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Phytochemical analysis by LC MS/MS and *in vitro* antioxidant activity of the Algerian endemic plant *Dianthus sylvestris* subsp. *aristidis* (Batt.) Greuter & Burdet

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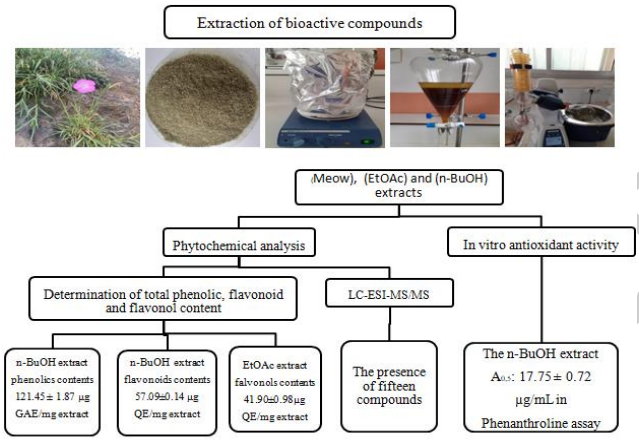
Graphical abstract



Abstract

The present work aimed to carry out the phytochemical analysis and antioxidant activity of hydro-methanolic, ethyl acetate and butanolic extracts of *Dianthus sylvestris* subsp, *aristidis* (Batt,) Greuter & Burdet leaves. Phenolics, flavonoids and flavonols contents were measured by the Folin-Ciocalteu reagent and the aluminum chloride ($AlCl_3$) method respectively. Phytochemical analysis was determined by using LC-ESI-MS/MS method. Antioxidant activity, *in vitro*, was evaluated by six different assays including: DPPH scavenging, ABTS cation radical, Ferrous ions chelating, Phenanthroline, Reducing power and Silver nanoparticle. The findings showed that the butanolic extract was found to be higher in phenolics and flavonoids contents (121.45 ± 1.87 µg GAE/mg extract and 57.09±0.14 µg QE/mg extract respectively). While ethyl acetate extract was found to be higher in falvonols contents (41.90±0.98µg QE/mg extract). The LC-ESI-MS/MS analysis of ethyl acetate and butanolic extracts revealed the presence of fifteen compounds among which: four phenolic acids, five flavonoids, one phenolic aldehyde and three vitamins, with P- coumaric acid, Hesperetin, Vanillin, Ascorbic Acid as major compounds respectively. The butanolic and ethyl acetate extracts exhibited the highest antioxidant activity in Phenanthroline assay ($A_{0,5}$: 17.75± 0.72 µg/mL and $A_{0.5}$: 21.02 ± 0.43 µg/mL respectively). On the basis of the significant findings, *D. sylvestris* subsp, *aristidis* would be recommended as a new potential source of natural antioxidant for pharmaceutical industries.

Keywords: Antioxidant activity, *Dianthus sylvestris* subsp. *aristidis* (Batt.) Greuter & Burdet, LC-ESI-MS/MS analysis

1. Introduction

Despite the fact that synthetic antioxidants are very effective, they can have side effects on our health over time (Liu and Mabury, 2020). Researchers are searching for natural and effective antioxidants based on the bioactive compounds of medicinal plants. The Caryophyllaceae family known as the pink family contains over 80 genera with more than 2600 species divided into 3 subfamilies, Caryophylloideae, Alsinoideae, and Paronychioideae (Jakimiuk *et al*., 2022). *Dianthus* L, is the second-largest genus in the Caryophyllaceae family represented by about 300 species with many taxonomically complex species groups (Fassou *et al*., 2022) mainly widespread in Africa, Eurasia, and North America (Mabberley, 2008). *Dianthus sylvestris* Wulfen is considered to be one of the most complex species. The flora of Algeria is characterized by the presence of the Caryophyllaceae family, the latter includes 25 endemic species and it is ranked second after the Asteraceae family (Quézel, 1964). The endemic flora of the state of Skikda represents more than 1/8 of the endemic flora of the northern Algeria with 407 taxa (Véla and Benhouhou, 2007). According to (Quézel and Santa, 1962), the genus *Dianthus* is represented in Algeria by 7 species distributed on the whole national territory, among which *Dianthus sylvestris* subsp, *aristidis* (Batt) Greuter et Burdet, an

