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Multicomponent pattern and biological activities of seven *Asphodeline* taxa: potential sources of natural-functional ingredients for bioactive formulations

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ABSTRACT

The current study was carried out to evaluate multicomponent pattern, biological and enzymatic activities of seven *Asphodeline* taxa root extracts as useful ingredients, due to the fact that these plants are commonly used as traditional food supplements in Turkish regions. The extracts were characterized for free anthraquinones and phenolics to obtain a specific chemical fingerprint useful for quality control. These analyzes were coupled to biological and enzymatic activities in order to obtain comprehensive information of the natural product. Free anthraquinones and phenolics were determined using validated HPLC-PDA methods. Antioxidant properties were determined by different procedures including free radical scavenging, reducing power, phosphomolybdenum and metal chelating assays. Ames assay was performed to evaluate mutagenic/antimutagenic properties. Enzyme inhibitory activities were tested against cholinesterase, tyrosinase, α-amylase and α-glucosidase. From the herein reported results, *Asphodeline* could be valuable for the production of bioactive products or food supplements for cosmetic and pharmaceutical industries.

List of abbreviations: ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); AChE: acetylcholinesterase; ADD: *A. damascena* subsp. *damascena*; ADG: *A. damascena* subsp. *gigantea*; ADO: *A. damascena* subsp. *ovoidea*; ADR: *A. damascena* subsp. *rugosa*; APR: *A. prismatocarpa*; ATT: *A. tenuior* subsp. *tenuiflora* var. *tenuiflora*; ATU: *A. turcica*; BChE: butyrylcholinesterase; CUPRAC: cupric ion reducing; DPPH: 2,2-diphenyl-1-picrylhydrazyl; en: endemic; FDA: Food and Drug Administration; FRAP: ferric ion reducing antioxidant power; GAE: gallic acid equivalents; HPLC-PDA: high performance liquid chromatography – photodiode array detector; RE: rutin equivalents; TE: trolox equivalents.

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Introduction

Recently, in many industrial fields the easy availability of natural derivatives can be very helpful in order to produce food supplements and/or natural products (also in combination) that can be used to obtain beneficial effects on human health. Many plants, across history and cultures, have been used for medicinal purposes as alternative therapies based on plants in order to avoid drug adverse effects, and over the past years, many articles were reported. To avoid this last drawback and, particularly, to obtain alternative biologically active products, an increased interest in natural products (or natural-like products) was observed[1,2]. When natural products were used, particular attention must be also paid to standardization process. The methods used in plant material extraction can influence the chemical composition of the resulting extracts and potentially the biological activity[3,4]. For these reasons, the actual challenge is to fully understand and characterize botanical preparations as "*multicomponent pattern*" in the context of modern food and health system in which consumers are often uneducated about the use and effective applications of these supplements[5]. Additionally, the Food and Drug Administration (FDA) specifies certain labeling requirements for foods, supplements, and drugs, and the European Union requires that standardized herbal substances are reported as content of constituents with known therapeutic activity.

For these reasons it is necessary a multidisciplinary approach in order to obtain chemical profiles and biological activities and, particularly, to evaluate possible correlations between these two aspects[6–13]. As biologically active compounds, generally were considered phenolics, flavonoids, anthraquinones and other secondary plant metabolites (Figure 1) that had show interesting biological activities as potential "*markers*" to characterize the extract.

The genus *Asphodeline* is conventionally used both as medicinal plants in Turkey folk medicine and as vegetables for human nutrition. *A. damascena* subsp. *damascena* and *A. tenuior* subsp. *tenuiflora* var. *tenuiflora* are often used to alleviate verrucae and heal lesions. Additionally, *A. damascena* is also used as salad vegetables. Modern studies show that *Asphodeline* could provide alimentary value due to the presence of anthraquinones, essential amino acids, and polyphenols[14].

Emodine, physcione, rhein and chrisophanol were used as colorants in food, drugs, and cosmetics. Additionally, they attracted attention especially for their anti-microbial, anti-cancer, anti-

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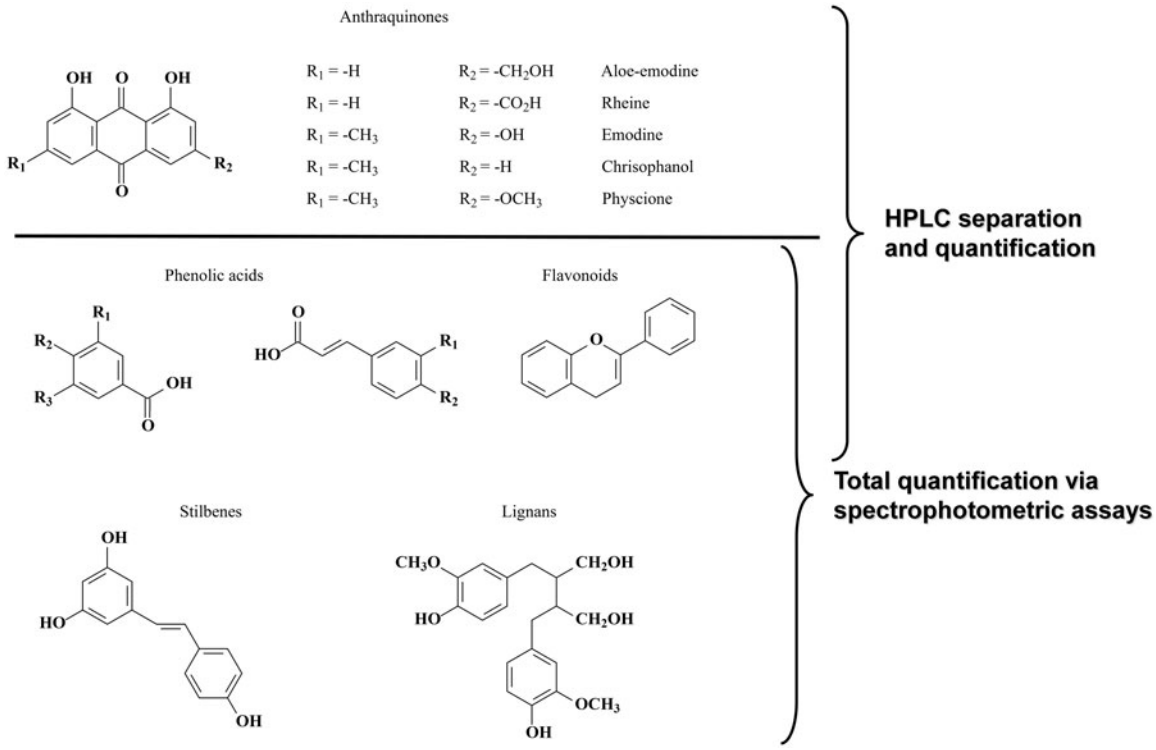

