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Parentucellia latifolia subsp. *latifolia*: A potential source for loganin iridoids by HPLC-ESI-MS^n technique

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Highlights

- *Parentucellia latifolia* subsp. *latifolia* extracts were probed for biopharmaceutical potentials.
- Antioxidant and enzyme inhibitory effects were investigated.
- Loganin and its isomers, rutin, and luteolin-*O*-hexoside were the most abundant compounds.
- *Parentucellia latifolia* subsp. *latifolia* can be considered as a potent plant that warrants further studies

Abstract

This study attempts to compare the pharmaceutical potential (antioxidant and key enzyme inhibition of clinical relevance) of organic and aqueous extracts of *Parentucellia*

latifolia (L.) Caruel subsp. *latifolia* (L.) Caruel as well as phytochemical composition. The phytochemical compounds were evaluated by spectrophotometric methods (for total amounts) and HPLC-ESI-DAD-MS^n (for individual compounds). The extracts were screened for antioxidant abilities by *in vitro* assays. Inhibition effects were also investigated against one set of enzymes linked to major health problems. Generally, the methanol (MeOH) and aqueous extracts displayed higher scavenging abilities on radicals and reductive effects when compared with ethyl acetate (EtOAc) extract. On the other hand, the EtOAc extract was the most active inhibitor on cholinesterases (1.81-1.88 mg GALAE/g), amylase (0.70 mmol ACAE/g), glucosidase (2.85 mmol ACAE/g) and lipase (33.24 mg OE/g). The highest TPC was observed in the aqueous extract (25.07 mg GAE/g) while MeOH extract possessed the highest level of TFC (44.15 mg RE/g) and TPAC (3.46 mg CE/g). LC-MS^n metabolite profiling indicated that loganin and its isomers, rutin, and luteolin-*O*-hexoside were the most abundant compounds. Our results suggest that *P. latifolia* may be valuable source of phyto-agents for the management of noncommunicable diseases.

Keywords: *Parentucellia latifolia*; antioxidants; enzyme inhibition; phenolic; flavonoid; loganin

1. Introduction

Oxidative stress is associated with some important disorders including diabetes mellitus, cancer, rheumatoid arthritis, cerebrovascular and cardiovascular diseases, chronic inflammation, aging, and other degenerative diseases. The increasing level of reactive oxygen species (ROS) is harmful for human macromolecules such as DNA, lipids, and proteins leading to mutations and carcinogenesis. Consequently, the regulation of cellular reactive oxygen species is vital to maintain cellular homeostasis At this point, natural antioxidant

compounds without side effects may hinder the destructive effects of the reactive oxygen species and the plant secondary metabolites are great sources of natural antioxidants [1].

During the last years, there has been increasing interest in the exploration of herbs in the pharmaceutical industries for potent novel drugs. Nonetheless, of the estimated number of plant species globally, just a few has been chemically investigated, whereas a much smaller fraction has been subjected to pharmacological screening [2]. Despite the lack of information on therapeutic effects of some plants, their medicinal uses are more acceptable as complementary and alternative medicines due to their low cost and less adverse effects [3-5]. With the development of modern and rapid isolation techniques, bioassay screening procedures, and spectroscopic methods, phytochemistry has now become an important branch in pharmaceutical studies [2]. Recently, the role of phytoconstituents has been extended to therapeutic uses, providing considerable health benefits [6].

The genus *Parentucellia* (Orobanchaceae) comprises of annual herbs, with sessile and generally opposite upper leaves and flowers which are arranged in racemes or spike-like [7]. Four species are distributed in Western Europe, Mediterranean, Central and Southwestern Asia. Morphologically, *P. latifolia* subsp. *latifolia* can be separated from *P. latifolia* subsp. *flaviflora* by its purple corolla and relatively thick stem [7].