Algerian endemic, rare and less studied plant (Sakhraoui *et al*., 2021). The prior studies revealed that several species of *Dianthus* are rich in phenolic and volatile compounds characterized by anticancer, antiviral, antibacterial, antifungal, insecticidal, repellent, antioxidant, reno-protective, anesthetic and analgesic effects (Al-Snafi, 2017). They were widely used in China, Korea, Iran, Turkey and Mongolia for millennia (Mutlu *et al*., 2016). *D. sylvestris* subsp, *aristidis* is one of the unknown subspecies regarding its phytochemical composition and biological effects. Therefore, the present study aims to determine the phytochemical analysis by using LC-ESI-MS/MS and to explore the in vitro antioxidant activitiy of hydromethanolic (MeOH), ethyl acetate (EtOAc) and butanolic (n-BuOH) extracts of the *D. sylvestris* subsp, aristidis subspecies harvested from the state of Skikda, using different assays.

2. Materials and methods

2.1. Plant material

The fresh leaves of *Dianthus sylvestris* subsp, *aristidis* were collected in November 2020 from the state of Skikda (North-East of Algeria: 36.881456, 6.927103), and identified by Pr, Sakhraoui Nora, Department of Sciences of Life and of Nature, University of 20 August 1955-Skikda. Afterwards, the leaves were cleaned and air-dried for two weeks. They were then grinded into fine powder with a coffee grinder. The samples were stored in jars made of glass protected from light.

2.2. Extraction of bioactive compounds

A quantity of 100 g of the plant leaves powder were macerated using 3 L of methanol–water (70:30 v/v) for three days; 1L per day through magnetic agitation in the dark at room temperature. After filtration through Whatman filter paper n°1, the filtrate was evaporated under pressure in a rotary evaporator at 50 °C to obtain the crude extract. A part of the said extract was diluted using 100 mL of distilled water and subjected to an extraction liquid–liquid in a separating funnel using different solvents of increasing polarity; Hexane (100mL×3), Ethyl acetate (100mL×3), and n-Butanol (100mL×3). The solvents were evaporated under pressure in a rotary evaporator at 50 °C. After evaporation, two fractions were obtained: ethyl acetate and n-butanol.

2.3. Determination of total phenolics, flavonoids and flavonols contents

2.3.1. Total phenolic content (TPC)

The total phenolic content of MeOH, EtOAc and n-BuOH extracts was determined using Folin-Ciocalteu reagent (Singleton and Rossi, 1965) and according to a microplate assay method described by (Müller *et al*., 2010). The protocol was based on mixing 20 µL of the extract solution (1mg/ml), 100 µL of Folin– Ciocalteu reagent and 75 µL of sodium carbonate (7.5%) one by one. The mixture was incubated at room temperature in the dark for 2 h. A blank was prepared in the same way by replacing the extract by the used solvent. The absorbance was read at 765 nm using a 96-well microplate reader (Perkin Elmer, Enspire). The findings were expressed as µg of gallic acid equivalents per mg of extract (µg GAE/mg) using the below calibration equation:

y=0.003x+0.104 with R^2 = 0.997.

2.3.2. Total flavonoid content (TFC)

The total flavonoid content of MeOH, EtOAc and n-BuOH extracts was determined using the microplate assay reported by (Topçu *et al*., 2007) with a slight modification. The method depends on forming a complex between flavonoids and Al^{3+}. A volume of 50 µL of the extract solution (1 mg/ml) was mixed with 130 µL of methanol, 10 µL of potassium acetate (1M) and 10 µL of aluminium nitrate (10%). The mixture was incubated for 40 min. A blank is prepared in the same way by replacing the extract by the used solvent. The absorbance was read at 415 nm using a 96-well microplate reader (Perkin Elmer, Enspire).The Findings were expressed as µg of quercetin equivalents per mg of extract (µg QE/mg) using the below calibration equation:

y=0.004X, with R2=0.997.

