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Exploring the antioxidant, anticancer and antimicrobial potential of *Amaranthus viridis* L. collected from Fayoum depression: Phytochemical, and biological aspects

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A B S T R A C T

Amaranthus viridis (AV) is an invasive weed designated for natural antioxidants. In the current endeavor, *A. viridis* aerial parts and soil samples were collected from six districts of Fayoum depression (FD), Egypt and were subjected to chemical and biological examination. Where, the organic extracts of the aerial parts of the six distinctive samples (AV1-AV6), were subjected to LC-HRESI-MS-assisted chemical profiling. Multivariate analysis of MS data revealed good clustering of *A. viridis* collected from three sites (1, 2 and 6) and empowered the hypothesis of chemical composition analogy among extracts AV1, AV2 and AV6. On the other hand, extracts AV3, AV4 and AV5 were not resemblant to each other and were apart from AV1, AV2 and AV6. Similarly, multivariate analysis of physico-chemical properties of *A. viridis*-associated soil samples showed good clustering of the same three sites (1, 2 and 6). The total phenolics contents (TPC) and total antioxidant capacity (TAC) were promising in all extracts, and they could be arranged in the order of AV6 > AV2 > AV5 > AV3 > AV4 > AV1. Moreover, the uniqueness of AV2, AV3 and AV4 extracts lies in guttiferic acid and the high content of alkaloids that granted the privilege of cytotoxicity toward human liver cancer cell line (HepG2) and human metastatic breast cancer cell line (MDA-MB-231). Regarding the antimicrobial activity, AV1 and AV6 showed the highest antibacterial activity against *S. aureus* meanwhile AV2, AV3 and AV5 depicted the highest values in case of *S. typhi* and *E. coli.* Also, the study revealed that AV1, AV4, and AV6 reflects their prodigious minimum inhibitory concentration (MIC) values against *E. coli, S. aureus, and S. typhi.* In terms of antifungal activity, AV6 extract recorded as the most inhibition performance against *A. flavus* and *A. niger*, whereas AV2 and AV5 inhibited *C. albicans* the least. According to biofilm activity, AV4 and AV5 were superior, remarkably against *E. coli and B. subtilis* respectively. Interestingly, the AV6 extract performed exceptionally well as an antibiofilm agent against *P. aeruginosa.* The potency of some extracts rather than others may rely on possible relationships between soil physicochemical characteristics and chemical composition of *A. viridis* which contributed to the observed biological properties. Such factors should be taken into consideration in assessing quality control of medicinal plants.

1. Introduction

Discovering bioactive compounds with novel modes of action has become an urgent necessity due to rapid increase in medical challenges including several serious health disorders (Newman and Cragg, 2016; Tammam et al., 2022). Structural characteristic and

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diversity of natural products has yielded well known footprints in both drug development and drug clinical application (Diab et al., 2022; Tammam and El-Demerdash, 2023).

Consequently, botanical naturally occurring compounds are well known for playing vital roles via several mode of actions that give them the ability to reduce risk of contracting several diseases (Salvamani et al., 2016a). Moreover, naturally occurring antioxidants are being considered as one of the tools for fighting against oxidative stress disorders for the purposed of improving quality of health (Nordberg and Arnér, 2001; Tziveleka et al., 2021).

Additionally, Weeds in holistic approach are regarded as a source of food, fodder, and medication (Radicetti and Mancinelli, 2021). Among them *Amaranthus viridis*, belonging to family *Amaranthaceae*, a family consist of more than 70 species of annual or perennial plants, including herbs, vines, and shrubs (Bang et al., 2021; Chaturvedi and Gupta, 2021). *Amaranthus,* a genus of this family comprises many species; some of which are cultivated as vegetables or ornamentals while others are listed as weeds and have become cosmopolitan all over the world (Rastogi and Shukla, 2013; Vincent et al., 2019). Additionally, several phytochemicals including phenolic acids, steroids, flavonoids, tannins, alkaloids, saponins and triterpenes have been isolated from various *Amaranthus* spp. with antioxidant properties and interesting pharmacological properties such as antimalarial, antimicrobial, anticancer, antipyretic, anti-inflammatory, and antiviral properties (Adegbola et al., 2020; Ahmed et al., 2013; Jin et al., 2013; Silva et al., 2021). Furthermore, it is considered as a good supplier of carbohydrate, proteins, fiber, vitamins, and minerals (Datta et al., 2019; Sarker and Oba, 2019).

