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SHORT COMMUNICATION

Comparative study of biological activities and multicomponent pattern of two wild Turkish species: *Asphodeline anatolica* and *Potentilla speciosa*

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Abstract

The multicomponent pattern and biological characterization of plant material are essential for pharmaceutical field, in the food supplements quality control procedures and to all plant-based products. These nutrients often show valuable effects related to their consumption due to the occurrence of secondary metabolites that show useful properties on health. In this framework, researches performed on this topic play a central role for human health and drug development process. The aim of this study was to compare phenolics and free anthraquinones multicomponent pattern of two wild Turkish species: *Asphodeline anatolica* and *Potentilla speciosa* using validated high-performance liquid chromatography–photogiode array (HPLC–PDA) assays, coupled to biological evaluation. Even if some variances related to biological and enzymatic inhibition activities can be ascribed to other phytochemicals, the reported data support traditional use of *Asphodeline anatolica* and *Potentilla speciosa* roots as valuable natural font for the development of novel natural-derived drug formulations and/or food supplements with health and nutritional benefits.

Keywords

Antioxidant, anthraquinones and phenolics pattern, *Asphodeline* and *Potentilla*, enzyme inhibitory effects, HPLC-PDA method

History

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Introduction

Several epidemiological, preclinical and clinical researches have highlighted the deep correlation between nutrition, natural active components related to the diet and healthiness. These findings improved the interest, especially from pharmaceutical and food industries, on new food supplements and/or natural plant-derived foods, which could be considered not merely as a nutrients source, but also as supplements helping conventional therapies against many diseases[1]. In this study, the availability of fundamental information on bioactive compounds pattern, biological activities, enzymatic inhibition evaluation, and valuable health benefits of plant-derived products related to their bioactive compounds content is reported. This could be achieved through a detailed characterization of their phytonutrients, multicomponent pattern (especially for quality control purposes) and biological and enzymatic activities.

Anthraquinones are well known for their biological activities on different targets and the analytical procedures able to their qualiquantitative analyses are recently reviewed[2] or described[3]. Furthermore, phenolic compounds have recently received particular attention owing to their possible preventive role in neurological disorders[4–7]. In particular, recently Karioti and Gidaro[8,9] reviewed that phenolics represent the main interesting biological activities on unconventional targets, such as carbonic anhydrases, among plant secondary metabolites.

Asphodeline is a genus of perennial plants in the family *Xanthorrhoeaceae* and for *Asphodeline anatolica E. Tuzlaci*, an endemic species widespread in Turkish regions, scientific studies are not reported on its biological activities and particularly on its multicomponent pattern, especially for phenolics. Additionally, many species of the *Asphodeline* genus have significant applications in Turkish traditional medicine as herbal remedy for inflammation and gastrointestinal disorders.

Potentilla is a genus of annual, biennial and perennial herbaceous flowering plants in the *Rosaceae* family. Similar to *Asphodeline*, also *Potentilla speciosa* Willd., native of the Balkan Peninsula, Turkey, Syria and northern Iraq, is used for the same reasons as natural therapy.

Due to the scarce information on these two endemic, relict and rare species, and to evaluate better their potential use as natural font for plant-derived drug formulations and food supplements with health and nutritional benefits, the reported results could be a useful material to create a scientific basis for ethnopharmacological use of *Asphodeline* and *Potentilla* species in the traditional medicine. Particularly for *Asphodeline anatolica* that represent a valid food supplement, as reported by Zengin and coworker[10] for its protein and essential amino acids content, and for *Potentilla*

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that represent one of the blending agent for highly saturated edible oils to provide oils with improved alimentary principles.

Using rugged and routinely validated HPLC-PDA procedure is also possible obtain quantitative information in order to standardize the extracts before further *in vivo* studies and for future food-supplements applications. In continuation to our studies[11–21], we report herein, for the first time, a comparative study on phenolics and free anthraquinones multicomponent pattern of these two wild Turkish species, coupled to a biological and enzymatic inhibitory activities evaluation in order to justify a possible correlation between considered phenolics and free anthraquinones on observed biological and enzymatic activities.