2.3.3. Total flavonol content (FLC)

The total Flavonol content of the MeOH, EtOAc and n-BuOH extracts was determined through the aluminum chloride ($AlCl_3$) method developed by (Kumaran and Karunakaran, 2007). 50 µL of extract solution (1 mg/ml) was mixed with 50 µL aluminum chloride 2% and 150 µL sodium acetate 5%. The mixture was incubated for 150 min at room temperature in the dark A blank is prepared in the same way by replacing the extract by the used solvent the absorbance was read at 440 nm using a 96-well microplate reader (Perkin Elmer, Enspire). The findings were expressed as µg of quercetin equivalents per mg of extract (µg QE/mg) using the bellow calibration equation:

y = 0.007X+0.022, (R^2 = 0.998).

2.4. Liquid chromatography-electrospray ionization-mass spectrometry analysis LC-ESI-MS/MS

The quantification of different phytochemical compounds in n-BuOH and EtOAc fractions of *D. sylvestris* subsp, *aristidis* leaves was achieved using an UPLC-ESI-MS-MS Shimadzu 8040 Ultra-High sensitivity with UFMS technology equipped with a binary bump Nexera XR LC-20AD. Separation was achieved with an Ultra-force C18 column (I,D, 150 mm × 4.6 mm, 3 µm particle size; Restek). The chromatographic separation was carried out using water, 0.1% formic acid as mobile phase A and methanol as mobile phase B. The following gradient elution program was applied: 80% A (0,1min to 1 min), 20%A (1 min to 30min), 0%A (30 min to 40min), 0%A (40 min to 45min) and 80%A (45 min to 60min). The flow rate was 0.3 mL/min, while the injection volume was 5 µL and the column temperature was fixed at 30°C, The ESI conditions employed in the LC-MS-MS are as follows: 230 KPs CID gas; − 6, 00 Kv conversion dynode; 350 °C interface temperature; 250 °C DL temperature; 3.00 L/min nebulizing gas flow, 400 °C heat block; and 15.00 L/min drying gas flow. The ion trap mass spectrometer was used

in both negative and positive ions in the MRM mode (multiple reaction monitoring).

2.5. In vitro antioxidant activity

2.5.1. DPPH free radical-scavenging assay

The free radical scavenging activity was determined according to (Blois, 1958). A volume of 40 µl of each extract (MeOH, EtOAc and n-BuOH) prepared in several concentrations was mixed with 160 µl of DPPH solution. The mixture was incubated for 30 min at room temperature in the dark and the absorbance was read at 517 nm using 96-well microplate reader. The results were compared to the standard antioxidants; butylated hydroxyanisole (BHA), butylated hydroxyltoluene (BHT), Trolox and Ascorbic acid. They were expressed as 50% inhibition concentration µg/mL (IC_{50}).

2.5.2. ABTS scavenging assay

The ABTS scavenging activity was determined according to the method developed by (Re *et al*., 1999). A volume of 40 µl of each extract (MeOH, EtOAc and n-BuOH) prepared in several concentrations was mixed with 160 µl of ABTS solution. The mixture was incubated for 10 min at room temperature in the dark and the absorbance was read at 734 nm using 96-well microplate reader. The results were compared to the standard antioxidants; BHA, BHT, Trolox, Ascorbic acid, and given as 50% inhibition concentration µg/mL (IC_{50}).

2.5.3. Ferrous ions chelating assay

The Ferrous ions chelating activity was measured by the method described by (Decker and Welch, 1990) with slight modifications. A volume of 40 µL of each extract prepared in several concentrations was mixed with 40 µL of methanol, 40 µL of Fe +2 (0.2 mM) and 80 µL of ferrozine (0.5 mM), The mixture was incubated for 10 min at room temperature in the dark and the absorbance was read at 593 nm using 96-well microplate reader. The findings were compared to the standard antioxidants; ethylenediamine tetra acetic acid (EDTA) and given as 50% inhibition concentration µg/mL (IC_{50}).