Figure 1. Biologically active compounds considered in this work.

oxidant, and anti-inflammatory activities and the intake of anthraquinones-rich plants[7,8] could be a valid preventive strategy in order to obtain health benefits.

In this paper, and in continuation to our studies on natural products analyzes[15–24] and instrument configurations[25–28], we report for the first time the free anthraquinones and phenolics pattern (*via* validated HPLC-PDA procedure), biological activities (ABTS, DPPH, CUPRAC, FRAP, phosphomolibdenum, metal chelating, and Ames assays), enzyme inhibitory activities (against cholinesterase, tyrosinase, α-amylase, and α-glucosidase). Particularly, for the first time was reported the mutagenic/anti-mutagenic evaluation of seven *Asphodeline* taxa, in particular with the aim to evaluate these extracts as valuable and safe sources for food supplements and/or for bioactive formulations.

Materials and methods

Plant material and methanol extracts

Asphodeline species were collected at flowering stage (May–July) in Turkey regions, and their information and localities are explained below. Voucher specimens were deposited in KONYA Herbarium (Department of Biology, Selcuk University, Konya, Turkey). The roots, air-dried at 45 °C (± 1 °C) for 48 h in the dark to obtain higher phenolics preservation[29,30], were finely triturated (5–10 g) and macerated overnight with 250 mL of methanol at room temperature (25 °C ± 1 °C). The concentrated extracts (under vacuum; 40 °C ± 1 °C) were stored at +4 °C (± 1 °C) in the dark until analyzes (extraction yields are reported in Table 1) **(en: Endemic).**

1. ***A. damascena* (Boiss.) Baker subsp. *damascena* (Boiss.) Baker:** Konya, Selcuk University, Alâeddin Keykubat Campus, Bağderesi location, 1170 m, 38° 02′ 51.6″ N, 32° 26′ 40″ E.
2. ***A. damascena* (Boiss.) Baker subsp. *gigantea* E. Tuzlaci**: Gaziantep, between Gaziantep and Narli road, Incesu village, 868 m, 37° 13′ 58″ N, 37° 18′ 06″ E. **(en)**
3. ***A. damascena* (Boiss.) Baker subsp. *ovoidea* E. Tuzlaci**: Kahramanmaras, between Kahramanmaras and Goksun road, 650 m, 37° 45′ 50″ N, 36° 43′ 52″ E. **(en)**
4. ***A. damascena* (Boiss.) Baker subsp. *rugosa* E. Tuzlaci**: Kayseri, between Yahyali and Sazak road, 1212 m, 38° 05′ 18″N, 35° 21′ 38″ E. **(en)**
5. ***A. prismatocarpa* J. Gay ex Baker**: Niğde, Çamardi-Mazmili Mountain, Dagdibi village, 1974 m, 37° 39′ 55″ N, 35°04′ 45″ E. **(en)**
6. ***A. tenuior* (Fischer) Ledeb. subsp. *tenuiflora* (C. Koch) E. Tuzlaci var. *tenuiflora* (Fischer) Ledeb.:** Malatya: between Malatya and Darende road, 1003 m, 38° 30′ 40′′ N, 37° 31′ 19′′ E.
7. ***A. turcica* Tuzlaci**: Antalya, Gebiz, Sanli Beli location, 1299 m 37° 20′ 01″ N, 31° 01′ 45″ E **(en)**

HPLC chemicals and reagents

Anthraquinones chemical standards (emodine, rhein, chrisophanol, aloe-emodine, and physcione; all purity ≥99%) were purchased from Extrasynthese (Genay, France). Phenolics as 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) and formic 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).

Table 1. Yields (%) and total phenolics content, total flavonoids content and radical (DPPH and ABTS) scavenging activities of seven *Asphodeline* extracts*.