Materials and methods

Plant material

Asphodeline and *Potentilla* species were collected at flowering stage (May to July) in different Turkey regions. Taxonomical information's and localities were as follows:

(1) *A. anatolica E. Tuzlaci*: *Isparta*, between *Sarkikaraagac* and *Yenisarbademli*, 38° 03′ 07″ N, 31° 17′ 51″ E, 1140 m (endemic).
(2) *P. speciosa* Willd.: Niğde, Çamardı, Mazmili mountain, 37°39′ 55″N, 35°04′ 45″ E 1974 m.

Voucher specimens of each taxon were deposited in KNYA Herbarium at the Department of Biology, Selcuk University, Konya, Turkey. The roots of these plant materials were separated and stored in the dark before further analyses.

Preparation of the methanol extracts

Raw collected materials were air-dried at 45 °C (±1 °C) for 48 h in the dark. In this way, as also reported in literature[22,23], it is possible to obtain the higher phenolics preservation before solvent extraction.

Methanol extracts were obtained by maceration with 250 mL of organic solvent at room temperature (25 °C ± 1 °C) overnight from finely triturated (500 mesh) air-dried roots samples (5–10 g). The concentrated extracts (obtained at 40 °C using a rotary evaporator) were stored at +4 °C until further analyses and the extraction yields were 12.2% and 6.8%, respectively.

HPLC-PDA chemicals and reagents

Anthraquinones chemical standards (emodine, rhein, chrysophanol, aloe-emodine and physcione (all purity >99%)) were purchased from Extrasynthese (Genay, France). Phenolics chemical standards (gallic acid, catechin, chlorogenic acid, *p*-hydroxy-benzoic acid, vanillic acid, epicatechin, syringic acid, 3-hydroxy-benzoic acid, 3-hydroxy-4-methoxybenzaldehyde, *p*-coumaric acid, rutin, sinapinic acid, *t*-ferulic acid, naringin, 2,3-dimethoxy-benzoic acid, benzoic acid, *o*-coumaric acid, quercetin dihydrate, *t*-cinnamic acid, naringenin (all purity > 98%)) were purchased from Sigma Aldrich (Milan, Italy). Methanol (HPLC grade), acetonitrile (HPLC grade) and acetic acid (99%) were obtained from Carlo Erba Reagenti (Milan, Italy). Double-distilled water was obtained using a Millipore Milli-Q Plus water treatment system (Millipore Bedford Corp., Bedford, MA).

Determination of total bioactive components

Total phenolics and total flavonoids content

The total phenolics and total flavonoids contents were evaluated by method reported in literature[24] and expressed as gallic acid equivalents (GAEs/g extract) and rutin equivalents (REs/g extract), respectively.

Free anthraquinones and phenolics HPLC-PDA chemical fingerprint

HPLC-PDA anthraquinones multicomponent pattern was evaluated by method reported in literature[3,25].

HPLC-PDA phenolics multicomponent pattern was performed on a Waters liquid chromatograph equipped with a model 600 solvent pump and a 2996 photodiode array detector, and Empower v.2 Software (Waters Spa, Milford, MA) was used for the acquisition of data. A C18 reversed-phase packing column (Prodigy ODS(3), 4.6 × 150 mm, 5 µm; Phemomenex, Torrance, CA) was used for the separation and the column was thermostated at 30 ± 1 °C using a Jetstream2 Plus column oven. The UV/Vis acquisition wavelength was set in the range of 200–500 nm. The quantitative analyses were achieved at maximum wavelength for each compound (see supplementary material section S.1 for validation parameters).

