-Hexane	0.369 ± 0.020^{b}
	CH_2Cl_2	1.409 ± 0.033^{d}
	AcOEt	0.336 ± 0.007^{b}
Inflorescences	Raw extract	6.561 ± 0.167^{f}
	n-Hexane	0.736 ± 0.045^{c}
	CH_2Cl_2	1.570 ± 0.027^{d}
	AcOEt	0.780 ± 0.009^{c}
Orlistat*		0.018 ± 0.001^{a}

Data are expressed as mean ± SE (n = 3). Different letters along column indicate statistically significant differences at $p < 0.05$ (Tukey's test).
* Positive control.

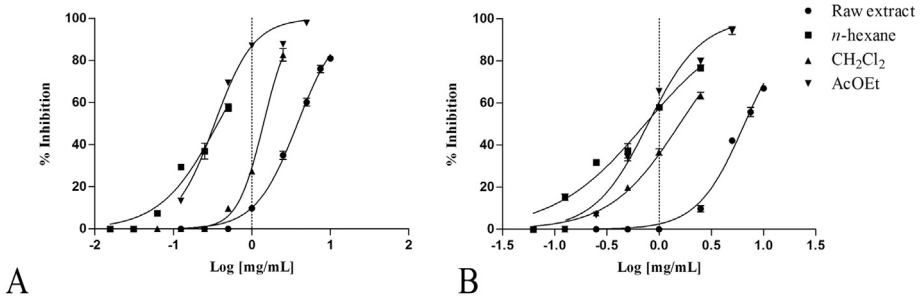

Fig. 5. Dose depending lipase inhibitory activity of leaves (A) and inflorescences (B) raw extracts and fractions.

such as metabolic syndrome, oxidative stress and inflammation (Menghini et al., 2010, 2016; Tabatabaei-Malazy et al., 2015). To the best of our knowledge, this is the first study concerning *L. comosa* (L.) Parl. aerial parts. The anti-obesity potential of leaves and inflorescences was evaluated testing their ability to inhibit pancreatic lipase activity *in vitro*. Both hydroalcoholic extracts and all their fractions were effective.

The metabolic profiling of leaves and inflorescences was also investigated. Obtained raw extracts were fractionated using solvents with increasing polarity such as *n*-hexane, dichloromethane and ethyl acetate, and the chemical composition of obtained fractions was analyzed by means of GC–MS and HPTLC. Obtained data were then analyzed through Principal Component Analysis (PCA), that pointed out a clear separation between the metabolic profile of leaves and inflorescences. In particular, the univariate analysis pointed out that cinnamic acid, myristic acid and palmitic acid were the only compounds characterized by no statistical differences between leaves and inflorescences organs, while the other identified metabolites were significantly different.

The biological activity of the bulbs of *L. comosa* has been already investigated. Pieroni et al. (2002) reported the free radical scavenging activity and the ability to inhibit bovine brain lipid peroxidation of the hydroalcoholic extract of this plant part. In a previous work (Marrelli et al., 2017), we also compared the antioxidant potential of wild and cultivated bulbs, underlining that extract from wild bulbs showed higher DPPH radical scavenging activity than extract obtained from cultivated ones. In the present study, the antioxidant potential of aerial parts from *L. comosa* was assessed for the first time. Leaves samples demonstrated the best radical scavenging potential, with an IC_{50} value equal to 154.8 ± 10.6 μg/m for the raw extract. The ethyl acetate samples were demonstrated to be the most effective fractions, particularly the one obtained from the leaves hydroalcoholic extract (IC_{50} = 86.09 ± 1.98 μg/mL). The ability to inhibit lipid peroxidation was evaluated by means of the β-carotene bleaching test, and leaves ethyl acetate fraction showed the best activity, with an IC_{50} value equal to 23.73 μg/mL.

To verify the anti-obesity potential of *L. comosa* (L.) Parl. extracts and fractions, porcine pancreatic lipase (type II) activity was measured using *p*-nitrophenyl caprylate (NPC) as a substrate. Al sample induced a dose-depending inhibition of the enzyme.

