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 in

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**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 However, despite some promising results, all of them have been withdrawn because of their serious adverse effects (Marrelli et al., 2016a).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...
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, Lilacaceae) grows in Central and Southern Europe, Northern Africa 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 (Peroni 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, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancreas, analytical grade were purchased from VWR International s.r.l. (Milan, Italy). Lipase Type II, crude, from porcine pancrea...
investigated by gas chromatography–mass spectrometry (GC–MS). A Hewlett-Packard 6890 gas chromatograph equipped with an SE-30 capillary column (100% dimethyl polysiloxane, 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 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 was utilized for the determination of the radical scavenging potency of antioxidant activity.

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. The antioxidant activity was measured in terms of successful prevention of β-carotene bleaching.

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 IC50 values (concentration providing 50% inhibition). Graphs were built using GraphPad Prism Software (version 6, San Diego, CA, USA).

Differences between IC50 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 ≤ 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 clustered 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 flavonoids 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,
quercitin, 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-enyl) 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).

Table 2

| Fatty acidsa | RTb | mg/g of extractc |
|-------------|-----|-----------------|
|            |     | Leaves | Inflorescences |
| Butanedioic acid | 9.878 | – | Trd |
| Caprylic acid | 10.129 | 0.3 | – |
| Azelaaldehdylic acid | 13.850 | 0.6 | – |
| Undecanoic acid | 14.713 | 0.1 | – |
| Lauric acid | 15.084 | 0.5 | – |
| Myristic acid | 16.845 | 1.3 | 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 | – |
| Lignoceric acid | 23.743 | 0.2 | 0.6 |

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

| Compounda | RTb | mg/g of extractc |
|-----------|-----|-----------------|
|            |     | Leaves | Inflorescences |
| Mevalonic acid lactone | 12.312 | 3.7 | – |
| Cinnamic acid | 13.850 | Trd | tr |
| Benzoic acid, 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 | – | 0.3 |
| Ferulic acid | 17.845 | – | 0.3 |
| 7-Hydroxy-3-(1,1-dimethylprop-2-enyl)coumarin | 18.919 | – | 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).
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\textsubscript{50} values equal to 154.8 ± 10.6 and 316.6 ± 11.69 μg/mL were obtained for leaves and inflorescences, respectively (Table 5). The best activity was detected for the two polar fractions, particularly the ethyl acetate fraction of leaves sample (IC\textsubscript{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\textsubscript{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\textsubscript{50} value equal to 53.35 ± 2.92 μg/mL.

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).
In this case, leaves samples, with IC50 values of 0.369 ± 0.020 mg/mL. The best anti-obesity potential was demonstrated by the two dichloromethane fractions. Any significant difference was observed between the lipase inhibitory activity of these two samples, with IC50 values of 1.409 ± 0.033 and 1.570 ± 0.027 mg/mL for leaves and inflorescences, respectively.

### 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 (IC50 = 3.819 ± 0.119 mg/mL) was more active than inflorescence sample (IC50 = 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 IC50 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 IC50 values equal to 0.736 ± 0.045 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 IC50 values of 1.409 ± 0.033 and 1.570 ± 0.027 mg/mL for leaves and inflorescences, respectively.

### 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 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 | 56.994 |
| Benzoic acid. 4-hydroxy-, methyl ester | 27.5 | 47.814 | 3.19E-02 | 54.961 |
| 3-ox-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 |
| 3/5-Ox-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 | 41.124 |
| Stigmasta-3-5-dien-7-one | 0.01875 | -5.737 | 5.00E-01 | 39.406 |
| Linoleic acid | 0.1907 | -23.906 | 0.000299 | 35.241 |
| Butanedioic acid | 0.075 | -3.737 | 0.000394 | 34.041 |
| Siringlic acid | 0.1875 | -2.415 | 0.000538 | 32.694 |
| Stearic acid | 0.28936 | -17.891 | 0.000697 | 3.157 |
| p-Hydroxyazelaic 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 |
| Lignoceric 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 |
| Eicoenoic 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 |

**Table 5**

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

| Sample | Fraction | IC50 (µg/mL) | DPPH test | β-Carotene bleaching test |
|--------|----------|--------------|-----------|-------------------------|
|        |          |              | 30 min    | 60 min                  |
| Leaves | Raw extract | 154.8 ± 10.6b | 44.46 ± 2.64b | >100                    |
|        | n-Hexane | >1000 | >100 | >100 |
|        | CH2Cl2 | >1000 | >100 | >100 |
|        | AcOEt | 86.09 ± 1.98a | 23.73 ± 1.11b | 84.08 ± 3.60c |
| Inflorescences | Raw extract | 316.6 ± 11.69b | 84.42 ± 2.42a | >100 |
|        | n-Hexane | >1000 | >100 | >100 |
|        | CH2Cl2 | 472.1 ± 24.67b | >100 | >100 |
|        | AcOEt | 102.4 ± 4.91a | 53.35 ± 2.92a | >100 |
| Ascorbic acid* | 2.00 ± 0.01c | - | - | - |
| Propyl gallate* | - | 1.00 ± 0.02a | 1.00 ± 0.02a | - |

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.

**Table 6**

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

| Sample | Fraction | IC50 (mg/mL) |
|--------|----------|--------------|
| Leaves | Raw extract | 3.819 ± 0.119b |
|        | n-Hexane | 0.369 ± 0.020b |
|        | CH2Cl2 | 1.409 ± 0.033d |
|        | AcOEt | 0.336 ± 0.007bd |
| Inflorescences | Raw extract | 6.561 ± 0.167d |
|        | n-Hexane | 0.736 ± 0.045c |
|        | CH2Cl2 | 1.570 ± 0.027ed |
|        | AcOEt | 0.780 ± 0.009f |
| Orlistat* | - | 0.018 ± 0.001a |

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.
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 acids 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 IC₅₀ value equal to 154.8 ± 10.6 μg/mL 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 (IC₅₀ = 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 IC₅₀ 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. All samples induced a dose-dependent inhibition of the enzyme.

Again, the biological activity of leaves hydroalcoholic extracts (IC₅₀ = 3.819 ± 0.119 μg/mL) was significantly higher than that of inflorescence sample (IC₅₀ = 6.561 ± 0.167 μg/mL). The best anti-obesity potential was demonstrated by the n-hexane and the AcOEt fractions, with IC₅₀ values of 0.369 ± 0.020 and 0.336 ± 0.007 mg/mL. The inhibitory activity of some fractions of the inflorescences hydroalcoholic extract were lesser, but still very important, with IC₅₀ 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 (IC₅₀ = 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.

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