Few phytochemical studies have been performed on the genus *Parentucellia*. For instance, Bianco et al. [8] isolated iridoid glucosides from *Parentucellia viscosa*. Terpenoids and malonic acid derivatives were also identified in *P. latifolia* [9, 10]. Furthermore, de Urbina, et al. [11] purified the antispasmodic peracetylated penstemonoside, aucubin, and catalpol from *P. latifolia*. Nonetheless, we found that there is still a dearth of pharmacological exploitation of plants from this genus. In this context, the present study aimed to investigate the biological and chemical properties of *Parentucellia latifolia* subsp. *latifolia* in an attempt to obtain valuable phytocompounds from this species for the management of chronic

disorders. The bioactivities were assessed based on antioxidant activity (reducing, chelating, and radical scavenging potentials) and enzyme inhibitory effects against enzymes associated with some noncommunicable diseases including neurodegenerative disorders (acetyl cholinesterase (AChE) and butyryl cholinesterase (BChE)), hyperpigmentation (tyrosinase), hyperglycemia and diabetes (α-amylase and α-glucosidase), and obesity (lipase). The phytochemical profile of the plant was also determined to identify any possible correlation with the observed biological activities.

2. Materials and methods

2.1. Plant material

Plant materials (n=20) were collected from same population during field study (spring 2016) in Konya, Turkey (between Alaeddin Keykubat Campus and Yukselen village road, 1100 m). The taxonomic identification was performed by the botanist Dr. Murad Aydin Sanda and one voucher specimen was deposited at the herbarium of Selçuk University, Konya, Turkey.

2.2. Extraction procedures

To prepare ethyl acetate and methanol extracts, the plant samples (dried-powdered aerial parts (about 2 mm)) were stirred overnight (24 h) at room temperature (10 g in 200 mL solvent (EtOAc and MeOH)). After filtration, the extracts were concentrated using a rotary evaporator under vacuum at 40 °C. For water extract, infusion was prepared (10 g of plants were kept in 200 mL of boiled water for 20 min). After that, the infusion was filtered and dried by using a lyophilizator. The restudies were stored at +4 °C until further analysis.

2.3. Total metabolite contents determination

Concerning our previous studies, the total amount of phenolics (TPC) (by standard Folin-Ciocalteu method), flavonoids (TFC) (by $AlCl_3$ method) and total phenolic acid content (TPAC) (by Arnow assay) were determined. The final results were expressed as equivalents of standard compounds (gallic acid (mg GAE/g) for TPC; rutin (mg RE/g) for TFC and caffeic acid (mg CE/g extract) for TPAC), respectively). The experimental details were summarized in previous papers [12, 13].

2.4. Antioxidant capacity measurements

Different experiments spectrophotometrically screened antioxidant capacity of the *Parentucellia* extracts as phosphomolybdenum, quenching of radicals (DPPH and ABTS), reduction potentials (FRAP and CUPRAC), and ferrous ion chelating. The findings were expressed as standard compounds equivalents (mg TE/g and mg EDTAE/g). The procedures of assays were already summarized reported in our earlier work [12].

2.5. Key enzyme inhibitory effects

The *Parentucellia* extracts were tested as sources of enzyme inhibitors on some enzymes, including α-amylase, α-glucosidase, cholinesterases, tyrosinase and lipase. The procedures of these assays were already summarized in our earlier work [12]. The enzyme inhibitor effects were evaluated as equivalents of orlistat (for lipase), acarbose (for α-amylase and α-glucosidase), galantamine (for AChE and BChE), and kojic acid (for tyrosinase).

2.6. Chromatographic conditions

An Agilent HPLC system (Series 1100 with a G1315B diode array detector) was employed for chromatographic analysis. It was connected to an Esquire 6000, Bruker Daltonics ion trap mass spectrometer with an electrospray interface. A reversed phase Luna Omega Polar C_{18} analytical column (150 x 3.0 mm and 5 µm particle size (Phenomenex)) and a Polar C_{18} Security Guard cartridge (Phenomenex) of 4 x 3.0 mm were used for analysis. The

HPLC-DAD-MS parameters have been previously reported [14]. 10 µL of sample (5 mg/mL in MeOH) was filtered through 0.45 µm PTFE membrane filters (Análisis Vínicos, Madrid, Spain) and then injected.