Invasive plants such as *Amaranthus* often have greater environmental adaptability than native ones (Rastogi and Shukla, 2013). *A. viridis* is renowned not only for being resilient to harsh environmental variables of temperature, drought, heavy metals, and pests (Fouad et al., 2023; Muhammad Javid Iqbal, 2012) but also containing pharmacologically active compounds displaying potent antioxidant and antimicrobial activities isolated from *Amaranthus* leaves and seeds (Reyad-ul-Ferdous, 2015; Thanikachalam and Jayaraj, 2020). Indeed, stem aqueous extract efficacy of *A. viridis* was demonstrated in curing diabetes and its complications (Pulipati, 2014). Antioxidant activity of *A. viridis* methanolic extract of leaves and seeds was good when estimated using DPPH free radical scavenging test. In a trial to evaluate some biological properties of *A. viridis*, the utilization of antioxidant capacities in many foods, pharmaceuticals, and agricultural applications was proved. *A. viridis* was used in the treatment of many diseases like hyperlipidemia, ulcer, diabetic, asthma, and inflammation and it was also used as antipyretic. It was found that, *A. viridis* aids with various actions as hepatoprotective, antihelmintic, hypolipidemic, antihyperglycemia and antifungal activity (Muhammad Javid Iqbal, 2012; Reyad-ul-Ferdous, 2015; Thanikachalam and Jayaraj, 2020).

Several secondary metabolites were isolated from different *Amaranthus* extract, obtained from its several parts, whereas root extract of *A. viridis* contains amasterol as a steroidal component, while chemical investigation of the methanolic extract leaves led to the isolation of different cholesterol derivatives including 24-methyllathosterol, 24-ethyllasosterol, 24-methyl-22-dehydrolathosterol, 24-ethyl cholesterol, while 24-ethyl-22-dehydrocholesterol represent the minor portion of sterol derived from *Amaranthus* extract, spinosterol was the major one. Also, rutin and quercetin were reported in *A. viridis* leaves methanolic extract (Pulipati, 2014).The hexane, ethyl acetate and ethanolic extracts of *A. viridis* leaves exhibited a virtuous inhibition against *Fusarium solani* meanwhile, dichloromethane, ethyl acetate along with ethanol extract was depicting a noticeable action against *Colletotrichum musae* (Carminate et al., 2012). Leaves and seeds methanolic extract yield a considerable amount of phenolic and flavonoid content, as well as radical scavenging ability and an appreciable antimicrobial efficiency in face of some bacterial and fungal strains. Although *A. viridis* antibacterial and antioxidant activities were stated in some reports, phytochemical content is affected by genetic variation, environmental and seasonal conditions (Bang et al., 2021; Carminate et al., 2012).

It is well known that ecological factors such as climate, soil, and surrounding habitat influence plant constituents including secondary metabolites produced through biosynthesis. These secondary metabolites are known to play key roles in chemical defense which give rise to their biological and medicinal properties and are mainly used as adaptive strategies for survival (Mohammed et al., 2023, 2021).*A. viridis* is the most common and highly noticed species in Fayoum Depression, Egypt (Abd El-Ghani et al., 2015; Al-Sherif et al., 2018), situated about 95 km southwest of Cairo at longitudes of 30° 23\ and 31° 5\ E, and latitudes of 29° 5\ and 29° 35\ N (Effat and El-Zeiny, 2017).

Herein, the soil samples and *A. viridis* aerial parts were gathered from six populations namely, Ibsheway, Atsa, Sinnuris, Tamiyah, Fayoum and Youssef Al-Seddik that covered the study area of Fayoum depression thoroughly. The study explored soil physicochemical properties from these districts as well as the profile of phytochemicals present in the plant aerial parts methanolic extract. Afterwards, the application of extracts for their potential antioxidant, anticancer and antimicrobial activities were tested. Lastly, this work tried to find a probable correlation between soil characteristics and plant chemical profiles at all samples from six aforementioned populations. The untargeted approach of our trial allowed us to identify and characterize a large number of metabolites, some of which have not been reported before in this plant.