Again, the biological activity of leaves hydroalcoholic extracts (IC_{50} = 3.819 ± 0.119 mg/mL) was significantly higher than that of inflorescence sample (IC_{50} = 6.561 ± 0.167 mg/mL). The best anti-obesity potential was demonstrated by the *n*-hexane and the AcOEt fractions, with IC_{50} values of 0.369 ± 0.020 and 0.336 ± 0.007 mg/mL. The inhibitory activity of same fractions of the inflorescences hydroalcoholic extract were lesser, but still very important, with IC_{50} values equal to 0.736 ± 0.045 and 0.780 ± 0.009 mg/mL for the *n*-hexane and the ethyl acetate samples, respectively.

The lipase inhibitory activity exerted by dichloromethane fraction of inflorescences could be related to the presence of *p*-coumaric and ferulic acids identified by GC–MS, as these two phenolic acids are known for their ability to inhibit pancreatic lipase (Cai et al., 2012).

The pancreatic lipase inhibitory activity observed for the AcOEt fractions could be in part related to the presence of the flavonoid glycoside rutin, detected in both leaves and inflorescences ethyl acetate samples. As a matter of fact, the interesting anti-obesity potential of this compound (IC_{50} = 0.10 ± 0.01 mg/mL) has been demonstrated in our previous work (Marrelli et al., 2016b).

5. Conclusions

To the best of our knowledge, this is the first study dealing with the potential health benefits of aerial parts of *L. comosa* (L.) Parl. Crude extracts and fractions obtained from leaves and inflorescences induced an interesting dose-depending inhibition of pancreatic lipase *in vitro*. It is worthwhile to further investigate these samples for their potential pharmacological effect in anti-obesity treatment. More studies are needed to correlate the activity of the two *n*-hexane samples to specific chemical constituents, with the aim to identify new lipid-lowering agent to be used as safer anti-obesity drugs.

Conflicts of interest

The authors do not have any conflicts of interest.

References

Araniti, F., Lupini, A., Sorgonà, A., Conforti, F., Marrelli, M., Statti, G.A., Menichini, F., Abenavoli, M.R., 2013. Allelopathic potential of *Artemisia arborescens*: isolation, identification and quantification of phytotoxic compounds through fractionation-guided bioassays. Natural Product Research 27, 880–887.

Araniti, F., Marrelli, M., Lupini, A., Mercati, F., Statti, G.A., Abenavoli, M.R., 2014. Phytotoxic activity of *Cachrys pungens* Jan, a mediterranean species: separation, identification and quantification of potential allelochemicals. Acta Physiologiae Plantarum 36, 1071–1083.

Araniti, F., Lupini, A., Sunseri, F., Abenavoli, M.R., 2017. Allelopatic potential of *Dittrichia viscosa* (L.) W. Greuter mediated by VOCs: a physiological and metabolomic approach. PLoS One 12, e0170161.

Birari, R.B., Bhutani, K.K., 2007. Pancreatic lipase inhibitors from natural sources: unexplored potential. Drug Discovery Today 129, 879–889.

Brunetti, L., Ferrante, C., Orlando, G., Recinella, L., Leone, S., Chiavaroli, A., Di Nisio, C., Shohreh, R., Manippa, F., Ricciuti, A., Mollica, A., Vacca, M., 2013. Orexigenic effects of endomorphin-2 (EM-2) related to decreased CRH gene expression and increased dopamine and norepinephrine activity in the hypothalamus. Peptides 48, 83–88.

Brunetti, L., Orlando, G., Ferrante, C., Recinella, L., Leone, S., Chiavaroli, A., Di Nisio, C., Shohreh, R., Manippa, F., Ricciuti, A., Vacca, M., 2014a. Peripheral chemerin administration modulates hypothalamic control of feeding. Peptides 51, 115–121.

Brunetti, L., Leone, S., Orlando, G., Ferrante, C., Recinella, L., Chiavaroli, A., Di Nisio, C., Shohreh, R., Manippa, F., Ricciuti, A., Vacca, M., 2014b. Hypotensive effects of omentin-1 related to increased adiponectin and decreased interleukin-6 in intrathoracic pericardial adipose tissue. Pharmacological Reports 66, 991–995.