2.7. Quantification of polyphenols

Calibration curves were prepared using the following standard reagents: ferulic acid, apigenin, kaempferol, loganin, luteolin, quercetin, rutin, and vicenin 2. Quantification was performed by UV signal, recording chromatograms at 320 and 350 nm for phenolic acids and flavonoids, respectively. When the exact analytical standard was available, it was used for the quantification. Otherwise, the corresponding analytical standard was used for the (semi)quantification of its derivatives (for instance, the aglycone for the corresponding glycosides).

2.8. Statistical analysis

All assays results were expressed as mean of three experiments with standard deviation (S.D). ANOVA assay (with Tukey's) were performed to evaluate the differences in the extracts. The statistical analysis were done by using SPSS v. 17.0 program.

3. Results and discussion

3.1. Phytochemicals content

Extraction of plant material is the first step in phytochemical studies and plays a crucial role. In the conventional method of extraction, several techniques have been employed such as hydrodistillation, soxhlet extraction and maceration. Among these, maceration is inexpensive and straightforward to extract phenolic components. This technique has been performed at room temperature and has a wide range of application including pharmaceutical and food areas. Secondly, the solvent selection is another critical step which frequently uses

most common solvents such as methanol, ethanol, ethyl acetate and water [15]. Taken together, we selected the maceration technique with three solvents (ethyl acetate, methanol, and water) to prepare *P. latifolia* subsp. *latifolia* extracts.

The total bioactive amounts of the extracts of *P. latifolia* were measured as TPC, TFC, and TPAC (Table 1). The highest TPC was observed in the aqueous extract (25.07 mg GAE/g) followed by the MeOH and EtOAc extracts (22.63 and 20.47 mg GAE/g, respectively). However, MeOH extract contained the highest level of TFC (44.15 mg RE/g) followed by the aqueous extract. With regards to TPAC, a similar pattern was observed and it was not detected in the EtOAc extract.

3.2. HPLC-ESI-MS^n

HPLC-ESI-MS^n (with the negative ion mode) was carried out to identify phytochemicals in analyzed extracts. The obtained chromatogram for the MeOH extract of *P. latifolia* is shown in Fig. 1 and the identified compounds could be seen in Table 2.

3.2.1. Iridoid and phenylethanoid glycosides

Compound **2**, with $[M-H]^-$ at m/z 375, exhibited MS^n fragment ions at m/z 213, 169, 151, and 125. This exact fragmentation pattern has been previously reported for the iridoid glycoside dihydrocominic acid [16].

Compound **6** was identified as loganin (formate adduct). Compound **5** and **7** presented the same fragmentation pattern, so they were characterized as loganin isomers. These iridoid glycosides were the most abundant compounds in the analyzed extracts.

Three phenylethanoid glycosides were characterized. On one hand, compound **22**, with [M-H]$^-$ at m/z 651, exhibited fragment ions at m/z 475, 457, and 329, in agreement with the fragmentation of martynoside [17]. On the other hand, compounds **10** and **13** presented the same fragmentation pattern as verbascoside [18] and were tentatively characterized as isomers.

3.2.2. Flavonoids

Compound **4** suffered the sequential neutral losses of 176 Da (glucuronide) and 308 Da (rutinoside) to yield quercetin at m/z 301 (the mass spectrum of quercetin was confirmed by comparison with an analytical standard). This fragmentation pattern is in agreement with quercetin-*O*-glucuronide-*O*-rutinoside. Compound **12** was identified as rutin by comparison with an analytical standard.

Compound **25** was identified as apigenin, and three related glycosides were also characterized (compounds **8**, **9**, and **18**). In all cases, apigenin was observed at m/z 269, and the attached sugars were glucuronide moieties (neutral losses of 176 Da).

Compound **11**, with [M-H]$^-$ at m/z 609, suffered two consecutive losses of hexoside moieties (162 Da), yielding the aglycone at m/z 285 (kaempferol). Hence, it was characterized as kaempferol-*O*-dihexoside.