The injection volume was 20 µL. The mobile phase was directly on line degassed by using Biotech DEGASi, mod. Compact (LabService, Anzola dell'Emilia, Italy). Gradient elution was performed using the mobile phase water-acetonitrile (93:7, *v:v*, 3% acetic acid) as reported in supplementary material section S.1. All the prepared sample solutions were centrifuged and the supernatant was injected into HPLC.

Biological activities determination

Phosphomolybdenum assay and radical-scavenging activity (DPPH and ABTS assays)

The total antioxidant and radical-scavenging activities were estimated by phosphomolybdenum, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS) methods according to the procedure described by Zengin et al.[26], respectively. The radical-scavenging activities were expressed as trolox equivalents (TEs/g extract).

Reducing power and metal chelating activity on ferrous ions

The reducing power and metal-chelating activities were estimated by cupric ion reducing (CUPRAC), ferric ion reducing antioxidant power (FRAP) and metal chelating on ferrous ions[27,28] methods. The reducing power was expressed as trolox equivalents (TEs/g extract), while the metal chelating activity was expressed as EDTA equivalents (EDTAEs/g extract).

Enzyme inhibitory activity determination

Cholinesterase (acetylcholinesterase (AChE) or butyrylcholinesterase (BChE)), α-amylase, α-glucosidase and tyrosinase inhibitory activities were estimated by the method described by Zengin et al.[29].

Statistical analysis

All the assays were carried out in triplicate. The results were expressed as mean values and standard deviations (mean ± SD). Statistical differences between the extracts were analyzed using Student's *t*-test and one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference *post hoc* test with $\alpha = 0.05$. All the analyses were carried out by using SPSS v14.0 software (Segrate (MI), Italy).

Results and discussion

Total phenolics, flavonoids, saponins and triterpenoids content

Total phenolics and flavonoids contents are presented in Table 1.

A. anatolica shows the lowest total phenolics content (24.21 mgGAEs/g extract) respect to *P. speciosa* (68.89 mgGAEs/g extract). Inverted findings are observed when total flavonoids content is considered. The lower total phenolics content herein reported for *A. anatolica*, respect to the value reported in literature by Zengin et al. (44.98 mgGAEs/g extract[26]) can be ascribed to the different extraction method (soxhlet and maceration) used. Values reported in Table 1 are equivalent to other *Asphodeline* and *Potentilla* species such as *A. lutea* (ranging from 17.26 mgGAEs/g to 22.45 mgGAEs/g, as reported by Lazarova et al.[30]).

Free anthraquinones and phenolics chemical fingerprint by HPLC-PDA

Total free-anthraquinones and phenolics multicomponent pattern are reported in Table 1 (for μg/mg of dried raw material). The most abundant and representative compounds are gallic acid (2.48 μg/mg) for *A. anatolica* and catechin for *A. anatolica* and *P. speciosa* (0.66 and 8.02 μg/mg, respectively), as reported in Table 2.

For *A. anatolica,* the most representative free anthraquinones are emodine (118.3 μg/mg) and rhein (68.9 μg/mg), while for *P. speciosa*-free anthraquinones, chemical fingerprint is very poor and only chrisophanol and physcione are quantified (1.4 and 1.2 μg/mg, respectively). Figure 1 reported the chromatographic separation for the 20 phenolics chemical standards, while in supplementary material section S.2 were reported the representative chromatograms at 278 nm for phenolics chemical fingerprint and at 435 nm for free anthraquinones.

These findings are also in agreement with the scarce data reported in literature for *Asphodeline* species[31–33] that shows the

Table 1. Extraction yields, total phenolics and flavonoids content for *Asphodeline anatolica* and *Potentilla speciosa*.

	Extraction Yield (%)	Total phenolics content* (mgGAEs/g extract)†	Total flavonoids Content* (mgREs/g extract)‡
A. anatolica	12.19	24.21 ± 0.67	11.39 ± 0.63
P. speciosa	6.8	68.89 ± 1.97	4.55 ± 0.05

*Values expressed are means ± SD of three parallel measurements.
†GAE: Gallic acid equivalents.
‡RE: Rutin equivalents.

