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Research Article

Phytochemical screening and *in vitro* biological activity of *Amaranthus viridis* growing in Northern Cyprus

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Abstract: In this study, phenolic and non-phenolic metabolites in different parts of *A. viridis* (from the Turkish Republic of Northern Cyprus) were characterized with reversed-phase high-performance liquid chromatography (RP-HPLC) with diode array detector and high-performance liquid chromatography (HPLC), respectively. In total, approximately twenty-five phenolic compounds including quercetin, chrysin, t-Ferulic acid, and sinapic acid as the most abundant secondary metabolites were identified. On the other hand, four organic acids as non-phenolic compounds quantitatively predominant were identified for the first time in *A. viridis* extracts. The seed and flower extract showed strong ferric-reducing capacity, radical scavenging activity for DPPH˙, phosphomolybdenum assay, metal chelating, and α-amylase inhibition activity by in vitro assays. Our results suggest that *A. viridis* widely used in the human diet in Cyprus is a source of numerous metabolites showing antioxidant and antibacterial potential.

1. INTRODUCTION

Leafy vegetables have been essential in completing both human and animal diets. Vegetables that can be eaten are living examples of the body's nutritious components. They are declared to possess bioactive substances that are physiologically active toward pathogens that lead to diseases (Iqbal *et al.*, 2012; Ahmed *et al.*, 2013; Sunday *et al.*, 2021).

The vegetable *Amaranthus viridis*, often known as "Lindo otu" in Turkish Republic of Northern Cyprus and a member of the Amaranthaceae family, is used for medicinal purposes. It is spread over the world's warmer regions. This plant is thought to have originated in Asia; however, it is occasionally referred to as a global weed that can be found in temperate, tropical, and subtropical regions (Asia, Africa, America, Australia, and Europe) of the world (Khan *et al.*, 2011; Iqbal *et al.*, 2012; Ahmed *et al.*, 2013; Pulipati *et al.*, 2014; Reyad-ul-Ferdous *et al.*, 2015; Sarker & Oba, 2019; Sunday *et al.*, 2021). The plant is a rising, short-lived perennial or

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annual herb that can reach heights of 1 m. It has alternating leaves with petioles that can reach a size of 10 cm. The hairless leaves of *A. viridis* have a dark green top surface and a light green bottom surface. Below 1 mm long, the subglobose fruits are small. The wrinkled, indehiscent, or erratically ruptured sepals are mature. The seeds are 1 mm long, glossy, and dark brown to black. The stem is cylindrical, light green, and has an unpleasant smell. It grows up and is herbaceous. The root's shape is cylindrical, has a strong smell, and is whitish. It has horizontal downward growth and rootlets. Stem and root feature fibrous fracture and a smooth texture (Khan *et al.,* 2011; Pulipati *et al.,* 2014; Reyad-ul-Ferdous *et al.,* 2015; Sunday *et al.,* 2021). It is gathered in the summer season. It is grown on fallow land, roadsides, and farming fields in Bangladesh (Sarker & Oba, 2019), but it grows healthier and more abundant in fertilized and irrigated agricultural land in Northern Cyprus. Due to its flavor, it is a highly very popular green leafy vegetable. Local communities harvest it and use its stem, leaf, flower, and seed for their diet. Broad bean - Lindo, cowpea - Lindo mixture can be boiled or fried with egg and consumed as a meal.

Both the leaves, the seeds, and the stems are very nourishing. Minerals such as phosphorus, iron, calcium, magnesium, zinc, copper, manganese, vitamin A, vitamin C (ascorbic acid), vitamin B_2 (riboflavin), vitamin B_1 (thiamin), fiber content, protein, and amino acids such as cystine, arginine, isoleucine, methionine, tryptophan, histidine, leucine, phenylalanine, tyrosine, lysine, valine, and threonine are among the nutrients found in the leaves. Protein and lipids are present in the seeds. Hence, *A. viridis* attracted a lot of attention due to its excellent nutritional content (Pulipati *et al.,* 2014; Sarker & Oba, 2019).