Compound **23**, luteolin, presented a typical fragment ion at m/z 243 and compound **14**, with an additional 162 Da loss, was characterized as luteolin-*O*-hexoside.

Compound **15** presented the deprotonated molecular ion at m/z 491 and, after the neutral loss of 176 Da, yielded the aglycone isorhamnetin at m/z 315 (characteristic 315→300 fragmentation). Therefore, it was characterized as isorhamnetin-*O*-glucuronide.

Finally, compound **26** was tentatively characterized as a methylated flavonoid due to the loss of 15 Da (methyl group) observed. Compounds **17**, **19**, and **20** had additional rutinoside,

hexoside, and glucuronide moieties in their structures, which indicated that they were probably glycosides of compound **26**.

3.2.3. Other compounds

Compound **3** could not be fully identified, but it seems a saccharide derivative, as it presented fragment ions at m/z 179, 161, 143, 119, and 113, typical of hexosides [19].Compound **16** displayed ferulic acid at m/z 193 (fragment ions at m/z 149 and 134), so it was characterized as a derivative. Similarly, compound **21** was characterized as a coumaric acid (163→119 fragmentation) derivative. Finally, compound **24** was characterized as oxo-dihydroxy-octadecenoic acid [20]. The structures of the main compounds could be found in Fig. 2.

3.3. Quantification by HPLC-DAD

Calibration graphs for each analytical standard (section 2.8) were constructed using six concentrations (0.3-100 µg/mL in MeOH), plotting peak area versus concentration, obtaining $R \geq 0.997$ in all cases. Quantitation limits varied between 0.3 and 1.0 mg L^{-1}, and the calibration graph was linear up to 100 mg L^{-1} for all analytical standards. Both the repeatability (same day, n=3) and the intermediate precision (3 different days, n=9) were evaluated, obtaining R.S.D. lower than 8% in all cases. The robustness of the method was assayed considering possible variations in UV wavelength (±2 nm) and stability of samples and standard solutions (stored at -20ºC for at least 1 month), observing no significant variations in the analytical signal.

Total individual phenolic content (TIPC) (51±1 mg/g DE) was defined as the sum of all the phenolics that were quantified by liquid chromatography. The results are shown in Table 3. Iridoid glycosides accounted for approximately 66% of the TIPC, followed by flavonoids, which represented approximately 33% of TIPC. On the other hand, the amount of phenolic

acid derivatives was almost residual (1.3% of TIPC). Among the iridoid glycosides, only loganin (compound **6**) and its two isomers (compound **5** and **7**) were characterized. Loganin was the most abundant compound in the extract, accounting for 47% of TIPC (24 ± 1 mg/g DE). Loganin is a usual ingredient of many tablets or pills due to its bioactivity. Its concentration has been reported in different plants, such as different *Lonicera* species and *Corni Fructus* [21-23]. In all of them, the levels of loganin were lower than 10 mg/g, which makes *P. latifolia* a potential source of loganin for pharmaceutical applications.

The most abundant flavonoids were rutin and luteolin-*O*-hexoside (compounds **12** and **14**), which accounted for almost 80% of the amount of flavonoids. In general, loganin and its isomers, rutin, and luteolin-*O*-hexoside were the most abundant compounds: ~92% of TIPC. Hence, they may considered as the responsible components for the bioactivity found in the analyzed extract, although loganin is the most abundant compound of all of them.

3.4. Antioxidant activity

In an effort to reduce oxidative damage, there has been extensive research conducted on many plant extracts and secondary metabolites. Phenolic compounds can terminate the oxidation damages of free radicals in human body by donating hydrogen atom, followed by the stabilisation and delocalization of the unpaired electron within its aromatic ring [24].

In this work, the antioxidant capacity of *P. latifolia* was investigated in terms of its free radical scavenging, reducing power, phosphomolybdenum, and metal chelating properties. As it could be seen in Table 4, the aqueous extract showed the best scavenging activity (37.80 (in DPPH) and 69.92 mg TE/g in (ABTS) while the EtOAc extract displayed the lowest activity. Similarly, the aqueous extract exerted the highest effect (61.73 mg TE/g) while the EtOAc extract was the weakest effective (24.94 mg TE/g) in FRAP assay. The high scavenging and FRAP activity of the aqueous extract is most probably due to its highest TPC as observed in this study [25].