2.5.4. Ferric reducing antioxidant power assay (FRAP)

The reducing power activity was determined according to the method of (Oyaizu, 1986) with slight modifications adapted to microplate reader. A volume of 10 µL of each extract (MeOH, EtOAc and n-BuOH) prepared in several concentrations was mixed with 40 µL of phosphate buffer (0,2 M, pH 6,6) and 50 µl of potassium ferricyanide $K_3Fe(CN)_6$ (1%); the plate was incubated at 50 °C for 20 min, Then, 50 µL of trichloroacetic acid TCA (10 %), 40 µL of distilled water and 10 µL of ferric-chloride $FeCl_3$ (0,1 %) were added. The absorbance of the resulting mixture was read at 700 nm using 96-well microplate reader and the findings were compared to the antioxidant standards; BHA, BHT, Trolox, Ascorbic acid, and given as absorbance $A_{0,50}$ µg/ mL which represented the concentration producing 0,5 absorbance.

2.5.5. Silver Nanoparticle Assay (SNP)

The silver nanoparticle activity was determined according to the method described by (Özyürek *et al*., 2012). A volume of 20 µl of each extract prepared in several concentrations was mixed with 130 µL of SNP solution and 50 µl of distilled water. Reaction mixtures were incubated at 25°C for 30 min and read at 423 nm using 96-well microplate reader and the results were compared to the standard antioxidants; BHA, BHT, Trolox, Ascorbic acid, and given as absorbance $A_{0,50}$ µg/mL which represented the concentration producing 0.5 absorbance.

2.5.6. Phenanthroline assay

The phenanthroline activity was carried out in accordance with the method described by (Szydłowska-Czerniak *et al*., 2008). A volume of 10 µl of each extract (MeOH, EtOAc and n-BuOH) at different concentrations was mixed with 50 µl of ferric chloride $FeCl_3$ (0.2%), 30 µl of phenanthroline (0.5%) solution and 110 µl of methanol, The obtained mixtures were incubated at 30 °C for 20 min. The absorbance was measured at 510 nm using a 96-well microplate reader and the findings were compared to the antioxidant standards; BHA, BHT, Trolox, Ascorbic acid, given as absorbance $A_{0,50}$ µg/mL which represented the concentration producing 0.5 absorbance.

2.5.7. Statistical analysis

Findings were reported as mean value ±SD of three measurements and analyzed by one-way ANOVA variance to detect significant differences at $p < 0, 05$ using IBM SPSS Statistics, version 25 followed by Tukey's test.

3. Results and discussion

In many African communities, the use of herbal remedies as alternative or supplemental therapeutic approaches against various diseases is a common practice (Akinrinde *et al*., 2018). Several species within the Caryophyllaceae family are widely used by many ethnic communities as traditional medicines worldwide (Chandra and Rawat, 2015) among them the species *Dianthus caryophyllus* L. and *Dianthus chinensis* L. known for their anticancer, antiviral, antibacterial and antioxydant activities (Al-Snafi, 2017; Chandra and Rawat, 2015). In this research we tried to highlight the phytochemical content of an endemic subspecies of the North-East of Algeria, *Dianthus sylvestris* subsp, *aristidis* and to determine its anioxydant activity.

3.1. Total phenolics, flavonoids and flavonols content

The findings presented in Table 1 showed that Polyphenols content in n-BuOH extract (121.45±1.87µg GAE/mg extract) is higher than the ethanolic extract of *Dianthus thunbergii* roots (28,01±0,37 mg GAE/g) (Akinrinde *et al*., 2018) and the aqueous extract of *Dianthus carmelitarum* (16,67±0,40 mg GAE/g) (Aliyazicioglu *et al*., 2017). Similar results were found with EtOAc and n-BuOH extracts of *Dianthus superbus* L (105,2 mg GAE /g and 80 mg GAE /g respectively) (Kim *et al*., 2019). n-BuOH extract was also found to be the richest extract in flavonoids contents (57,09±0,14 µg QE/mg extract), which is almost similar to the ethanolic extract of *Dianthus Thunbergii* (62,21 mg QE/g) (Akinrinde *et al*., 2018), EtOAc (69,11 mg QE/g) and n-BuOH (62,50 mg QE / g extracts) of *Dianthus superbus L* (Kim *et al*., 2019).