2. Materials and methods

2.1. Sample collection and identification

Ostensibly, *Amaranthus viridis* is pervading throughout Fayum depression-Egypt. To harness this wide distribution, sampling sites cover all Fayoum districts. Plant specimens as well as soil samples were collected from Ibsheway, Atsa, Sinnuris, Tamiyah, Fayoum and Youssef Al-Seddik districts (Table S2 and Fig. S2) during summer, which is the growing season of the examined species. Subsequently, samples were transferred to the lab in paper bags. The plant aerial parts were cut, rinsed with distilled water, and were allowed to be dried at 35 °C in open air according to the modified method of Chaturvedi and Gupta (2021) and according to the standard shade drying protocols until complete dryness (Nurhaslina et al., 2022). The examined samples have been coded as AV1 to AV6, a collection kept in CAI (Cairo University Herbarium) and the plant material was deposited as herbarium voucher No: CAI. 25. 91. 262. 232.

2.2. Extraction process

Air dried aerial parts of six collected *A. viridis* samples (100 g for each sample) were individually extracted four times with methanol (250 mL) at room temperature (25 ± 2 °C). The obtained crude extracts were concentrated using Rota Vapour (Büchi R-300) under reduced pressure at (40 °C) (Ghareeb et al., 2019). The total extractable content (TEC %) are 12.31 %, 13.6 %, 10.44 %, 11.67 %, 13.12 % and 13.37 % for *A. viridis* 1, 2, 3, 4, 5, and 6, respectively, which were calculated according to the formula: TEC (%) = Dry extract weight/ dry powder weight × 100.

2.3. Secondary metabolite profiling by LC-DAD-Q-TOF-MS/MS

Extracts were subjected to a solid phase extraction pre-cleaning method to remove salts, polar acids and bases that usually interfere with mass spectrometry ionization. 1 mg of extract was first dissolved in water and passed through a C18 SPE column (Phenomenex)

("Solid Phase Extraction (SPE) Method Development Tool from Phenomenex," n.d.), followed by 100 % MeOH, dried, resuspended in 1.0 mL MeOH, centrifuged and analysed by LC-DAD-Q-TOF-MS/MS. High resolution mass spectrometric data were acquired using Acquisition Mode AutoMS2 system (Agilent, Santa Clara, California, USA) ("LC Q-TOF MS, target/suspect screening, Quadrupole Time of Flight | Agilent," n.d.), ion source (Dual AJS ESI; MS, MS/MS (*m/z* 100–2000) connected to an Agilent HPLC system (Accela PDA detector, Accela PDA autosampler, and Quaternary Pump) ("Liquid Chromatography | Agilent," n.d.). Chromatographic separations were carried out on a Phenomenex ("Phenomenex UHPLC, HPLC, SPE, GC - Leader in Analytical Chemistry Solutions," n.d.), Luna Phenyl-hexyl, 5 μm, 150 × 4.6 mm column at 1mL/min using a gradient mobile phase from 0–100 % MeOH in 12 min then kept at 100 % MeOH until 22 min followed by equilibration until 27 min before the next injection. Mass spectrometry conditions used were as follows: capillary voltage of 45 V, sheath gas temperature of 380 °C, sheath gas flow 12 l/min, spray voltage 4.5 kV, and mass range 100–2000 amu (MS resolution:60,000; MS/MS resolution: 30,000). LC-DAD-Q-TOF-MS were analyzed using Agilent Mass Hunter (MH Qual 10.0 G3336-60122) ("Robust Mass Spectrometry Application Software, MassHunter | Agilent," n.d.), software with associated peak picking and alignment algorithm. Only masses between 0 to 22 min were considered for dereplication using database, Antibase2012 ("chem IT Services - Antibase," n.d.), to avoid late eluting compounds which are mostly fats and lipids and are usually of no interest in drug discovery. Both positive and negative ionization modes were used as it is known that these could account for more than 90 % of secondary metabolites in the extract (Nielsen et al., 2011).