With regards to the reducing power against Cu (II) (CUPRAC assay), the MeOH extract (89.08 mg TE/g) exhibited the best effect followed by the water extract (87.62 mg TE/g). EtOAc extract depicted the least activity (57.59 mg TE/g). High TFC and TPC in the MeOH extract might be responsible for its strong reducing activity.

Interestingly, the EtOAc extract displayed the highest effect in phosphomolybdenum (1.82 mmol TE/g) and metal chelating assays (19.29 mg EDTAE/g) and the aqueous extract showed the least activity. On the other hand, the MeOH extract had the weakest metal chelating effect (12.17 mg EDTAE/g). It is important to highlight that the observed biological activity of EtOAc extract was not related to the level of total bioactive compounds since it contained the lowest TPC, TFC, and TPAC among the three solvent extracts. Therefore, other phytochemicals are responsible for these activities. In addition, different classes of phenolics and flavonoids have different biological effects and it is possible that specific compounds, responsible for the strong activity in the phosphomolybdenum and metal chelating assay, are present in a higher amount in the EtOAc extract. These compounds may also act in combination to produce a synergistic or additive effect to contribute to their overall observed bioactivity [26].

3.5. Enzyme inhibitory activity

Currently, enzyme inhibition has become an attractive approach in the treatment of various noncommunicable diseases. The enzyme inhibitory activity of *P. latifolia* extracts are summarized in **Table 5**. In the tested extracts, the EtOAc extract had the superior enzyme inhibition, displaying the highest anticholinesterase (1.88 and 1.81 mg GALAE/g against AChE and BChE, respectively), anti-amylase (0.70 mmol ACAE/g), anti-glucosidase (2.85 mmol ACAE/g), and lipase inhibitory activity (33.24 mg OE/g). In addition, the highest tyrosinase inhibition was exhibited by the MeOH extract (60.84 mg KAE/g extract). The high content of flavonoids in the MeOH extract could have contributed to its tyrosinase inhibitory

activity since a study by Wang et al. [27] found a positive correlation between TFC and tyrosinase inhibition. On the other hand, the weak enzyme inhibitory effects were displayed by the aqueous extract. In addition, no BChE and lipase inhibition was observed by the aqueous extract.

The highest cholinesterase activity exerted by the EtOAc of *P. latifolia* tends to justify its potential therapeutic use in the management of neurodegenerative diseases. Acetylcholine is hydrolyzed by AChE. So, its inhibition could increase acetylcholine level and improvement of memory and brain function. Late stages of Alzheimer's is related with increasing of BChE activity [28]. Also, the highest lipase inhibition displayed by the EtOAc extract indicates its potential use to replace the synthetic drug orlistat in the management of obesity. Indeed, orlistat has been linked to some important adverse gastrointestinal effects [29].

Additionally, the EtOAc extract also exhibited the most potent anti-diabetic activity. α-Glucosidase and α-amylase inhibitors have gained a wide application in the control of postprandial hyperglycemia which results in a decline of carbohydrate digestion to absorbable monosaccharides. α-Amylase is responsible for the hydrolysis of large polysaccharides to smaller carbohydrates, which are then digested to liberate glucose by intestinal α-glucosidase. In this way, inhibition of these enzymes will ultimately diminish the postprandial hyperglycemia. Currently, acarbose and miglitol are the leading glucosidase inhibitors. Nonetheless, they are often associated with serious adverse effects [30].

It is important to note that although the aqueous extract of *P. latifolia* showed the highest activity in most antioxidant assays, it displayed the weakest enzyme inhibitory effects.