Table 2. Free anthraquinones and phenolics multicomponent pattern of *Asphodeline anatolica* and *Potentilla speciosa* expressed as total amount (μg/mg extract).

	A. anatolica	*P. speciosa*
Free anthraquinones		
Aloe-emodine	15.1 ± 2.1	–
Rhein	68.9 ± 1.6	–
Emodine	118.3 ± 0.4	–
Chrysophanol	–	1.4 ± 0.1
Physcione	8.8 ± 2.6	1.2 ± 0.1
Phenolics		
Gallic acid	2.48 ± 0.04	–
Catechin	0.66 ± 0.01	8.02 ± 0.09
Chlorogenic acid	–	–
p-OH benzoic acid	–	–
Vanillic acid	–	0.18 ± 0.04
Epicatechin	0.17 ± 0.03	–
Syringic acid	–	0.17 ± 0.02
3-OH benzoic acid	–	–
3-OH-4-MeO benzaldehyde	0.10 ± 0.02	–
p-Coumaric acid	–	–
Rutin	–	–
Sinapinic acid	0.11 ± 0.01	–
t-Ferulic acid	–	–
Naringin	0.34 ± 0.06	–
2,3-diMeO benzoic acid	0.17 ± 0.02	–
Benzoic acid	–	–
o-Coumaric acid	–	–
Quercetin dihydrate	–	–
t-Cinnamic acid	–	–
Naringenin	–	–

*Values expressed are means ± SD of three parallel measurements.