Asphodeline species (abbreviation)	Yield (%)	Total phenolics content (mg GAEs/g extract)[a]	Total flavonoids content (mg REs/g extract)[b]	DPPH scavenging (mg TEs/g extract)[c]	ABTS scavenging (mg TEs/g extract)[c]
A. damascena subsp. *damascena* (ADD)	14.84	22.54 ± 0.80	11.67 ± 0.39	24.14 ± 1.37	72.01 ± 0.76
A. damascena subsp. *gigantea* (ADG)	7.57	34.03 ± 0.97	23.88 ± 0.39	32.52 ± 1.03	117.37 ± 1.16
A. damascena subsp. *ovoidea* (ADO)	7.57	31.34 ± 0.70	23.92 ± 0.60	32.01 ± 1.16	101.70 ± 0.29
A. prismatocarpa (APR)	10.24	27.12 ± 0.45	24.21 ± 0.51	29.86 ± 1.30	89.89 ± 0.72
A. damascena subsp. *rugosa* (ADR)	12.93	18.61 ± 0.31	11.87 ± 0.18	23.14 ± 0.98	63.41 ± 0.22
A. tenuior subsp. *tenuiflora* var. *tenuiflora* (ATT)	3.98	27.57 ± 0.91	27.69 ± 1.36	35.87 ± 0.93	103.11 ± 2.97
A. turcica (ATU)	12.06	26.39 ± 0.24	10.33 ± 0.26	26.66 ± 1.00	78.28 ± 1.50

*Values expressed are means ± SD of three parallel measurements. [a]GAEs: gallic acid equivalents; [b]REs: rutin equivalents; [c]TEs: trolox equivalents.

Determination of total bioactive components

Total phenolics and flavonoids

The total phenolics content was determined by a reported method[31] with slight modification and expressed as gallic acid equivalents (GAEs/g extract), while total flavonoids content was determined by a reported method[32] with slight modification and expressed as rutin equivalents (REs/g extract).

Free anthraquinones and phenolics HPLC-PDA pattern

HPLC-PDA free anthraquinones and phenolics pattern was evaluated by validated methods reported in literature[8,26,33].

Biological activities evaluation

The activities was evaluated by phosphomolybdenum method[34] and expressed as trolox equivalents (TEs/g extract). The reducing power measured using cupric ion reducing (CUPRAC) and ferric ion reducing antioxidant power (FRAP). Metal chelating activity on ferrous ions, determined by the method described by Zengin et al.[31], was expressed as EDTA equivalents (EDTAEs/g extract). Acetylcholinesterase (AChE) or butyrylcholinesterase (BChE), α-amylase, α-glucosidase and tyrosinase inhibitory activities were carried out by the method described by Zengin et al.[31]. Mutagenic and antimutagenic properties were determined by Ames assay[35].

Results and discussion

Total bioactive compounds

The total phenolic contents of the *Asphodeline* extracts were detected by Folin-Ciocalteu assay. Sample solution (0.25 mL) was mixed with diluted Folin-Ciocalteu reagent (1 mL, 1:9, v:v) and shaken. After 3 minutes, Na_2CO_3 solution (0.75 mL, 1%) was added, and the sample absorbance was read at 760 nm after 2 h of incubation at room temperature (25 °C ± 1 °C). The results are presented in Table 1. The highest phenolic content was found in ADG (34.03 mg GAEs/g extract), followed by ADO (31.34 mg GAEs/g extract) and ATT (27.57 mg GAEs/g extract). The lowest content detected in the ADR (18.61 mg GAEs/g extract).

Flavonoid contents were spectrophotometrically determined. The sample solution (1 mL) was mixed with aluminum trichloride (2%) in methanol. Similarly, blank sample was prepared by adding sample solution (1 mL) to methanol (1 mL) without $AlCl_3$. The sample and blank absorbances were read at 415 nm after 10 minutes of incubation at room temperature (25 °C ± 1 °C). The blank sample absorbance was subtracted from the sample, and the total flavonoids content was expressed as equivalents of rutin (REs). Flavonoid contents of the extracts ranged from 10.33 mg REs/g extracts for ATU to 27.69 mg REs/g extract for ATT (Table 1). From these results, flavonoids constitute a major part of phenolics in *Asphodeline* extracts tested. The total phenolics and flavonoids content were comparable to our previous report for eight *Asphodeline* root extracts and the content were found to be 13.5–49.2 mg GAEs/g extract and 10.6–30.9 mg REs/g extract, respectively[7]. Similar contents were reported for *A. lutea*[14] and *A. anatolica*[34].

Free anthraquinones and phenolics HPLC pattern

Using validated HPLC-PDA assay[26] the anthraquinones pattern was obtained for each *Asphodeline* sample considered in this study. The obtained results (Table 2) show a possible correlation between biological activity and chemical profile. Particularly, we evidenced that aloe-emodine, rheine and chrisophanol abundance was inversely proportional to α-amylase and α-glucosidase inhibitory activities. A higher chrisophanol content brought to an increased chelating power, and with minor exception, also with a higher tyrosinase inhibitory activity. Our obtained results also reveal that ATT extract shows lower phosphomolybdenum, chelating power, AChE, and BChE inhibitory activity, and simultaneously shows lower aloe-emodine, rheine and chrisophanol abundance. In the same way, ATT shows higher emodine and physcione abundances that reveals, coupled to higher total flavonoids, a better and increased DPPH, CUPRAC, α-amylase and α-glucosidase inhibitory activities. These findings were in accordance with just reported papers on other *Asphodeline* extracts[9].

Additionally from the extracts, we can highlight that chrisophanol and physcione are certainly the most abundant anthraquinones present in this taxon, as shown in Figure 2. Among phenolics (see ***Supplementary materials section S(0).1*** for the maximum wavelength, retention times and SST chromatograms), it can be observed that gallic acid, vanillic acid, and benzoic acid are the most representative compounds in these seven *Asphodeline* spp. Particularly, gallic acid was generally from 2 to 10-folds more concentrated than the others, as reported in Table 2.