4. Conclusion

This is the first report on the inhibitor (on key enzymes linked to global health problems) and antioxidant effects of *Parentucellia latifolia* subsp. *latifolia*. The extracts exhibited

significant free radical scavenging, reductive, chelating, as well as enzyme inhibitor effects. Metabolite profiling of the plant indicated that loganin and its isomers, rutin, and luteolin-*O*-hexoside were the most abundant compounds. The results suggest that *P. latifolia* can be a great potential as a source of bioactive compounds for possible uses as functional foods and natural pharmaceuticals. However, further works (*in vivo* or bioavailability, etc) on this species would help to us establish a greater degree of accuracy on this matter.

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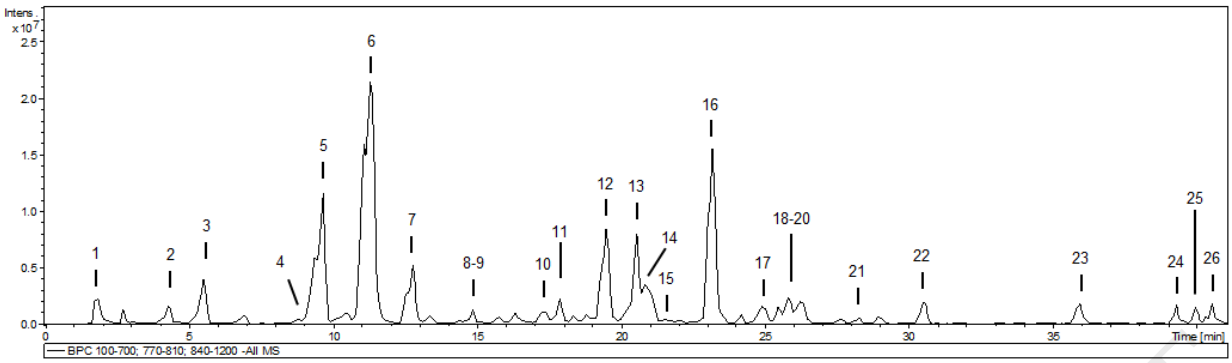

Fig. 1. HPLC-ESI-MS^n base peak chromatograms (BPC) of the methanolic extract of *P. latifolia*

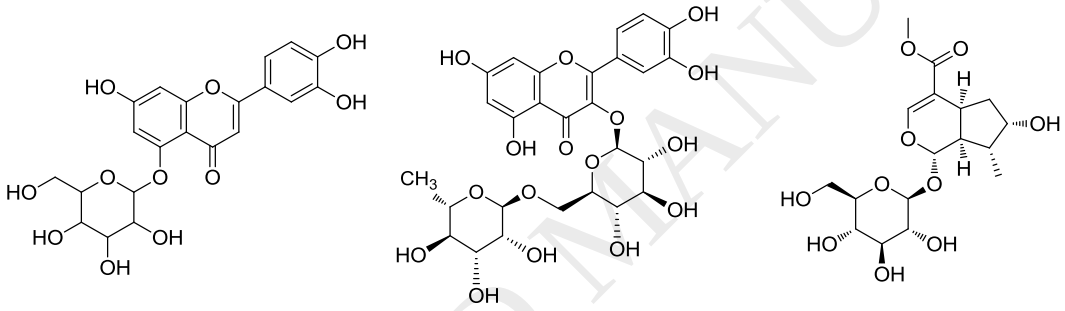

Fig.2. Major components identified in *P. latifolia* by LC-MS^n technique.

Table 1. Total bioactive compounds of the samples. [A]

Extracts	TPC (mg GAE/g extract)	TFC (mg RE/g extract)	TPAC (mg CE/g extract)
EtOAc	20.47±0.41[c]	7.14±0.13[c]	nd
MeOH	22.63±0.30[b]	44.15±0.22[a]	3.46±0.57[a]
Water	25.07±0.17[a]	34.22±0.22[b]	1.31±0.19[b]

[A] Values expressed as means ± S.D. of three parallel measurements. CE: caffeic acid equivalents; GAEs: Gallic acid equivalents; REs: Rutin equivalents; TFC: Total flavonoids content; TPAC: Total phenolic acids content; TPC: Total phenolic content. nd: non detected. Different letters refer statistically significant differences in the extracts (p<0.05).