In addition to these, it is a superb and exceptional source of antioxidant phytochemicals like flavonoids, phenolics, β-carotene, and antioxidant leaf pigments like betalain, β-xanthin, and β-cyanin, as well as a source of other pigments like chlorophylls, anthocyanin, and carotenoids. It was crucial for the food industry because the majority of these chemicals are natural antioxidants that detoxify reactive oxygen species (ROS) in the human body. Significant free radical-scavenging activity is possessed by the betalain, β-xanthin, β-cyanin, amaranthine, and carotenoid pigments. It is very adaptable to many abiotic conditions, including salinity and drought (Iqbal *et al.,* 2012; Ahmed *et al.,* 2013; Torres *et al.,* 2018; Sarker & Oba, 2019; Chen *et al.,* 2022).

Products obtained from *A. viridis* are known to have crucial pharmacological activities. It is believed that the leaves are useful for both cleaning and decreasing tissue swelling. *A. viridis* has antipyretic and analgesic qualities. Therefore, in conventional medicine, it is used to relieve fever and pain. Additionally, this plant has been used to treat ulcers, intestinal bleeding, diarrhea, dysentery, and excessive menstrual flow. The tea prepared from its leaves is used to treat these issues. The plant's seeds have been used to alleviate gastrointestinal issues and decrease labour pain. The plant's leaves are used to treat eczema, burns, wounds, boils, psoriasis, and rashes. The leaves, flowers, and fruits of *A. viridis* perform as a laxative, antileprotic, and anti-inflammatory urinary tract agent, diuretic, antiemetic, antirheumatic, appetite enhancer, respiratory and asthma treatment, ophthalmiatrics, as well as a vermifuge in venereal disorders. They also help to lower cholesterol and excessive blood sugar levels. Moreover, *A. viridis* is utilized for its antidiabetic, antioxidant, hepatoprotective, anti-nociceptive, anti-phytopathogenic, and anthelmintic properties. Other medical uses include those for diaphoretic, galactagogues, gonorrhea, earaches, hemorrhoids, bronchitis, and snake venom antidotes. This plant also has ribosome-inactivating protein, ß-carotene, and antiviral capabilities in addition to antiproliferative and antifungal qualities. These purported antipyretic and analgesic effects, however, are not sufficiently supported by scientific research (Ahmed *et al.,* 2013; Pulipati *et al.,* 2014; Reyad-ul-Ferdous *et al.,* 2015; Olarewaju *et al.,* 2018; Datta *et al.,* 2019; Sarker & Oba 2019; Sunday *et al.,* 2021; Naeem *et al.,* 2022; Zaware *et al.,* 2022).

Since there haven't been any studies on *A. viridis* in Türkiye or Northern Cyprus this study is special and valuable. Hence, the goal of this study is to explore the *A. viridis* methanolic extract's antioxidant, antibacterial, and antidiabetic characteristics.

2. MATERIAL and METHODS

2.1. Gathering and Preparing Plant Material

A. viridis stems, leaves, seeds, and flowers were gathered in July 2022 from Northern Cyprus' Kyrenia region (35°20´35´´N 33°17´09´´E 23 m). The plant was identified via Pl@ntNet. The overall wet weight of the samples was 640 grams. They were then gently split into small pieces and dried for six hours in a 50°C oven. The samples weighed 160 grams in total dry weight. For the analysis, the powdered dried samples were kept in a refrigerator at +4 °C.

2.2. Extraction of Samples

For 1 hour at room temperature, 80% of methanol (1:10 [w/v]) was used to extract the different parts of *A. viridis* on a magnetic stirrer. After that, the Whatman No. 4 paper was used to filter the extract. For biological and chemical investigations, the extracts were stored in a refrigerator at +4 °C.

2.3. Analyses of Antioxidant Activity

2.3.1. *DPPH˙ (1,1-diphenyl-2-picrylhydrazyl) radical scavenging capacity*

Blois (1958), Ucan Turkmen & Mercimek Takci, (2018) stated that the free radical scavenger method relies on the ability of samples to transfer electrons or protons to render the DPPH reagent colorless. 100 µL of the extract was dissolved in 3.9 mL of DPPH˙ reagent (0.025 g/L in methanol) generated in methanol (0.1 mM). To enable the chemical reaction, this mixture was placed in the dark at room temperature for 30 minutes. The mixture's absorbance was measured spectrophotometrically at 517 nm after incubation (Biochrom, Libra S60, B, England). In terms of Trolox equivalent (mg TE/g), DPPH˙ scavenging activity was measured against a methanol blank.