2.4. Multivariate statistical analysis

The processed MS data were exported as an Excel sheet of list of annotated metabolites that were subsequently were subjected to multivariate analysis (MVA) with the aid of the MetaboAnalyst 5.0 software (Xia et al., 2009). Supervised and unsupervised statistical representation of the MS data were carried out in terms of principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA), which helped to identify and highlight compounds likely associated with the extracts' observed antibacterial activity. There was a $\log_{10}$ transformation applied to the signal strength of all variables. Principal Component Analysis (PCA) is a technique employed for unsupervised clustering which aims to reduce the complexity of multivariate data, comprising 282 variables, while retaining a significant amount of the data's variance. The primary elements derived from this process can be visually displayed through a plot known as a "scores" plot. This type of visualization assists in identifying potential clusters within the dataset. PCA models are formulated by incorporating all the samples available in the study. The term 'loadings' refers to the coefficients applied to the original variables in order to compute the Principal Components (PCs). The magnitude of a variable's loading on a PC is indicative of the degree to which the variable is associated with that specific component.

2.5. Total phenolic content (TPC) estimation

The total phenolic content (TPC) of the investigated extracts was estimated via using Folin-Ciocalteu's assay according to the reported procedures of Kumar et al. (2008) and Yu et al. (2002). The TPC was presented as mg gallic acid equivalent (GAE) per g dry extract.

2.6. Antioxidant activity

2.6.1. Free radical masking activity estimation using DPPH test

The free radical masking activity of the tested extracts was estimated via using DPPH assay according to the reported procedures of Shirwaikar et al. (2006). Antioxidant results were expressed in IC_{50} values.

2.6.2. Free radical masking activity estimation using ABTS test

The ABST free radical scavenging activity of the investigated extracts was evaluated via using ABTS antioxidant assay according to the reported procedures of Kaur et al. (2011).

2.6.3. Total Antioxidant Capacity (TAC) estimation

The total antioxidant capacity (TAC) of the tested extracts was estimated via using phosphomolybdenum assay according to the reported procedures of Prieto et al. (1999). The TAC value was presented as the number of ascorbic acid equivalent (AAE) according to the formula: TAC= A_{Sample}/ $A_{ascorbic\ acid}$ X 1000.

2.7. Determination of the cytotoxic effects of the examined extracts

2.7.1. Human cancer cell lines

Two cell lines namely, human liver cancer cell line or hepatocellular carcinoma cell line (HepG2) and human metastatic breast cancer cell line (MDA-MB-231) were attained in liquid nitrogen (−180 °C) from VACSERA (The Egyptian Company for Production of Vaccines, Sera and Drugs). Both cell lines were originally acquired from the American Type Culture Collection (ATCC), the propagation of the cells have been done in the (NCI). In DMEM augmented with 10 % FBS and 1 % penicillin-streptomycin, the two studied cell lines were preserved as mono-layer cultures.

2.7.2. Cell viability assay by SRB (Sulforhodamine-B)

Cytotoxicity was determined using sulforhodamine-B (SRB) method according to Skehan et al. (1990). Cells were seeded in 96-well microtiter plates at a concentration of 4 × 10^3 cells/well. They were left to attach for 24 h before incubation with drugs. The cells were treated for 48 h with different concentrations (0, 10, 20, 40 and 80 μg/mL) of the six examined extracts of *A. viridis*, using paclitaxel as a positive control in concentration ranging from (0–15) μg/mL and using DMSO as a negative control, each concentration was applied as triplicate. Each well was deliberated for its optical density at 570 nm using an ELISA microplate reader (TECAN Sunrise, Germany). The mean values were calculated as the percentage of cell viability as follows: O.D (treated cells) / O.D (control cells) × 100. The IC_{50} value of each tested extract was valued using dose-response curve-fitting models (Graph-Pad Prism software, version 7).

2.8. Determination of antimicrobial effect

2.8.1. Microbial strains

For the antimicrobial testing, four bacterial strains including: Gram-negative bacteria (*Escherichia coli* ATCC 25955, *Pseudomonas aeruginosa* ATCC 10145, *Salmonella typhi* ATCC 6539), Gram-positive bacteria (*Staphylococcus aureus* NRRL B-767), one strain of yeast *Candida albicans* (Robin) Berkhout (AUMC13447) and three strains of fungi (*Aspergillus niger* ATCC 16404), *Rhizoctonia solani* Kuhn (AUMC14447) and *A. flavus* Link (AUMC 9797) were obtained from Assuit University Mycological Center (AUMC), Assuit, Egypt.