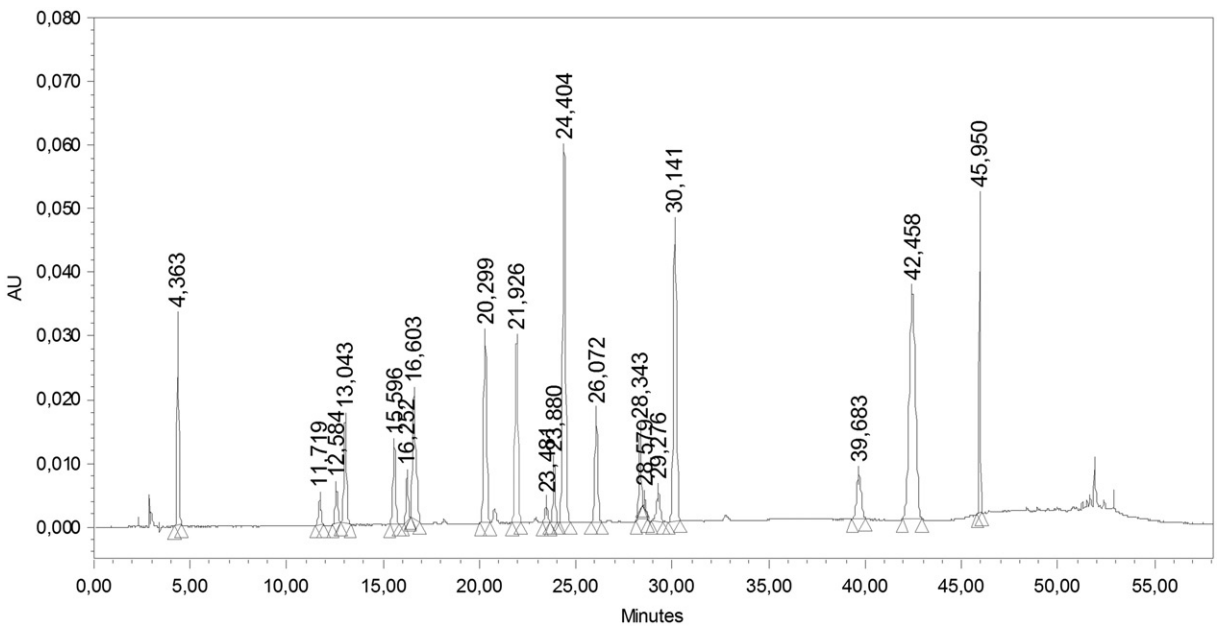

Figure 1. Chromatogram of phenolics standards (gallic acid, catechin, chlorogenic acid, *p*-hydroxy-benzoic acid, vanillic acid, epicatechin, syringic acid, 3-hydroxy-benzoic acid, 3-hydroxy-4-methoxybenzaldehyde, *p*-coumaric acid, rutin, sinapinic acid, *t*-ferulic acid, naringin, 2,3-dimethoxy-benzoic acid, benzoic acid, *o*-coumaric acid, quercetin dehydrate, *t*-cinnamic acid, naringenin; wavelength, 278 nm; flow-rate, 1 mL/min; injection volume, 20.0 μL; 5 μg/mL each).

Table 3. Free radical scavenging, reducing power, antioxidant and metal chelating activity of *Asphodeline anatolica* and *Potentilla speciosa*.

	Free radical-scavenging activity* (mgTEs/g extract)†		Reducing power* (mgTEs/g extract)†		Phosphomolybdenum*	Metal chelating activity* (mgEDTAEs/g extract)‡
Samples	DPPH	ABTS	FRAP	CUPRAC	(mmolTEs/g extract)	Ferrous ion chelating
A. anatolica	27.52 ± 1.08	137.60 ± 2.11	58.11 ± 1.25	72.61 ± 0.99	1.76 ± 0.15	15.09 ± 0.52
P. speciosa	257.65 ± 4.86	507.80 ± 20.44	133.35 ± 1.84	189.24 ± 3.86	2.23 ± 0.15	4.32 ± 0.90

*Values expressed are means ± SD of three parallel measurements.
†TE: Trolox equivalents.
‡EDTAE: EDTA equivalents.

Table 4. Enzyme inhibitory activity of *Asphodeline anatolica* and *Potentilla speciosa*.

	Cholinesterase inhibition*		Antidiabetic assay*		Skin care effects*
Samples	AChE inhibition (mg GALAEs/g extract)†	BChE inhibition (mg GALAEs/g extract)†	α-amylase inhibition (mmol ACEs/g extract)‡	α-glucosidase inhibition (mmol ACEs/g extract)‡	Tyrosinase inhibition (mg KAEs/g extract)¶
A. anatolica	1.73 ± 0.03	1.76 ± 0.07	0.50 ± 0.02	2.42 ± 0.08	22.27 ± 2.64
P. speciosa	3.18 ± 0.20	0.38 ± 0.01	1.72 ± 0.17	49.48 ± 0.75	124.35 ± 2.82

*Values expressed are means ± SD of three parallel measurements.
†GALAE: Galantamine equivalents.
‡ACAE: Acarbose equivalents.
¶KAE: Kojic acid equivalents.

presence of sesquiterpene lactones, flavonoids, anthraquinones and oxepine derivates.

No additional considerations can be addressed on multi-component pattern for *Potentilla speciosa* due to the presence in literature of papers reporting only the composition of essential oil[34,35]. In these studies, γ- and α-tocopherol are the major constituents, and fatty acids represent an important element. Other interesting review paper reports a summary of phytochemical and pharmacological profile[36]. The herein reported free anthraquinones and phenolics multicomponent pattern for the *Potentilla speciosa* are the first results reported in literature, but are in agreement with general composition for this genus.

Biological activities

Phosphomolybdenum assay, radical scavenging activity (DPPH and ABTS assays), reducing power (FRAP and CUPRAC assays) and chelating activity on ferrous ion

In the phosphomolybdenum assay, the *A. anatolica* extract showed the highest total antioxidant capacity, with 1.76 mmol TEs/g respect to *P. speciosa*.