In Figure 2 were reported chromatograms at 278 nm obtained from the phenolics chemical fingerprint (see ***Supplementary materials section S.2*** for the chromatograms at 278 nm and marked phenolics). At this wavelength, all considered compounds can be identified, even if each phenolic compound was quantified to its maximum wavelength, as previously reported in literature[33]. The correct phenolics and anthraquinones identification was carried out using co-elution procedure with references standards, while quantitative analyzes were obtained by external matrix-matched calibration based on HPLC-PDA validated method[26,33].

The obtained phenolics profiles could be a valuable starting point in order to justify the observed biological activities. High phenolics content, particularly gallic acid, vanillic acid, and benzoic acid, are responsible for well-known health benefits[9,14,36].

Biological activities

Antioxidant properties of *Asphodeline* extracts were evaluated using different assays including free radical scavenging (ABTS and DPPH), reducing power (CUPRAC and FRAP), phosphomolybdenum and ferrous ion chelating tests.

Table 2. Free anthraquinones and phenolics chemical fingerprint of the seven *Asphodeline* taxa expressed as total amount (μg/mg extract)*.

		ADD	ADG	ADO	APR	ADR	ATT	ATU
Free Anthraquinones	Aloe-emodine	24.5 ± 2.0	14.6 ± 1.2	4.3 ± 0.3	24.3 ± 1.9	9.4 ± 0.8	5.0 ± 0.4	15.3 ± 1.2
	Rhein	43.3 ± 3.5	71.4 ± 5.7	19.4 ± 1.6	55.9 ± 4.5	26.3 ± 2.1	25.5 ± 2.0	24.8 ± 2.0
	Emodine	3.9 ± 0.3	1.7 ± 0.1	5.5 ± 0.4	–	2.3 ± 0.2	25.6 ± 2.0	0.7 ± 0.1
	Chrisophanol	470.1 ± 37.6	196.1 ± 15.7	182.5 ± 14.6	794.8 ± 63.6	171.7 ± 13.7	249.1 ± 19.9	129.5 ± 10.4
	Physcione	47.3 ± 3.8	29.7 ± 2.4	82.5 ± 6.6	46.8 ± 3.7	100.7 ± 8.1	258.3 ± 20.7	15.0 ± 1.2
Phenolics	Gallic acid	3.03 ± 0.08	11.50 ± 1.57	6.77 ± 0.01	3.38 ± 1.02	9.34 ± 1.01	1.08 ± 0.34	1.26 ± 0.54
	Catechin	BLD	–	–	–	–	0.10 ± 0.01	–
	Chlorogenic acid	0.09 ± 0.01	0.08 ± 0.03	–	0.67 ± 0.14	0.26 ± 0.03	–	–
	p-OH-benzoic acid	BLD	0.08 ± 0.02	BLD	BLQ	0.10 ± 0.15	0.24 ± 0.10	–
	Vanillic acid	0.95 ± 0.16	1.05 ± 0.32	2.04 ± 0.98	1.15 ± 0.24	3.73 ± 0.84	0.66 ± 0.16	0.93 ± 0.21
	Epicatechin	BLD	–	BLD	BLQ	BLQ	–	0.30 ± 0.13
	Syringic acid	0.08 ± 0.01	0.12 ± 0.03	0.20 ± 0.04	0.82 ± 0.12	–	–	–
	3-OH benzoic acid	0.09 ± 0.02	0.18 ± 0.02	0.27 ± 0.09	0.37 ± 0.08	0.20 ± 0.03	–	0.36 ± 0.11
	3-OH-4-MeO-benzaldehyde	–	–	–	–	0.24 ± 0.04	0.06 ± 0.01	0.08 ± 0.01
	p-coumaric acid	–	–	–	BLQ	–	–	–
	Rutin	BLD	–	0.22 ± 0.10	BLQ	–	BLD	BLQ
	Sinapinic acid	0.09 ± 0.01	0.08 ± 0.01	0.12 ± 0.02	0.09 ± 0.01	–	0.08 ± 0.01	0.10 ± 0.02
	t-ferulic acid	–	–	–	–	BLD	–	–
	Naringin	0.15 ± 0.06	0.44 ± 0.12	0.18 ± 0.01	–	–	0.11 ± 0.05	0.48 ± 0.11
	2,3-diMeO-benzoic acid	–	–	BLD	–	1.01 ± 0.89	–	–
	Benzoic acid	0.52 ± 0.19	1.84 ± 0.98	1.44 ± 0.55	0.88 ± 0.31	BLD	–	0.89 ± 0.31
	o-coumaric acid	–	–	–	–	–	BLD	–
	Quercetin dihydrate	–	–	–	–	–	–	–
	t-cinnamic acid	–	–	–	–	–	–	–
	Naringenin	–	–	–	–	–	–	–

*Values expressed are means ± SD of three measurements; BLD: below Limit of Detection; BLQ: below Limit of Quantification.

The DPPH scavenging ability showed the highest value in ATT (35.87 mg TEs/g extract), followed by ADG (32.52 mg TEs/g extract), ADO (32.01 mg TEs/g extract) and APR (29.86 mg TEs/g extract). The weakest activity was observed in ADR with 23.14 mg TEs/g extract. Similarly to DPPH assay, the ABTS scavenging activity of *Asphodeline* extracts was in the following descending order: ADG > ATT > ADO > APR > ATU > ADD > ADR (Table 1). The radical scavenging activity of *Asphodeline* extracts showed a similar trend with both total phenolics and flavonoids content. Thus, the phenolics in the extracts could be responsible for the radical scavenging activities. These findings were consistent with several studies on *Asphodeline* species[9,14,32].