2.3.2. *FRAP (Ferric reducing antioxidant power)*

The ability of antioxidant compounds in the extract to reduce Fe^{3+} to Fe^{2+} was evaluated in this analysis (Oyaizu, 1986). When $FeCl_3$ was added to the reaction mixture, the Prussian blue color that resulted was tested for absorbance. The extract (1 mL) was mixed with 2.5 mL of 1% potassium $K_3Fe(CN)_6$ and 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6). For 20 minutes, this mixture was incubated at 50 °C. 10% trichloroacetic acid (TCA) was added to stop the activity, and the mixture was centrifuged for 10 minutes at 2500 rpm. To 2.5 mL of supernatant, an equal volume of distilled water and 0.5 mL of $FeCl_3$ (0.1%) were added. At 700 nm, the mixture's absorbance was recorded (Biochrom, Libra S60, B, England). The extract's reducing capacity was defined in Trolox equivalents (µg TE/g).

2.3.3. *Performance of metal chelating activity (MCA)*

Dinis *et al.*, (1994) devised a technique to appraise the extract's Fe^{2+} chelating potential. This method's base is the conflict between the extract's metal-binding molecules and ferrozine (a potent iron-chelating agent). Compounds that have a high metal ion binding capacity hinder the red ferrozine/Fe^{2+} complex formation. 100 µL of 2 mM $FeCl_2$, 3.7 mL of distilled water, and 1 mL of extract were combined. 200 µL of 5 mM ferrozine was added to the reaction after it had been incubated at room temperature for 30 minutes. The mixture was then agitated for 10 minutes. The absorbance of the mixture was obtained at 562 nm (Biochrom, Libra S60, B, England). Chelating activity as a percent of inhibition (%) was determined using the formula below.

$$\% \text{ chelating activity} = (1-(A_{sample}/A_{control})) \times 100$$

2.3.4. *Phosphomolybdenum technique*

The phosphomolybdenum technique was used to determine the total antioxidant capacity (TAC) (Zengin *et al.*, 2014). 3 mL of a reactive solution containing 4 mM ammonium molybdate, 28 mM sodium phosphate, and 0.6 M sulfuric acid were promptly added to 300 µL of extract. After 90 minutes of incubation at 95 °C, the absorbance was measured at 695 nm (Biochrom, Libra S60, B, England). The total antioxidant capacity was referred to using Trolox equivalents (µg/TE g). At least three times each analysis was performed.

2.4. Total Phenolic (TPC) and Total Flavonoid Content (TFC)

The Folin-Ciocalteu colorimetric procedure was applied to determine the total phenolic content (TPC). Total phenolic content was expressed as gallic acid equivalents (mg GAE)/g (Stanković, 2011). By using the Sharma and Vig method, the total flavonoid content was calculated. Total flavonoid content was expressed as mg of routine equivalent (mg RE)/g (Sharma & Vig, 2013).

2.5. Antibacterial Activity

The extract's antibacterial activity was assessed on Mueller Hinton Agar (MHA) using the approved technique, which adhered to the Kirby-Bauer disc diffusion method, according to Clinical Laboratory Standard Institute (CLSI) recommendations. (CLSI, 2012; Owusu *et al.*, 2021). Turbidity was set to the McFarland standard reference range of 0.5 for the overnight bacterial cultures. 10 µL of each microbial suspension was added to MHA with a pipette and then uniformly applied to the surface with a wooden cotton applicator stick. Placed apart from one another were the sterile blank discs that had been impregnated with 20 µL of the extract. After inoculation, Petri plates were incubated at 37°C for 12 to 24 hours. The inhibition zones surrounding the discs were subsequently measured. Methicillin (M; 5 µg/disc) for *Staphylococcus aureus* ATCC 25923, and Erythromycin (E; 15 µg/disc) for *Salmonella typhimurium* ATCC 14028 and *Escherichia coli* ATCC 25922 were the positive controls. As a negative control, methanol was utilized.