Table 2. Characterization of the compounds found in the analyzed extracts of *Parentucellia latifolia*.

No.	t_R (min)	$[M-H]^-$ *m/z*	m/z (% base peak)	Assigned identification
1	1.9	495	MS^2 [495]: 477 (18), 379 (100) MS^3 [495→379]: 169 (100)	Unknown
2	4.3	375	MS^2 [375]: 213 (100), 169 (31), 125 (16) MS^3 [375→213]: 169 (100), 151 (18), 125 (47) MS^4 [375→213→169]: 151 (100), 125 (3)	Dihydrocominic acid
3	5.5	433	MS^2 [433]: 387 (59), 225 (74), 179 (100) MS^3 [433→179]: 161 (100), 143 (34), 119 (17), 113 (100)	Saccharide derivative
4	8.8	785	MS^2 [785]: 609 (100) MS^3 [785→609]: 301 (100) MS^4 [785→609→301]: 255 (44), 179 (100), 151 (10)	Quercetin-*O*-glucuronide-*O*-rutinoside
5	9.7	435	MS^2 [435]: 227 (100) MS^3 [435→227]: 101 (100)	Loganin isomer (formate adduct)
6	11.3	435	MS^2 [435]: 389 (100), 227 (70) MS^3 [435→389]: 227 (100) MS^4 [435→389→227]: 101 (100)	Loganin* (formate adduct)
7	12.8	435	MS^2 [435]: 389 (29), 227 (100) MS^3 [435→227]: 101 (100)	Loganin isomer (formate adduct)
8	14.9	643	MS^2 [643]: 467 (100) MS^3 [643→467]: 269 (100) MS^4 [643→467→269]: 225 (100)	Apigenin-*O*-glucuronide derivative
9	14.9	621	MS^2 [621]: 445 (100), 269 (47) MS^3 [621→445]: 269 (100)	Apigenin diglucuronide
10	17.5	623	MS^2 [623]: 461 (24) MS^3 [623→461]: 315 (100), 285 (48), 161 (11), 143 (27), 135 (71)	Verbascoside isomer
11	17.9	609	MS^2 [609]: 447 (90), 285 (100) MS^3 [609→447]: 285 (100) MS^4 [609→447→285]: 255 (82), 241 (100)	Kaempferol-*O*-dihexoside
12	19.5	609	MS^2 [609]: 301 (100) MS^3 [609→301]: 179 (100), 151 (63)	Rutin*
13	20.5	623	MS^2 [623]: 461 (100) MS^3 [623→461]: 315 (49), 161 (54), 143 (8), 135 (100)	Verbascoside isomer
14	20.8	447	MS^2 [447]: 285 (100) MS^3 [447→285]: 243 (100), 175 (67)	Luteolin-*O*-hexoside
15	21.5	491	MS^2 [491]: 315 (100) MS^3 [491→315]: 300 (100)	Isorhamnetin-*O*-glucuronide
16	23.2	399	MS^2 [399]: 311 (61), 285 (47), 193 (100) MS^3 [399→193]: 149 (47), 134 (100)	Ferulic acid derivative
17	24.9	607	MS^2 [607]: 299 (100), 284 (41)	Methyl-flavonoid-*O*-rutinoside
18	25.4	445	MS^2 [445]: 269 (100) MS^3 [445→269]: 225 (100), 151 (25)	Apigenin-*O*-glucuronide
19	25.8	461	MS^2 [461]: 299 (100) MS^3 [461→299]: 284 (100)	Methyl-flavonoid-*O*-hexoside