The reducing powers of *Asphodeline* extracts are illustrated in Table 3. ADG, ADO and ATT were more effective in both FRAP and CUPRAC assays compared to other *Asphodeline* extracts. In addition, ADR had the weakest activity in the assays. This is not surprising since the extract contained significantly lower phenolic content than other extracts. This proved that the phenolics have as effective and potent reductive abilities. Similar approaches were observed for *Asphodeline* and other plant extracts[36].

The total antioxidant capacities of *Asphodeline* extracts were evaluated by phosphomolybdenum method. In a descending order can be ranked as ADO (1.53 mmol TEs/g extract) > ATU (1.42 mmol TEs/g extract) > ADD (1.32 mmol TEs/g extract) > APR (1.32 mmol TEs/g extract) > ADG (1.30 mmol TEs/g extract) > ADR (1.28 mmol TEs/g extract) > ATT (1.17 mmol TEs/g extract) (Table 3), which contrast to total phenolic content. According to these results, the observed activity may be explained with the presence of non-phenolic reducing agents such as vitamin C and tocopherol. These findings were supported by several reports[37,38]. Transition metals (especially iron) play an important role as prooxidants of oxidation process including lipid peroxidation. In this sense, metal chelating ability is considered as one of antioxidant mechanism as it reduces the formation hydroxyl and hydroperoxide radical in lipid peroxidation.

Thus, the ferrous ion chelating abilities of the extracts were evaluated and the results are shown in Table 3. ADO possesses the best metal chelating ability with 22.79 mg EDTAEs/g extracts, followed by APR (22.38 mg EDTAEs/g extract), ADG (19.90 mg EDTAEs/g extract), ADD (19.90 mg EDTAEs/g extract) and ATU (19.71 mg EDTAEs/g extract). The order of the extracts was different from that observed for other antioxidant assays. For example, ATT had the highest DPPH scavenging activity among extracts but it exhibited the lowest metal chelating ability (8.19 mg EDTAEs/g extract). Moreover, the extract contained considerable amounts of phenolic compounds.

The observed differences may be explained with the presence of non-phenolic chelators and synergic or antagonistic actions of phytochemicals. Our approaches are in agreement with previous findings[39,40], in which negative correlation was observed between metal chelating and other antioxidant activities. In addition, our previous studies showed metal chelating abilities of several *Asphodeline* species[9,14,32].

Enzyme inhibition activities

The treatment and management strategies of several diseases are important subjects in scientific area. The inhibition of key enzymes is one of the most accepted pharmacological approaches[41–46]. Several synthetic inhibitors, developed for the treatment of several diseases, show side effects such as gastrointestinal disturbances[47–49]. The side effects of synthetic inhibitors have driven the search for novel and safe inhibitors, especially from natural sources.

The enzyme inhibitory activities of *Asphodeline* extracts were investigated by spectrophotometric methods against AChE, BChE, tyrosinase, α-amylase and α-glucosidase. The results are summarized in Table 4. In both AChE and BChE inhibitory assays, ADD, ADR, ADO and ATU had the strongest inhibitory activity. The weakest activity for both enzymes was found in ATT. The AChE and BChE inhibitory activities were found to be 0.41–2.09 and 0.36–1.30 mg GALAEs/g extracts, respectively. As regards their tyrosinase inhibitory activity, the following order could be extrapolated: APR > ADR > ATU > ADO > ATT > ADD > ADG. Apparently, the tyrosinase inhibitory activity of ADG (1.45 mg KAEs/g extract)