2.6. Assay of α-Amylase Inhibition

The mixture consisted of 1 mL of extract, 1 mL of 20 mM sodium phosphate buffer (pH: 6.9), and 1 mL of 1% w/v starch solution, which was incubated at 37°C for 5 min. The reaction was then started by adding the α-amylase solution (1 mL) to this mixture. After incubation for 30 min., the reaction was terminated with 1 mL of color reagent (96 mM 3,5-dinitrosalicylic acid solution, 2 M NaOH, and 5.31 M sodium potassium tartrate solution). The absorbance of this mixture boiled for 5 minutes, at 540 nm was measured (Biochrom, Libra S60, B, England) (Başyiğit *et al., 2*020). All analyses were repeated three times.

2.7. Phenolic Content

Phenolic components were extracted by adding 100 mL of methanol to approximately 25 g of plant and mixing with extraction solvent (methanol/chloroform/water; 7:2:1) in magnetic stirrers at room temperature. This process was repeated three times. The Whatman filter paper was used to filter the obtained extracts and the filtrates were collected. Then, the solvent was removed at 60 °C in a Buchi, R300 model evaporator. The residues formed at the bottom of the volumetric flask were dissolved in methanol (Zhang *et al.*, 2018).

The reversed-phase high-performance liquid chromatography analysis (Agilent, 1260 Infinity RP-HPLC, USA) was performed to determine the chromatograms of each standard phenolic chemical compound in the extract. The separation of phenolic components was performed on a C18 reverse phase (110 Å, 5 µm, 4.6 x 250 mm, ACE Generix) HPLC column. In the separation process, 10 µl injection volume, mobile phase A (0.1% phosphoric acid-water solution) and B (100% acetonitrile) gradient system, oven temperature 30ºC, and DAD (diode array detector) detector were used (Gupta *et al.*, 2012). The data analysis was done using Agilent Lab Advisor software the phenolic compounds were defined by comparing them with

the standards of each identified compound using retention time. The data obtained are expressed as (mg/kg) dry weight. The analyzes were repeated three times.

2.8. Organic Acid Content

The extraction of organic acids was carried out by the method described by Gallardo-Guerrero *et al.*, (2010). Approximately 1 g of the samples was homogenized in 25 mL deionized water/methanol (7/3, v/v) using a high-speed shredder (IKA, T18 model). The mixture was held in a water bath at 80°C for 30 min. Then, this extract was centrifuged at 10.000 rpm for 10 minutes at 4°C. The supernatant was filtrated through a 0.45 µm filter and transferred into a vial and 20 µL was used for the analysis.

Organic acid analysis was performed by an HPLC (Shimadzu brand, Prominence Modular LC20A model) according to Zong *et al.*, (2016). Separation was done using a Rezex brand, model of ROA-Organic Acid H+ (8%), LC Column 300 x 7.8 mm at 50°C with a UV detector set at 210 nm. The mobile phase was 0.01 N H_2SO_4 at a flow rate of 0.7 mL/min using isocratic flow. The quantification of organic acids was calculated according to the authentic standards and expressed as mg/kg dry weight. All analyses were performed in triplicate.

3. RESULTS

Table 1 presents the results of the methanol extracts of *A. viridis* for total phenolic and flavonoid content, antioxidant activities, and α-amylase inhibition activity. The TPC values of stem, leaf, and seed-flower extracts were calculated as 0.329, 0.665, and 0.516 mg GAE/g, respectively. TFC values of stem, leaf, and seed-flower extracts were calculated as 0.012, 0024, and 0.024 mg RE/g, respectively. DPPH˙ radical scavenging activity of extracts was evaluated by comparing it with the standard antioxidant activity of Trolox equivalent (mg TE/g). DPPH˙ of stem, leaf, and seed-flower extracts was determined as 67, 78, and 82%, respectively (0.43, 0.50, and 0.53 mg TE/g). FRAP results of stem, leaf, and seed-flower extracts were calculated as 23.98, 26.07, and 32.56 µg TE/g, respectively. Metal chelating activity results of stem, leaf, and seed-flower extracts were calculated as 63, 27, and 62%, respectively. Phosphomolybdenum assay results of stem, leaf, and seed-flower extracts were calculated as 191, 210, and 127 µg TE/g, respectively. α-amylase activities of stem and leaf extracts were not detected. On the other hand, α-amylase activities of seed-flower extracts were determined as 2%. The antibacterial efficacy of extracts from *A. viridis* against all tested microorganisms is shown in Table 2.