Cai, S., Wang, O., Wang, M., He, J., Wang, Y., Zhang, D., Zhou, F., Ji, B., 2012. In vitro inhibitory effect on pancreatic lipase activity of subfractions from ethanol extracts of fermented oats (*Avena sativa* L.) and synergistic effect of three phenolic acids. Journal of Agricultural and Food Chemistry 60, 7245–7251.

Casoria, P., Menale, B., Muoio, R., Orto botanico, 1999. *Muscari comosum*, Liliaceae, in the food habits of south Italy. Economic Botany 53, 113–115.

Conforti, F., Perri, V., Menichini, F., Marrelli, M., Uzunov, D., Statti, G.A., Menichini, F., 2012a. Wild Mediterranean dietary plants as inhibitors of pancreatic lipase. Phytotherapy Research 26, 600–604.

Conforti, F., Marrelli, M., Statti, G., Menichini, F., Uzunov, D., Solimene, U., Menichini, F., 2012b. Comparative chemical composition and antioxidant activity of *Calamintha*

nepeta (L.) Savi subsp. *glandulosa* (Req.) Nyman and *Calamintha grandiflora* (L.) Moench (Labiatae). Natural Product Research 26, 91–97.
Ferrante, C., Orlando, G., Recinella, L., Leone, S., Chiavaroli, A., Di Nisio, C., Shohreh, R., Manippa, F., Ricciuti, A., Vacca, M., Brunetti, L., 2016a. Central inhibitory effects on feeding induced by the adipo-myokine irisin. European Journal of Pharmacology 791, 389–394.
Ferrante, C., Orlando, G., Recinella, L., Leone, S., Chiavaroli, A., Di Nisio, C., Shohreh, R., Manippa, F., Ricciuti, A., Vacca, M., Brunetti, L., 2016b. Central apelin-13 administration modulates hypothalamic control of feeding. Journal of Biological Regulators and Homeostatic Agents 30, 883–888.
Hasani-Ranjbar, S., Jouyandeh, Z., Abdollahi, M., 2013. A systematic review of anti-obesity medicinal plants-an update. Journal of Diabetes and Metabolic Disorders 12, 28.
Krentz, A.J., Fujioka, K., Hompesch, M., 2016. Evolution of pharmacological obesity treatments: focus on adverse side-effect profiles. Diabetes, Obesity and Metabolism 18, 558–570.
Lucas, K.H., Kaplan-Machlis, B., 2001. Orlistat-a novel weight loss therapy. Annals of Pharmacotherapy 35, 314–328.
Marrelli, M., Loizzo, M.R., Nicoletti, M., Menichini, F., Conforti, F., 2013. Inhibition of key enzymes linked to obesity by preparations from Mediterranean dietary plants: effects on α-amylase and pancreatic lipase activities. Plant Foods for Human Nutrition 68, 340–346.
Marrelli, M., Loizzo, M.R., Nicoletti, M., Menichini, F., Conforti, F., 2014. *In vitro* investigation of the potential health benefits of wild Mediterranean dietary plants as anti-obesity agents with α-amylase and pancreatic lipase inhibitory activities. Journal of the Science of Food and Agriculture 94, 2217–2224.
Marrelli, M., Cristaldi, B., Menichini, F., Conforti, F., 2015. Inhibitory effects of wild dietary plants on lipid peroxidation and on the proliferation of human cancer cells. Food and Chemical Toxicology 86, 16–24.
Marrelli, M., Conforti, F., Araniti, F., Statti, G.A., 2016a. Effects of saponins on lipid metabolism: a review of potential health benefits in the treatment of obesity. Molecules 21, 1404.
Marrelli, M., Argentieri, M.P., Avato, P., Menichini, F., Conforti, F., 2016b. Inhibitory effect on lipid absorption and variability of chemical constituents from *Capparis sicula* subsp. *sicula* and *Capparis orientalis*. Chemistry & Biodiversity 13, 755–761.
Marrelli, M., Menichini, F., Conforti, F., 2016c. Hypolipidemic and antioxidant properties of hot pepper flower (*Capsicum annuum* L.). Plant Foods for Human Nutrition 71, 301–306.