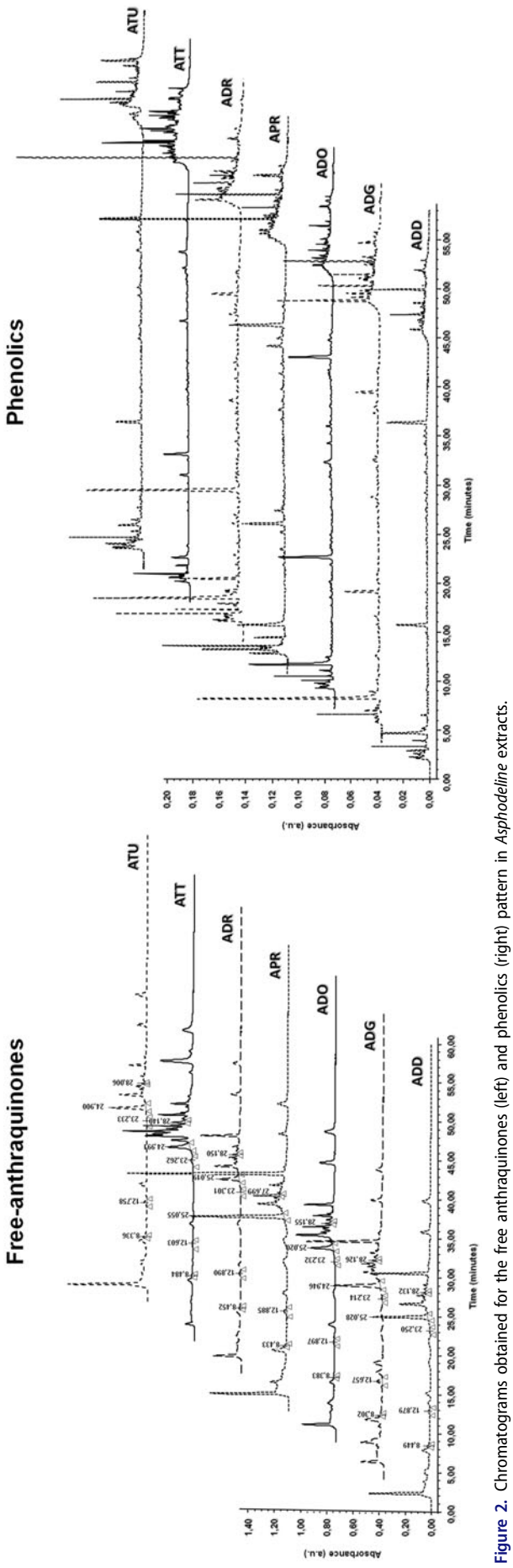

Figure 2. Chromatograms obtained for the free anthraquinones (left) and phenolics (right) pattern in *Asphodeline* extracts.

Table 3. Phosphomolybdenum assay, reducing power (by CUPRAC and FRAP assays) and metal chelating activities of seven *Asphodeline* extracts.*

Asphodeline species	Phosphomolybdenum (mmol TEs/g extract)[a]	CUPRAC (mg TEs/g extract)[a]	FRAP (mg TEs/g extract)[a]	Metal Chelating Activity (mg EDTAEs/g extract)[b]
ADD	1.32 ± 0.02	59.70 ± 1.15	45.39 ± 2.15	19.90 ± 0.82
ADG	1.30 ± 0.06	73.76 ± 0.70	73.66 ± 1.54	19.90 ± 0.35
ADO	1.53 ± 0.07	70.58 ± 1.27	65.56 ± 3.78	22.79 ± 0.60
APR	1.32 ± 0.07	71.86 ± 1.23	62.35 ± 1.03	22.38 ± 0.58
ADR	1.28 ± 0.06	57.07 ± 1.19	43.48 ± 2.40	12.91 ± 0.23
ATT	1.18 ± 0.02	79.29 ± 1.86	64.34 ± 1.71	8.19 ± 0.09
ATU	1.42 ± 0.01	57.87 ± 1.59	45.40 ± 0.49	19.71 ± 0.03

*Values expressed are means ± SD of three parallel measurements. [a]TEs: trolox equivalents; [b]EDTAEs: ethylenediamine tetraacetic acid equivalents.

Table 4. Enzyme inhibitory activities of seven *Asphodeline* extracts.*

Asphodeline species	Acetylcholine-sterase (mg GALAEs/g extract)[a]	Butyrylcholine-sterase (mg GALAEs/g extract)[a]	Tyrosinase (mg KAEs/g extract)[b]	α-amylase (mmol ACAEs/g extract)[c]	α-glucosidase (mmol ACAEs/g extract)[c]
ADD	1.61 ± 0.05	1.27 ± 0.06	10.79 ± 0.73	0.79 ± 0.03	4.12 ± 0.39
ADG	1.36 ± 0.10	0.41 ± 0.07	1.45 ± 0.44	0.75 ± 0.02	4.79 ± 1.18
ADO	1.98 ± 0.02	0.95 ± 0.02	16.98 ± 0.14	0.67 ± 0.02	10.62 ± 0.26
APR	1.64 ± 0.03	0.82 ± 0.02	23.70 ± 0.85	0.80 ± 0.02	10.50 ± 0.35
ADR	2.09 ± 0.05	0.95 ± 0.03	20.69 ± 0.62	0.63 ± 0.02	4.95 ± 0.11
ATT	0.41 ± 0.05	0.36 ± 0.02	14.78 ± 1.28	0.85 ± 0.03	23.70 ± 0.14
ATU	1.81 ± 0.02	1.30 ± 0.02	20.54 ± 0.68	0.67 ± 0.03	5.17 ± 0.08

*Values expressed are means ± SD of three parallel measurements. [a]GALAEs: galantamine equivalents; [b]KAEs: kojic acid equivalents; [c]ACEs: acarbose equivalents.

was about 16 folds lower than APR (23.70 mg KAEs/g extract). In our previous study, the activity was reported as 18.57–33 mg KAEs/g extracts[9]. As reported in Table 4, ATT was the most active on both α-amylase and α-glucosidase with 0.85 mmol ACAEs/g extract and 23.70 mmol ACAEs/g extract, respectively. APR was more effective on these enzymes compared to other extracts. Similarly to our results, these activities were found to be 0.34–1.24 mmol ACAEs/g extract for α-amylase and 1.10–4.99 mmol ACAEs/g extract for α-glucosidase in our first study[9]. Enzyme inhibitory activities were also reported for *A. lutea* in another previous study[50]. These activities were 2.04 mg GALAEs/g extract for AChE, 2.93 mg GALAEs/g extract for BChE, 0.268 mmol ACAEs/g extract for α-amylase, 0.839 mmol ACAEs/g extract for α-glucosidase and 10.31 mg KAEs/g extract for tyrosinase. The differences in the inhibitory activities of *Asphodeline* species may be due to the different phytochemical composition or the interactions among their components.