The total flavonol content were significantly higher in EtOAc extract (41,90±0,98 µg QE/mg) than the other tested extracts and the ethanolic extract (13,06 mg QE/g) of *Dianthus Thunbergii* (Akinrinde *et al*; 2018).

Table 1. Total phenolic, flavonoid and flavonol content of various extracts of *D. sylvestris* subsp. *Aristidis*

Extracts	Total phenolics (µg GAE/mg)[1]	Flavonoids (µg QE/mg)[2]	Flavonols(µg QE/mg)[2]
MeOH extract	23.02 ± 0.17 [c]	21.18±0.14[c]	13.78±0.64 [c]
EtOAc extract	93.51±0.16[b]	49.58±0.15[b]	41.90±0.98[a]
n-BuOH extract	121.45± 1.87 [a]	57.09±0.14[a]	28.99±1.44[b]

Values were expressed as means ± SD of three parallel measurements. The values with different superscripts (a. b or c) in the same columns are significantly different (p < 0, 05)

[1]Total phenolics were expressed as µg Gallic acid equivalent /mg of extract (µg GAE/mg)

[2]Flavonoids and Flavonols content were expressed as µg quercetin equivalent /mg of extract (µg QE/mg)

Table 2. Phenolic profile determined by LC-MS-MS of EtOAc and n-BuOH fractions of *D. sylvestris* subsp. *aristidis*

Compound	Rt (min)	Molécular Weight	Precursor ion	Product ion	Charge (+/-)	Voltage CE (v)	Max intensity		Area %	
							EtOAc	n-BuOH	EtOAc	n-BuOH
Naringenin	48.10	272.25	273.10	147.15	+	-25	4827	4531	0.49	0.49
Maleic Acid	47.90	116.07	117.10	85.20	+	-10	39153	54800	4.00	5.97
p-Coumaric Acid	1.42	164.15	165.10	101.20	+	-10	429312	434367	43.88	47.35
Keampferol	26.25	286.24	287.10	225.25	+	-8	13488	14305	1.38	1.56
Quercetin	48.07	302.23	303.10	85.05	+	-41	3738	3556	0.38	0.39
Beta-Carotene	42.62	536.87	537.20	23.10	+	-49	5325	7377	0.54	0.80
Butylated Hydroxytoluene	17.90	220.33	221.00	203.25	+	-8	8794	9052	0.90	0.92
Ascorbic Acid	48.15	176.12	174.90	131.10	-	16	127519	45043	13.03	4.91
Chlorogenic Acid	20.40	354.31	355.00	73.15	+	-34	6893	6879	0.70	0.75
Gallic acid	18.30	170.12	168.80	125.10	-	16	346	325	0.04	0.04
Hesperetin	37.60	302.28	300.90	255.25	-	10	40541	54296	4.14	5.92
Folic acid	38.40	441.14	442.90	323.45	+	-26	50015	49748	5.12	5.42
Vanillin	48.20	152.15	153.10	71.15	+	-22	30490	27698	3.15	3.02
4-Hydroxy Coumarin Acid	47.90	162.14	160.80	117.10	-	22	1444	1444	0.15	0.16
Benzoic acid	47.65	122.12	123.10	91.20	+	-12	216559	203997	22.13	22.24