Marrelli, M., La Grotteria, S., Araniti, F., Conforti, F., 2017. Investigation of the potential health benefits as lipase inhibitor and antioxidant of *Leopoldia comosa* (L.) Parl.: variability of chemical composition of wild and cultivated bulbs. Plant Foods for Human Nutrition:1–6 https://doi.org/10.1007/s11130-017-0618-1.
Menghini, L., Genovese, S., Epifano, F., Tirillini, B., Ferrante, C., Leporini, L., 2010. Antiproliferative, protective and antioxidant effects of artichoke, dandelion, turmeric and rosemary extracts and their formulation. International Journal of Immunopathology and Pharmacology 23, 601–610.
Menghini, L., Ferrante, C., Leporini, L., Recinella, L., Chiavaroli, A., Leone, S., Pintore, G., Vacca, M., Orlando, G., Brunetti, L., 2016. A natural formula containing lactoferrin, *Equisetum arvensis*, soy isoflavones and vitamin D3 modulates bone remodeling and inflammatory markers in young and aged rats. Journal of Biological Regulators and Homeostatic Agents 30, 985–996.
Menichini, G., Alfano, C., Marrelli, M., Toniolo, C., Provenzano, E., Statti, G.A., Nicoletti, M., Menichini, F., Conforti, F., 2013. *Hypericum perforatum* L. subsp. *perforatum* induces inhibition of free radicals and enhanced phototoxicity in human melanoma cells under ultraviolet light. Cell Proliferation 46, 193–202.
Meydani, M., Hasan, S.T., 2010. Dietary polyphenols and obesity. Nutrients 2, 737–751.
Mocan, A., Zengin, G., Uysal, A., Gunes, E., Mollica, A., Degirmenci, N.S., Alpsoy, L., Aktumsek, A., 2016. Biological and chemical insights of *Morina persica* L.: a source of bioactive compounds with multifunctional properties. Journal of Functional Foods 25, 94–109.
Mocan, A., Zengin, G., Simirgiotis, M., Schafberg, M., Mollica, A., Vodnar, D.C., Crişan, G., Rohn, S., 2017. Functional constituents of wild and cultivated Goji (*L. barbarum* L.) leaves: phytochemical characterization, biological profile, and computational studies. Journal of Enzyme Inhibition and Medicinal Chemistry 32, 153–168.
Pieroni, A., Janiak, V., Dürr, C.M., Lüdeke, S., Trachsel, E., Heinrich, M., 2002. *In vitro* antioxidant activity of non-cultivated vegetables of ethnic Albanians in southern Italy. Phytotherapy Research 16, 467–473.
Savran, A., Zengin, G., Aktumsek, A., Mocan, A., Glamoćlija, J., Ćirić, A., Soković, M., 2016. Phenolic compounds and biological effects of edible *Rumex scutatus* and *Pseudosempervivum sempervivum*: potential sources of natural agents with health benefits. Food & Function 7, 3252–3262.
Singh, G., Suresh, S., Bayineni, V.K., Kaeppagari, R.K., 2015. Lipase inhibitors from plants and their medical applications. International Journal of Pharmacy and Pharmaceutical Sciences 7, 1–5.
Tabatabaei-Malazy, O., Larijani, B., Abdollahi, M., 2015. Targeting metabolic disorders by natural products. Journal of Diabetes & Metabolic Disorders 14, 57.
Trigueros, L., Peña, S., Ugidos, A.V., Sayas-Barberá, E., Pérez-Álvarez, J.A., Sendra, E., 2013. Food ingredients as anti-obesity agents: a review. Critical Reviews in Food Science and Nutrition 53, 929–942.
Xia, J., Sinelnikov, I.V., Han, B., Wishart, D.S., 2015. MetaboAnalyst 3.0—making metabolomics more meaningful. Nucleic Acids Research 43, W251–W257.
Zengin, G., Uysal, A., Aktumsek, A., Mocan, A., Mollica, A., Locatelli, M., Custodio, L., Neng, N.R., Nogueira, J.M.F., Aumeeruddy-Elalfi, Z., Mahomoodally, M.F., 2017. *Euphorbia denticulata* Lam.: a promising source of phyto-pharmaceuticals for the development of novel functional formulations. Biomedicine & Pharmacotherapy 87, 27–36.