Mutagenic/anti-mutagenic evaluation

The results of the preliminary range finding tests for *Asphodeline* extracts gave no toxic effect to tester strain *S. typhimurium* TA98 and TA100 at doses of 5000, 2500, and 1000 μg/plate in the presence and absence of S9, respectively. Based on the results of the range finding test, the doses mentioned above were determined as the highest doses. As shown in Table 5, TA98 and TA100 strains did not increase in the number of revertant colonies compared to the negative control when the bacterial strain was treated with *Asphodeline* extracts at 5000, 2500, and 1000 μg/plate concentrations both with and without metabolic activation enzymes (S9). *Asphodeline* extracts were not found to be mutagenic for TA98 and TA100 strains. On the contrary, the positive control substances obviously increased revertant colonies in comparison with negative control. Hence, all extracts tested were found to be

Table 5. Mutagenic activity expressed as mean number of revertants/plate ± standard deviation of extracts of *Asphodeline* taxa towards *S. typhimurium* TA98 and TA100 strains with and without S9.

		Number of His$^+$ Revertants/plate			
		TA98		TA100	
Samples	Concentration (μg/plate)	S9 (−)	S9 (+)	S9 (−)	S9 (+)
*Negative Control	100 μl/plate	23 ± 3	35 ± 3	139 ± 9	135 ± 7
®Positive Control		637 ± 37	2866 ± 31	1835 ± 45	2632 ± 33
	0	32 ± 4	40 ± 4	156 ± 24	170 ± 11
ADG	5000	25 ± 5	32 ± 3	195 ± 1	159 ± 8
	2500	29 ± 1	39 ± 3	197 ± 16	157 ± 15
	1000	30 ± 1	45 ± 3	164 ± 17	133 ± 1
ATT	5000	42 ± 6	41 ± 7	179 ± 4	199 ± 8
	2500	32 ± 3	43 ± 2	168 ± 11	176 ± 6
	1000	37 ± 3	40 ± 2	181 ± 12	183 ± 8
ADD	5000	42 ± 5	45 ± 0	195 ± 11	167 ± 8
	2500	37 ± 4	51 ± 5	185 ± 9	146 ± 10
	1000	33 ± 2	37 ± 5	171 ± 7	150 ± 13
ADR	5000	30 ± 1	39 ± 3	168 ± 9	156 ± 13
	2500	28 ± 1	45 ± 7	160 ± 7	182 ± 6
	1000	27 ± 3	46 ± 2	169 ± 7	147 ± 4
ADO	5000	34 ± 0	32 ± 3	184 ± 4	198 ± 4
	2500	34 ± 7	39 ± 2	162 ± 3	193 ± 17
	1000	25 ± 3	35 ± 3	170 ± 10	173 ± 12
ATU	5000	29 ± 6	40 ± 2	196 ± 8	182 ± 4
	2500	34 ± 4	42 ± 6	185 ± 5	144 ± 13
	1000	35 ± 0	49 ± 7	155 ± 9	173 ± 2
APR	5000	27 ± 1	34 ± 5	182 ± 7	116 ± 18
	2500	37 ± 3	45 ± 2	185 ± 2	116 ± 18
	1000	28 ± 4	35 ± 1	188 ± 1	121 ± 11

*Negative control: DMSO (100 μl/plate) was used for *S. typhimurium* TA98 and TA100 both in the presence and absence of S9.
®Positive controls: 2-Aminofluorene (7.5 μg/plate) was used as positive indirect mutagen in the presence of S9 mix; 4-nitro-*o*-phenylenediamine (5 μg/plate) was used as positive direct mutagen in the absence of S9 mix for *S. typhimurium* TA98 strain; 2-aminoanthracene (5 μg/plate) was used as positive indirect mutagen in the presence of S9 mix; sodium azide (5 μg/plate) was used as positive direct mutagen in the absence of S9 mix for *S. typhimurium* TA100.

non-mutagenic at the highest doses on *S. typhimurium* TA98 and TA100 without metabolic activation in the Ames Assay.

The antimutagenic effect of each extract was assessed from the mean number of revertants/plate, the standard deviation (SD) and the percent inhibition (I%) of the mutagenic activity of 4-NPDA (4-nitro-*o*-phenylenediamine) and 2-AF (2-aminofluorene) for TA98 strain; SA (sodium azide) and 2-AA (2-aminoanthracene) for TA100 strain on treatment with the three concentrations of the plant extracts. Decreasing colony numbers and inhibition rates for TA98 and TA100 were displayed in Table 6.

According to the results obtained from assays, these extracts of *Asphodeline* spp. revealed antimutagenicity ratio ranging between moderate to strong activity against 4-NPDA at concentrations of 5000 μg/plate for TA98 strain in the absence of S9 mix. When combined with 4-NPDA, all test doses of the ADO extracts exhibited 43%, 41% and 44% inhibition, respectively, and can be considered strong antimutagenic for TA98 strain. With the addition of metabolic activation enzymes ADG, ATT, APR extracts manifested excellent inhibition ratios against 2-AF at all test doses.

ADG extract showed the highest antimutagenic activity with a ratio of 93% at a dose of 5000 μg/plate against positive mutagen for TA98 strain. ADD, ADO and ATU extracts revealed very strong antimutagenic activity at concentrations of 5000 and 2500 μg/plate (92/77%, 92/86% and 91/75%, respectively). Associated with SA treatment, extracts of APR, ATU, ADO, and ADG were described as strong antimutagenic (51%, 49%, 47%, and 41%, respectively) at a dose of 5000 μg/plate for TA100 in the absence of S9 mix. Also, 2500 and 1000 μg/plate doses of APR, ADO, ADG extracts induced the inhibition greater than 25%, reaching 38/31%, 37/33%, and 36/34% respectively, in the absence of S9 for TA100 and ranking them as moderately antimutagenic (Table 6). Although ATU extract exhibited strong antimutagenic activity with a rate of 46% at a dose of 2500 μg/plate, it revealed moderately antimutagenic action at a concentration of 1000 μg/plate against SA with a ratio of 33%

Table 6. Antimutagenicity and inhibition ratios of *Asphodeline* extracts towards *S. typhimurium* TA98 and TA100 strains with and without metabolic activation (S9) against direct and indirect mutagens.