RT: retention time

3.2. Liquid chromatography-electrospray ionization-mass spectrometry analysis

To the best of our knowledge, this is first time that an LC-MS/ MS has been used to analyze the major and secondary metabolites of the *Dianthus sylvestris* subsp, *aristidis* leaves. The LC-ESI/MS analysis of EtOAc and n-BuOH fractions of the plant leaves revealed the presence of fifteen compounds (Table 2) among which four phenolic acids, five flavonoids, one phenolic aldehyde and three vitamins were detected and represented by p-Coumaric acid, Hesperetin, Vanillin ,and Ascorbic Acid as major compounds respectively. Our findings are quite close to those reported by Aliyazicioglu *et al*. (2017) where p-Coumaric acid, Chlorogenic acid, Gallic acid, Benzoic acid and Vanillin were isolated from the aqueous extract of *Dianthus carmelitarum* using the RP-HPLC analysis. On the other hand, Ding *et al*. (2013) isolated benzoic acid, kaempferol, quercetrin, and trans-p-coumaric acid from the EtOAc fraction of the ethanolic extract of *Dianthus superbus* using Sephadex LH-20 chromatographic method. There was not a fully overlapping between our chemical composition findings and the literature data of *Dianthus* genus (Yusupova *et al*., 2022; Liu *et al*., 2022). This may have arisen from the plant species as well as the number and type of the used standards. We believe that the chemical composition of *D. sylvestris* subsp*, aristidis* could be more revealed with further standard compounds in the future investigations.

3.3. In vitro antioxidant activity

This is the first research exploring the antioxidant properties of *D. sylvestris* subsp, *aristidis.* In our study, the antioxidant activity of different extracts was determined by using six different assays including: DPPH scavenging, ABTS cation radical, Ferrous ions chelating, Phenanthroline, Reducing power and Silver nanoparticle. Knowing that the difference between these assays is in the mode of action and the reaction mechanism; two mechanisms are available for the action of the antioxidants: the hydrogen-atom transfer (HAT) and the single-electron transfer (SET) (Srief *et al*., 2023). The findings of the antioxidant activities are shown in Table 3, expressed in terms of IC_{50} and $A_{0,5}$ and compared to antioxidant standards (BHT, BHA, Trolox, Ascorbic acid, and EDTA). The DPPH scavenging method shows that the EtOAc and n-BuOH extracts exhibited a weak antioxidant activity (IC_{50}: 290.3 ± 2.9 µg/mL and IC_{50}: 291.4± 0.2

µg/mL, respectively), while the MeOH extract did not give any antioxydant activity at 800 µg/mL. The data analysis of the ABTS assay shows that the EtOAc and n-BuOH extracts presented a good antioxydant activity (IC_{50}: 69.2±0.5 µg/mL and IC_{50} and 89.5 ±0.0 µg/mL, respectively) compared with the standards. Furthermore, the MeOH extract exhibited a weak activity (479.2±0.6 µg/mL). The results of DPPH scavenging and ABTS assays of *Dianthus superbus L* MeOH, EtOAc and n-BuOH extracts recorded by Kim *et al*. (2019) (IC_{50}: 54,3 µg/mL, 47,1 µg/mL and 42,4 µg/mL) and (IC_{50}: 5,2 µg/mL, 4,99 µg/mL and 5,02 µg/mL) respectively, were found to be higher than the values reported in the present work

For the Ferrous ions chelating assay all the extracts showed no antioxydant activity compared to the ethanolic and the hydro ethanolic extracts of *Dianthus basuticus* (IC_{50}: 60,65±1,09 mg/mL and 6,95±1,44 mg/mL respectively) (Kazeem and Ashafa; 2015). On the other hand, the results of Phenanthroline assay of the n-BuOH extract presented the highest antioxidant activity ($A_{0,5}$: 17.7±0.7 µg/mL) not far compared with ascorbic acid activity ($A_{0,5}$: 8.3±0.7 µg/mL) followed by EtOAc extract ($A_{0,5}$: 21.0 ±0.4µg/mL); the MeOH extract however exhibited a moderate activity ($A_{0,5}$: 113.0±0.8 µg/mL). Moreover, the results of reducing power assay showed that the EtOAc extract exhibited a moderate activity ($A_{0.5}$: 117.9±1.6 µg/mL) followed by n-BuOH extract ($A_{0,5}$: 167,9 ±1.5 µg/mL) which is more than the BHT standard (no activity at >200µg/mL) and lower than the other standards; the MeOH extract however, had zero activity. The obtained findings of reducing power assay are however better than the aqueous extract of *Dianthus carmelitarum* $A_{0,5}$: 238 ± 2,89µM TE / g (Aliyazicioglu *et al*., 2017). Regarding Silver nanoparticle assay, a moderate antioxidant activity was found with the EtOAc extract ($A_{0.5}$: 195.40±1.71 µg/mL) which is more potent than BHT, ascorbic acid (no activity at> 200 µg/mL) and the other extracts; MeOH and n-BuOH (no activity at < 400 µg/mL).