DPPH and ABTS free radicals are used to evaluate the natural compounds or plant extracts radical scavenging activity. In the present study, *Potentilla speciosa* (257.65 mgTEs/g for DPPH and 507.80 mgTEs/g for ABTS) exhibited higher performance against these radicals as compared to *A. anatolica* (27.52 mgTEs/g for DPPH and 137.60 mgTEs/g for ABTS). The highest radical scavenging activities could be enlightened with the presence of higher phenols level in *P. speciosa*. These results are in good accordance with literature, which showed that there is a positive correlation between phenolics content and radical-scavenging activity[37,38]. From these results, phenolics and flavonoids could be considered ones of the main responsible for the hydrogen-donating properties of tested extracts.

Plant extract-reducing power is generally related to its electron transfer ability and could provide an important antioxidant activity marker. Ferric ion reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC) assays were employed to evaluate reducing abilities of tested extracts. Both reveal that *A. anatolica* had a lower potency respect to *P. speciosa.* The higher reducing power activities observed in *P. speciosa* could be related to higher phenolics contents. This finding shows also that flavonoids and anthraquinones, if present, could improve this ability, as reported by Zengin et al[28]. and by Yen et al[39].

Chelating ferrous ion (Fe^{2+}) was considered significant to characterize the *A. anatolica* and *P. speciosa* subsp. *speciosa* extracts ability to chelate ferrous ion. All the extracts confirmed the capacity to chelate ferrous ions (Table 3). Conversely to radical-scavenging and reducing power assays, potent chelation ability was detected in *A. anatolica* (15.09 mgEDTAEs/g) respect to *P. speciosa* (4.32 mgEDTAEs/g). In this direction, Yen et al.[39] ascribe the extracts chelating abilities also to anthraquinones (emodine, rhein and chrisophanol, etc.) and not only merely to phenolics.

Enzyme Inhibitory activities

Recently, key enzymes inhibition involved in human diseases is considered as one of the available therapeutic strategies in diseases treatment. Many synthetic inhibitors have been developed, but they could show some adverse effects such as gastrointestinal disturbances and liver damage[40,41]. For this reason, there is an increasing attention in plant-derived natural enzyme inhibitors in order to avoid negative effects. The enzyme inhibitor activities of the *P. speciosa* subsp. *speciosa* are reported in Table 4 for the first time.

The *A. anatolica* extract shows an intermediate activity with respect to other *Asphodeline* spp[28]. and all the plant extracts show an inhibitory activity against AChE and BChE. The differences observed between the two extracts could be explained by changes in the phytochemicals composition of the *A. anatolica* respect to *P. speciosa*.

Table 4 also shows the higher α-amylase, α-glucosidase and tyrosinase inhibitory activities of *P. speciosa* extract than *A. anatolica* extract, probably justified by the highest level of phenolics. These findings are in accordance with the observed strong relationship between enzyme inhibitory activities and phenolics in plant-derived extracts[42–44].

Conclusions

The herein reported novel HPLC-PDA method for phenolics multicomponent pattern is simple, accurate (precision and trueness) and selective for separation and quantification of 20 phenolics in the *Asphodeline* and *Potentilla* roots samples. The assay was also reasonably sensitive for the direct quantitative determination of these compounds in extracts of real matrices. Thanks to the herein reported HPLC-PDA methods for free anthraquinones and phenolics multicomponent pattern, and the classic spectrophotometric assays, are possible to better elucidate the pathway that occurring when plant-derived extracts were tested against different biological tests and it can provide a possible explanation of the observed enzyme inhibition activities, contributing to a global vision in these plant extracts characterization. The information on *A. anatolica* and *P. speciosa* subsp. *speciosa* is scarce and the current need for the availability of new plant-derived bioactive molecules is nowadays fundamental. Due to interesting biological activities showed by these plants and herein reported for the first time, *A. anatolica* and *P. speciosa* could be an interesting starting point for new pharmaceutical formulations development process.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article. The authors gratefully acknowledge the financial support given for this research from the Selcuk University, Science Faculty, Department of Biology and from University ''G. d'Annunzio'' of Chieti-Pescara.

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Supplementary material available online