		Number of His$^+$ Revertants/plate							
		TA 98				TA 100			
Samples	Concentration (μg/plate)	S9 (−)	%I	S9 (+)	%I	S9 (−)	%I	S9 (+)	%I
*Negative Control	100 μl/plate	36 ± 9		37 ± 3		162 ± 26		127 ± 14	
®Positive Control		504 ± 38	0	3622 ± 139	0	1428 ± 88	0	4430 ± 181	0
	0	27 ± 3		32 ± 5		160 ± 9		139 ± 10	
ADG	5000	216 ± 4	60	292 ± 10	93	909 ± 46	41	418 ± 4	93
	2500	381 ± 6	26	414 ± 16	89	975 ± 74	36	539 ± 2	91
	1000	409 ± 25	20	835 ± 39	78	1002 ± 82	34	1190 ± 46	76
ATT	5000	130 ± 7	78	361 ± 18	91	963 ± 23	37	447 ± 2	93
	2500	368 ± 29	29	623 ± 33	84	950 ± 65	38	523 ± 4	91
	1000	431 ± 41	15	1024 ± 52	72	1151 ± 56	22	713 ± 30	87
ADD	5000	358 ± 24	52	320 ± 24	92	1006 ± 86	33	454 ± 8	93
	2500	397 ± 3	22	846 ± 19	77	1250 ± 77	14	574 ± 33	90
	1000	480 ± 16	5	3147 ± 123	13	1317 ± 35	9	1250 ± 57	74
ADR	5000	339 ± 10	35	593 ± 23	84	1131 ± 162	23	461 ± 36	92
	2500	374 ± 13	27	3062 ± 45	16	1144 ± 60	22	2210 ± 112	52
	1000	381 ± 15	26	3136 ± 59	14	1260 ± 41	13	2639 ± 83	42
ADO	5000	299 ± 2	43	326 ± 24	92	828 ± 40	47	599 ± 44	89
	2500	315 ± 16	41	531 ± 8	86	953 ± 20	37	754 ± 26	86
	1000	294 ± 14	44	2379 ± 41	35	1010 ± 50	33	969 ± 16	81
ATU	5000	330 ± 8	37	369 ± 18	91	809 ± 37	49	480 ± 28	92
	2500	397 ± 1	23	918 ± 33	75	839 ± 29	46	700 ± 14	87
	1000	421 ± 16	18	2505 ± 77	31	1009 ± 46	33	1125 ± 106	77
APR	5000	370 ± 19	28	380 ± 13	90	777 ± 52	51	521 ± 14	91
	2500	401 ± 8	22	412 ± 21	89	945 ± 41	38	783 ± 28	85
	1000	455 ± 17	10	1321 ± 42	64	1033 ± 53	31	802 ± 48	85

%I: % Inhibition; *Negative control: DMSO (100 μl/plate) was used as negative control for *S. typhimurium* TA98 and TA100 both in the presence and absence of S9.
®Positive controls: 2-Aminofluorene (7.5 μg/plate) was used as positive indirect mutagen in the presence of S9 mix; 4-nitro-*o*-phenylenediamine (5 μg/plate) was used as positive direct mutagen in the absence of S9 mix for *S. typhimurium* TA98 strain; 2-Aminoanthracene (5 μg/plate) was used as positive indirect mutagen in the presence of S9 mix; Sodium azide (5 μg/plate) was used as positive direct mutagen in the absence of S9 mix for *S. typhimurium* TA100.

inhibition. In an interesting manner, all the extracts of *Asphodeline* taxa showed inhibition exceeding 42% and all the concentrations in the range of 5000–1000 μg/plate achieved inhibition ranging between 42 to 93%, making the extracts a very strong antimutagen in the presence of metabolic activation system for TA100 against 2-AA. Against 2-AA in the presence of S9 mix, 5000 μg/plate concentrations of ADG, ATT and ADD extracts showed more than 90% inhibition and the highest concentration attained 93% for all extract ranking them as strongly antimutagenic (Table 6).

Overall, it can be stated from the study that S9 metabolic enzyme system increased the inhibition rate, reaching 93% of mutagenic effects against known chemicals both for TA98 and TA100 strains. These results suggest that extracts of *Asphodeline* taxa, with high antimutagenic activity in the presence of S9, should be suitable for evaluation concerning CYP450 modulations effects[51]. As a result, it was determined that *Asphodeline* taxa, tested in this study, had significant antimutagenic capacities and they could be used in drug and food industries.

Conclusion

In summary, the present work investigated the free-anthraquinones and phenolics chemical fingerprint and biological activities (antioxidant, enzyme inhibitory and mutagenic/antimutagenic activity) of seven *Asphodeline* root extracts. To the best of our knowledge, these findings have not been reported before for these *Asphodeline* species with the aim to evaluate these extracts as valuable sources for food supplements and/or for plant-based bioactive formulations.

The analyzed species exhibited notable antioxidant, enzyme inhibitory and anti-mutagenic properties. All extracts did not show any mutagenic effect in Ames test, and are rich sources of phenolics, flavonoids and anthraquinones, which contributed to the observed biological activities. Consequently, the *Asphodeline* species could be considered as promising sources of natural-functional agents for bioactive formulations.

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Disclosure statement

Authors declare no conflict of interests.

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