The evaluation of the antioxidant activity showed that the EtOAc and n-BuOH extracts were the most active (Table 3) with no significant difference (p > 0. 05) in DPPH activity and with significant difference (p > 0.05) in ABTS, Phenotroline and FRAP activities. This may be due to the number and the type of bioactive molecules well-known for their antioxidant and biological activities such as: p-coumaric acid (4-hydroxy-cinnamic acid) (Shen *et al*., 2019), chlorogenic acid (Wang *et al*., 2022), kaempferol (Jakimiuk *et al*., 2022), quercetin, naringenin (Nishimura *et al*., 2013), and ascorbic acid (Gęgotek and Skrzydlewska, 2022) detected and identified by the LC-ESI-MS/MS analysis as previously indicated in Table 2 or their synergy effect, in addition to the sensitivity and the mode of action of the used reagents.

The variation in total phenolic, flavonoid, flavonol content and the antioxidant activity findings between species of the same *Dianthus* genus might be related to various factors including the genetic potential of the species, the geographic location, the environmental conditions, the harvest season, the post-harvesting, the extraction methods and the used solvent polarity (Zahnit *et al*., 2022).

Table3. Antioxydant activity of various extracts of *D. sylvestris* subsp. *aristidis*

Extracts	DPPH assay IC_{50}(µg/mL)	ABTS assay IC_{50}(µg/mL)	Ferrous ions chelating assay IC_{50}(µg/mL)	Phenanthroline assay $A_{0.5}$(µg/mL)	Reducing power assay $A_{0.5}$(µg/mL)	SNP assay $A_{0.5}$(µg/mL)
MeOH extract	>800	479.20 ± 0.69^{a}	NA	113.04±0.8^{a}	NA	>400
EtOAc extract	290.39±2.90^{a}	69.26±0.56^{c}	NA	21.02±0.4^{b}	117.97±1.65^{b}	195.43±1.73^{a}
n-BuOH extract	291.45±0.22^{a}	89.59±0.08^{b}	NA	17.75±0.7^{c}	167.92±1.59^{a}	>400
BHA	6.14±0.41^{c}	1.81±0.10^{e}	NT	0.93±0.0^{g}	9.29± 0.22^{c}	73.4 7±0.88^{b}
BHT	12.99±0.41^{b}	1.29±0.30^{e}	NT	2.24±0.1^{f}	>200	<200
Trolox	5.12±0.21^{c}	3.21±0.06^{d}	NT	5.21±0.2^{e}	5.25±0.20^{d}	34.17±1.23^{c}
Ascorbic acid	4.39±0.01^{d}	3.04±0.05^{d}	NT	8.30.±0.7^{d}	3.62±0.29^{d}	>200
EDTA	NT	NT	8.88±0.47	NT	NT	NT

IC50 and A0.5 represent the means ± S.D of three parallel measurements, calculated by linear regression analysis. The values with different superscripts (a.b.c.d.f.or g) in the same columns are significantly different (p < 0, 05)
BHA: butylated hydroxyanisole. BHT: butylated hydroxyltoluene. EDTA: ethylenediamine tetraacetic acid
NT: not tested. NA: not absorbance

4. Conclusion

Our findings add new information to the literature data of the Caryophyllaceae family. To the best of our knowledge, this is the first publication about photochemical profile of the subspecies *Dianthus sylvestris* subsp*, aristidis* by using LC-ESI-MS/MS technique which proved the presence of bioactive compounds with interesting pharmacological activities. Therefore, *Dianthus sylvestris* subsp, *aristidis* could be identified as a potential plant exhibiting antioxidant properties.

Conflict of Interest

The authors declare no conflicts of interest.

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