20	26.2	475	MS^2 [475]: 299 (100), 284 (8)	Methyl-flavonoid-*O*-glucuronide
21	28.2	783	MS^2 [783]: 619 (22), 483 (100) MS^3 [783→483]: 337 (46), 319 (79), 163 (100) MS^4 [783→483→163]: 119 (100)	Coumaric acid derivative
22	30.5	651	MS^2 [651]: 651 (100), 505(3), 475 (23), 457 (9), 193 (8) MS^3 [651→475]: 329 (100), 161 (57)	Martynoside
23	35.8	285	MS^2 [285]: 243 (84), 241 (100)	Luteolin*
24	39.2	327	MS^2 [327]: 291 (45), 229 (24), 211 (33), 171 (100)	Oxo-dihydroxy-octadecenoic acid
25	39.9	269	MS^2 [269]: 269 (100)	Apigenin*
26	40.5	299	MS^2 [299]: 284 (100)	Methyl-flavonoid

* Identified by comparison with analytical standards

Table 3. Quantification of phenolic compounds in *P. latifolia* (mg/g DE).

No.	Assigned identification	Concentration (mg/g DE)
Iridoid glycosides		
5	Loganin isomer	7.1 ± 0.5
6	Loganin	24 ± 1
7	Loganin isomer	3.5 ± 0.3
Total		34.6 ± 1
Flavonoids		
4	Quercetin-*O*-glucuronide-*O*-rutinoside	0.21 ± 0.02
8+9	Apigenin di-glucuronide + derivative	1.1 ± 0.1
11	Kaempferol-*O*-dihexoside	0.71 ± 0.05
12	Rutin	6.2 ± 0.1
14	Luteolin-*O*-hexoside	6.3 ± 0.1
18	Apigenin-*O*-glucuronide	0.40 ± 0.03
23	Luteolin	0.84 ± 0.05
25	Apigenin	0.39 ± 0.02
Total		15.8 ± 0.1
Phenolic acids		
16	Ferulic acid derivative	0.61 ± 0.08
21	Coumaric acid derivative	0.06 ± 0.01
Total		0.67 ± 0.01
TIPC*		51 ± 1

* Total individual phenolic content

Table 4 Antioxidant properties of the samples. [A]

Extracts	Phosphomolybdenum (mmol TE/g extract)	DPPH (mg TE/g extract)	ABTS (mg TE/g extract)	CUPRAC (mg TE/g extract)	FRAP (mg TE/g extract)	Metal chelating activity (mg EDTAE/g extract)
EtOAc	1.82±0.11[a]	2.50±0.71[c]	14.05±0.95[c]	57.59±0.57[b]	24.94±0.87[c]	19.29±0.19[a]
MeOH	1.45±0.12[b]	33.71±0.43[b]	47.43±0.82[b]	89.08±1.77[a]	50.61±1.11[b]	12.17±0.52[b]
Water	0.96±0.01[c]	37.80±0.84[a]	69.92±0.47[a]	87.62±2.25[a]	61.73±1.14[a]	18.35±0.05[a]

[A] Values expressed are means ± S.D. of three parallel measurements.
TE: Trolox equivalent; EDTAE: EDTA equivalent. Different letters refer statistically significant differences in the extracts (p<0.05).

Table 5. Enzyme inhibitory effects of the samples. [A]

Extracts	AChE inhibition (mg GALAE/g extract)	BChE inhibition (mg GALAE/g extract)	Tyrosinase inhibition (mg KAE/g extract)	Amylase inhibition (mmol ACAE/g extract)	Glucosidase (mmol ACAE/g extract)	Lipase (mg OE/g extract)
EtOAc	1.88±0.09[a]	1.81±0.09[a]	48.74±1.11[b]	0.70±0.03[a]	2.85±0.30[a]	33.24±0.71[a]
MeOH	1.30±0.11[b]	0.83±0.13[b]	60.84±0.85[a]	0.41±0.03[b]	1.08±0.08[b]	12.78±2.15[b]
Water	0.28±0.03[c]	na	15.54±0.93[c]	0.10±.0.01[c]	0.87±0.05[c]	na

[A] Values expressed are means ± S.D. of three parallel measurements. GALAE: Galantamine equivalent; KAE: Kojic acid equivalent; ACAE: Acarbose equivalent; OE: orlistat equivalent; na: not active. Different letters refer statistically significant differences in the extracts (p<0.05).