Table 1. The total phenolic, total flavonoid content, antioxidant, and antidiabetic activities of *A. viridis* methanol extracts.

	Stem extract	Leaf extract	Seed and flower extract
Total phenolic content (mg GAE/g)	0.329±0.002	0.665±0.014	0.516±0.007
Total flavonoid content (mg RE/g)	0.012±0.0003	0.024±0.0007	0.024±0.0003
DPPH (%/mg TE/g)	67±0.022% 0.43±0.022 mg TE/g	78±0.002% 0.50±0.002 mg TE/g	82±0.005% 0.53±0.005 mg TE/g
Ferric reducing capacity (µg TE/g)	23.98±0.066	26.07±2.068	32.56±1.155
Metal (Fe^{2+}) chelating activity (%)	63±0.012	27±0.0005	62±0.006
Phosphomolybdenum (Total antioxidant capacity) (µg TE/g)	191±0.529	210±4.696	127±1.257
α-amylase activity (%)	ND	ND	2±0.025

Values are mean ± Standard deviation (SD) of three replicate analyses. ND: Not detected.

Table 2. Diameter of the inhibition zone (mm) of *A. viridis* extracts.

	A. viridis stem extract	*A. viridis* leaf extract	*A. viridis* seed and flower extract	Positive Control	Negative Control
Staphylococcus aureus ATCC 25923	-	-	-	- (Methicillin)	-
Salmonella typhimurium ATCC 14028	-	-	-	18 (Tetracycline)	-
Escherichia coli ATCC 25922	-	-	-	18 (Tetracycline)	-

(-) represents a no-inhibition zone against microorganisms.

Table 3 displays the identified compounds with their retention times and the amount (mg/kg) of the phenolic content of the methanol extracts of *A. viridis* by reversed-phase high-performance liquid chromatography. In RP-HPLC, twenty-five, twenty-four, and twenty-one compounds were identified for stem, leaf, and seed and flower extracts, respectively. The most abundant compounds in stem extract were quercetin (125.05 mg/kg) and chrysin (112.45 mg/kg). Moreover, the most abundant compounds in leaf extract were catechine hydrate (608.24 mg/kg), t-Ferulic acid (344.8 mg/kg), sinapic acid (233.6 mg/kg), and quercetin (167.2 mg/kg). On the other hand, the most abundant compounds in seed and flower extract were catechine hydrate (674.25 mg/kg), catechine (547.63 mg/kg), t-Ferulic acid (337.7 mg/kg), and sinapic acid (269.13 mg/kg). Apart from these compounds, all compounds found in different amounts are listed in Table 3.

Table 3. The retention time (min) (R.T.) and amount (mg/kg) of the phenolic content of *A. viridis* methanol extracts.

Compound	Stem extract		Leaf extract		Seed and flower extract	
	R.T. (min)	Amount mg/kg	R.T. (min)	Amount mg/kg	R.T. (min)	Amount mg/kg
Chlorogenic acid	6.119	-	6.114	10.64	6.147	13.37
Catechine	6.479	60.75	6.469	54.64	6.668	547.63
Catechine hydrate	6.479	73.6	6.469	608.24	6.668	674.25
Caffeic acid	9.285	9.85	9.657	48.64	9.687	27.37
Vanillic acid	9.843	8.71	9.657	60.08	9.687	33.77
Gentisic acid	10.419	-	10.204	32.24	10.419	-
4-hydroxy benzoic acid	13.776	10.45	13.758	6	13.738	0
Vanillin	16.470	17.95	16.547	21.2	16.453	12.17
p-Coumaric acid	17.727	25.85	17.713	9.92	17.309	-
Rutin	19.409	-	19.075	0	19.483	15.6
Sinapic acid	20.048	58.7	20.055	233.6	20.090	269.13
t-Ferulic acid	20.669	32.6	20.055	344.8	20.090	337.7
Hyperoside	20.669	44.6	20.757	64.8	20.944	-
Isoquercitrin	21.764	14.95	21.796	24.8	21.900	14
Hdroxy cinnamic acid	23.274	17.85	23.686	8.8	23.378	-
Naringin	27.697	9.65	27.716	34.96	27.498	-
o-Coumaric acid	28.462	22.6	28.688	27.44	28.477	20.57
Protocatechuic acid ethyl ester	29.951	7.95	30.165	16.8	30.152	10.51
Rosmarinic acid	30.393	6.2	30.165	8	30.152	5.49
Salicylic acid	31.526	3.55	31.961	27.04	31.960	13.66
Resveratrol	32.436	6.47	32.478	14.4	32.475	2.80
Baicalin	32.802	16.8	32.478	3.52	32.475	6.29

Oleuropein	34.787	-	34.787	-	34.787	-
Quercetin	34.966	125.05	34.965	167.2	34.971	71.2
t-Cinamic acid	35.759	-	35.759	-	35.771	31.6
Naringenin	36.313	12.1	36.312	-	36.413	2.29
Hesperidin	36.850	2.89	36.759	-	36.593	8.40
6-hydroxy flavone	38.113	6.95	37.944	-	37.944	-
Chrysin	39.357	112.45	39.278	30.64	39.502	10.86
Curcumin	39.973	-	39.973	-	39.973	-
Flavones	40.909	2.2	40.909	17.6	40.770	-

Table 4 exhibits the identified compounds with their retention times and the amount (mg/kg) of the organic acid of *A. viridis* methanol extracts by reversed-phase high-performance liquid chromatography. In HPLC, 3 compounds were identified for all extracts: Succinic acid (28112.65 mg/kg), malic acid (12513.75 mg/kg), and shikimic acid (269. mg/kg) were present in stem extract. Additionally, leaf and seed-flower extracts contained shikimic acid (837.2 and 291.58 mg/kg), succinic acid (72992.48 and 30212.26 mg/kg), and formic acid (3943.84 and 2727.23 mg/kg).

Table 4. The retention time (min) (R.T.) and amount (mg/kg) of organic acid of *A. viridis* methanol extracts.

Compound	Stem extract		Leaf extract		Seed and flower extract	
	R.T. (min)	Amount mg/kg	R.T. (min)	Amount mg/kg	R.T. (min)	Amount mg/kg
Malic acid	10.622	12513.75	10.541	-	10.541	-
Shikimic acid	12.835	269.3	12.860	837.2	12.864	291.58
Succinic acid	12.835	28112.65	12.860	72992.48	12.864	30212.26
Formic acid	14.501	-	14.913	3943.84	14.914	2727.23

4. DISCUSSION and CONCLUSION

The secondary metabolites have anti-inflammatory, antioxidant, immune system stimulation, detoxification enzyme modulation, steroid metabolism, antiviral, anticancer, and antibacterial activities as natural compounds in plants. Table 1 shows the antioxidant activity and total phytochemical contents of methanol extracts of different parts of *A. viridis*. Leaf extract showed a high level of total phenolics (0.665±0.014 mg GAE/g) and had pronounced levels of radical scavenging (78±0.002%), and ferric reducing (26.07±2.068 µg TE/g) antioxidant activities. Despite the highest DPPH scavenging (82±0.005%) and ferric reducing (32.56±1.155 µg TE/g) capacities, seed and flower extracts had a lower total phenolic content (0.516±0.007 mg GAE/g) than that of leaf extract. The stem, seed, and flower extracts showed good metal-binding potential in the presence of ferrozine (Table 2), % values were 63±0.012 and 62±0.006, respectively. These results indicated that the methanolic extracts belonging to different parts of *A. viridis* contained a strong antioxidant capacity, and their efficacy was comparable with the literature studies.

Some previous studies reported that the high phenolic constituents in *A. viridis* contribute to antioxidant activity (Datta *et al.*, 2019; Sarker & Oba, 2019; *Sunday et al.*, 2021) and this was the case in this study. The superior levels of antioxidant activities of the leaf, seed, and flower extracts with high phenolic content partly confirmed this hypothesis. Pulipati *et al.*, (2014) found that the extracts prepared from the seeds of *A. viridis* had the highest DPPH antioxidant activity, which is consistent with our findings. Similarly, Abdel-alim *et al.*, (2023) declared the high DPPH (123±8.9 mg VCE/g DW) and FRAP (1.5±0.04 mM FE^{2}+ equivalent/g DW) antioxidant values of *A. viridis* ethanolic extract. Our results were similar with the observation of Popoola, (2022) and Sarker & Oba, (2020) that *A. viridis* seed extracts included radical scavenging agents reacting directly to DPPH. Kumari *et al.*, (2018) also indicated similar

antioxidant results for *A. viridis* extracts, showing that the high DPPH scavenging potential ranged from 53.31±1.08% to 65.2±1.41%. Datta *et al.,* (2019) reported that the DPPH (13.126±0.263%), FRAP (1.553±0.004 TE, mg/g DE) and MCA (22.359±0.491%) capacities of *A. viridis* extracts were quite lower than those of our antioxidant results.

The antioxidant activity of plant foods is now widely acknowledged to be primarily sourced from phenolics. Thus, it is crucial to ascertain the extract's phenolic content. The TP and TF contents of methanolic extracts for *A. viridis* different parts are of the order: for stem 0.329±0.002 mg GAE/g and 0.012±0.0003 mg RE/g, for leaf 0.665±0.014 mg GAE/g and 0.024±0.0007 mg RE/g, and for seed and flower 0.516±0.007 mg GAE/g and 0.024±0.0003 mg RE/g. These values were higher than the reported levels of green Amaranth leaves (ranging from 11.24±00.5 to 20.13±0.04 GAE μg/g) (Sarker *et al.,* 2020). In another study, the total phenolic contents of the *A. viridis* extracts, especially the leaf extract correlated to its high antioxidant activity with 57.25 mg GAE/g (Swarnakumari *et al.,* 2021). Similarly, Popoola, (2022) noted that the amount of total phenolic and flavonoid compounds found in the raw extract from the seed parts of *A. viridis* were 107.9±1.04 mg TA/g and 24.1±0.89 mg RE/g, respectively. The other stated total phenolic contents (TPC) for the methanolic extracts of the leaves *A. viridis* ranged from 40.26±0.27, 43.24±0.32, and 46.72±0.22 GAE μg/g (Sarker & Oba, 2019). The presence, absence, and formation of available phenolic constituents in *A. viridis* vary depending on environmental and genetic factors.

One Gram-positive and two Gram-negative bacterial strains were tested for the antibacterial activity of the *A. viridis* methanol extracts. The antibacterial activity of the extract could not be observed against the tested microorganisms. However, literature studies pointed out that the variety of microorganisms in which *A. viridis* extracts show antimicrobial activity was quite much (Akbar *et al.,* 2018; Rose *et al.,* 2021; Zahir *et al.,* 2021).

A promising therapeutic approach for the management and treatment of chronic health disorders including diabetes and obesity is the inhibition of digestive enzymes like α-amylase. Therefore, the α-amylase activity of extracts was examined. *A. viridis* seed-flower methanol extracts exhibited α-amylase inhibitory activity of 2±0.025%. Oluwagunwa *et al.,* (2021) reported a strong α-amylase inhibition activity (68.45%) in the aqueous leaf extract of *A. viridis* in contrast with our data. Similarly, Mareshvaran *et al.,* (2020) noted the high inhibitory capacity (73.85%) of the α-amylase enzyme of *A. viridis* dichloromethane extract. The findings of earlier investigations demonstrated that *A. viridis* extracts exhibit α-amylase inhibitory activity.

Plants include a large number of phenolic compounds and flavonoids, which have biological properties like antidiabetic (e.g., chlorogenic acid, caffeic acid, and rutin), antimicrobial (e.g., ferulic acid, and sinapic acid), anti-inflammatory (e.g., vanillic acid, ferulic acid, and quercetin), antioxidant (e.g., sinapic acid, p-coumaric acid, and quercetin), antianxiety (e.g., sinapic acid), antihistamine (quercetin) and anticarcinogenic (e.g., p-coumaric acid, chlorogenic acid, ferulic acid, sinapic acid, quercetin, and rutin) (Sadia *et al.,* 2016; Datta *et al.,* 2019;). The commonly identified components of *A. viridis* are flavonoids, cyanogenic glycosides, saponins, tannin, and phlobatannins (Iqbal *et al.,* 2012). Table 3 presents the qualitative results of phytochemicals in different parts of *A. viridis.*

The dominant phenolic compounds of the *A. viridis* extract were catechine, catechine hydrate, and sinapic acid, followed by quercetin. Sarker and Oba (2020), similar to our phenolic content varying amounts depending on the plant parts, identified salicylic acid, gentisic acid, vanillic acid, chlorogenic acid, ferulic acid, t-cinnamic acid, quercetin, p-coumaric acid, caffeic acid, rutin, sinapic acid, naringenin, and catechin by using HPLC. A study conducted by Abdel-alim *et al.,* (2023) expressed the presence of rutin, quercetin, and naringin as major phenolics in the stem and leaves of *A. viridis.* Kumari *et al.,* (2018) and Zaware *et al.,* (2022) reported identifying chlorogenic acid and ferulic acid in *A. viridis* extract.

The phytochemical results from the current study express that the methanolic extracts of stem, leaf, and seed-flower of *A. viridis* included pharmacologically active compounds having varied biological potential. Especially, the most popular among the biologically active compounds in the chemical composition of plants are organic acids. In this study, the efficiency of this method for the extraction of organic acids from *A. viridis* was evaluated for the first time. The potential bioactive organic acid profile of *A. viridis* was determined in scientific reports using different extraction methodologies (Javed *et al.*, 2018). Javed *et al.*, (2018) reported the different organic acid components (citric, malic, fumaric, oxalic, and glutamic acids) of *A. viridis* root exudates in contrast with our data.

The current study reported the phenolic and non-phenolic profiles, antioxidant activity, antibacterial activity, and antidiabetic properties of extracts obtained from different parts of *A. viridis* (Cyprus). The metabolic profile of the extracts obtained from different parts of *A. viridis* was determined by RP-HPLC and nearly thirty-one phenolic compounds were characterized. Contents varying depending on plant parts, two compounds, catechin, and sinapic acid were at the highest level in the phenolic metabolites. Organic acids, non-phenolic compounds, were quantitatively predominant, opposite phenolics. This indicates that the ripening of plant parts is a complex mechanism affecting the quantitative profile of phytochemicals. The phenolic and non-phenolic metabolites identified in *A. viridis* extracts were the source of strong biological activity, in particular phosphomolybdenum activity, metal chelating activity, free radical scavenging capacity, ferric reducing antioxidant power, antibacterial activity, and anti-diabetic impact performed *in vitro* models. Our results highlighted the potential of *A. viridis* as a source of antioxidant phytochemical contents that need more scientific information to explain the molecular mechanisms in biological activities through *in vivo* studies.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Melis Sümengen Özdenefe: Concept, Design, Supervision, Resources, Materials, Data collection and/or Processing, Analysis and/or Interpretation, Literature Research, Writing, and Critical Reviews. **Fikret Büyükkaya Kayış**: Concept, Design, Supervision, Analysis and/or Interpretation and Critical Reviews. **Ümit Haydar Erol:** Materials, Data collection and/or Processing, and Analysis and/or Interpretation. **Hatice Aysun Mercimek Takcı:** Concept, Design, Supervision, Resources, Materials, Data collection and/or Processing, Analysis and/or Interpretation, Literature Research, Writing, and Critical Reviews.

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