2.8.2. Determination of antimicrobial effect

In brief, the test was conducted using 96-well flat polystyrene plates. 10μl of test extracts (final concentration of 645 μg/ml) were added to 80μl of either nutrient broth (NB) for bacteria or potato dextrose broth (PDB) for fungi, then 10μl of bacterial or fungal culture suspension in log phase was added. The plates were incubated at 37 °C for 24 h for bacteria and yeast, and at 28 ± 2 °C for 48–72 h for fungal strains. Following incubation, the antibacterial efficacy of the tested extracts was assessed by detecting clear wells, whereas the absence of such impact was shown by opaque growth media in the wells. Moreover, the plates were further incubated at 37 °C for 30 min before reading the minimum inhibitory concentration (MIC) for bacteria. The last well with clear inhibition of bacterial growth was recorded as the MIC. The MIC were determined in two independent experiments. The MIC were reported as the average of the lowest concentrations with no observable growth of microorganisms. The control consisted of untreated pathogens. The absorbance was measured 24 h later using a Spectrostar Nano Microplate Reader (BMG LABTECH GmbH, Allmendgrun, Germany) at OD600. Dimethyl sulfoxide (dmso) was used as negative control while ciprofloxacin (10 μg/mL) and fluconazole (5 μg/mL) served as positive control for antibacterial and antifungal activity respectively (Abdelgawad et al., 2022; Alhadrami et al., 2021; Qader et al., 2021).

2.8.3. Biofilm inhibitory activity

The biofilm inhibitory activity of the obtained organic extracts was measured via microtiter plate assay (MTP) in 96 well-flat bottom polystyrene titre plates against four clinical bacteria (*P. aeruginosa* ATCC 10145, *S. aureus* NRRL B-767, *E. coli* ATCC 25955 and *B. subtilis* ATCC 6633) according to Abd-Elsalam et al. (2022), Hussein et al. (2022), Mostafa et al. (2022). Briefly, 180 μL of LB broth (containing of 10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl) were poured into each well, and 10 μL of an overnight pathogenic bacterial culture was then added. Subsequently, 10 μL of the chosen samples or a blank control was added, and the wells were incubated at 37 °C for 24 h. Following incubation, the contents of the wells were removed and washed with 200 μL of phosphate buffer saline (PBS) at pH 7.2 to eliminate any free-floating bacteria. The adherence of the sessile bacteria was fixed using 2 % sodium acetate, then samples was stained with 0.1 % crystal violet. The excess stain was eliminated using deionized water, and the plates were then allowed to dry. Afterwards, the optical density (OD) was determined at 595 nm using a microtitre plate reader (BMG LABTECH GmbH, Allmendgrün, Germany) after the dried plates had been cleaned with 95 % ethanol.

2.9. Sampling and physiochemical analysis of soil

From each sampling site, soil samples were collected at 20cm depth. Subsequently, samples were transferred to lab in plastic bags, allowed to be air dried and standing for analysis.

After gently crushing each sample using a wooden pestle and mortar, they were carefully mixed with distilled water terminating to soil paste. Electric conductivity, pH and sodium were measured as the described method in Scrimgeour (2008). Some macroelements (Ca, Mg and K) along with Pb were determined using inductively coupled plasma mass spectroscopy (Agilent 7500a, USA). Probable contamination should be avoided throughout the digestion and analytical procedure using reagent blanks (Stoltz and Greger, 2002). The experimental work was extended to quantify total organic matter (TOC) obeying (FAO, 2019) and Calcium carbonate percentage using a modified pressure calcimeter method (Sherrod et al., 2002).

2.10. Statistical analysis

All data were collected and coded to facilitate data manipulation and double entered into Microsoft Access and data analysis performed using the Statistical Package of Social Science (SPSS) software version 22 in windows 7 (SPSS Inc., Chicago, IL, USA). Simple descriptive analysis in the form of arithmetic means as central tendency measurement, standard deviations as a measure of dispersion of quantitative parametric data. One-way ANOVA test used to compare quantitative measures between more than two independent groups of quantitative data. The *P-value* < 0.05 was considered as statistically significant, quantitative data included in the study of total phenolics, flavonoids, antioxidant, and antimicrobial activities, first tested for normality by One-Sample Kolmogorov-Smirnov test in each study group then inferential statistic tests selected. The data for the cytotoxicity experiment were analysed using two-way ANOVA followed by Tukey-Kramer test for the cytotoxicity examination. Physiochemical measured parameters of soil-derived data were subjected

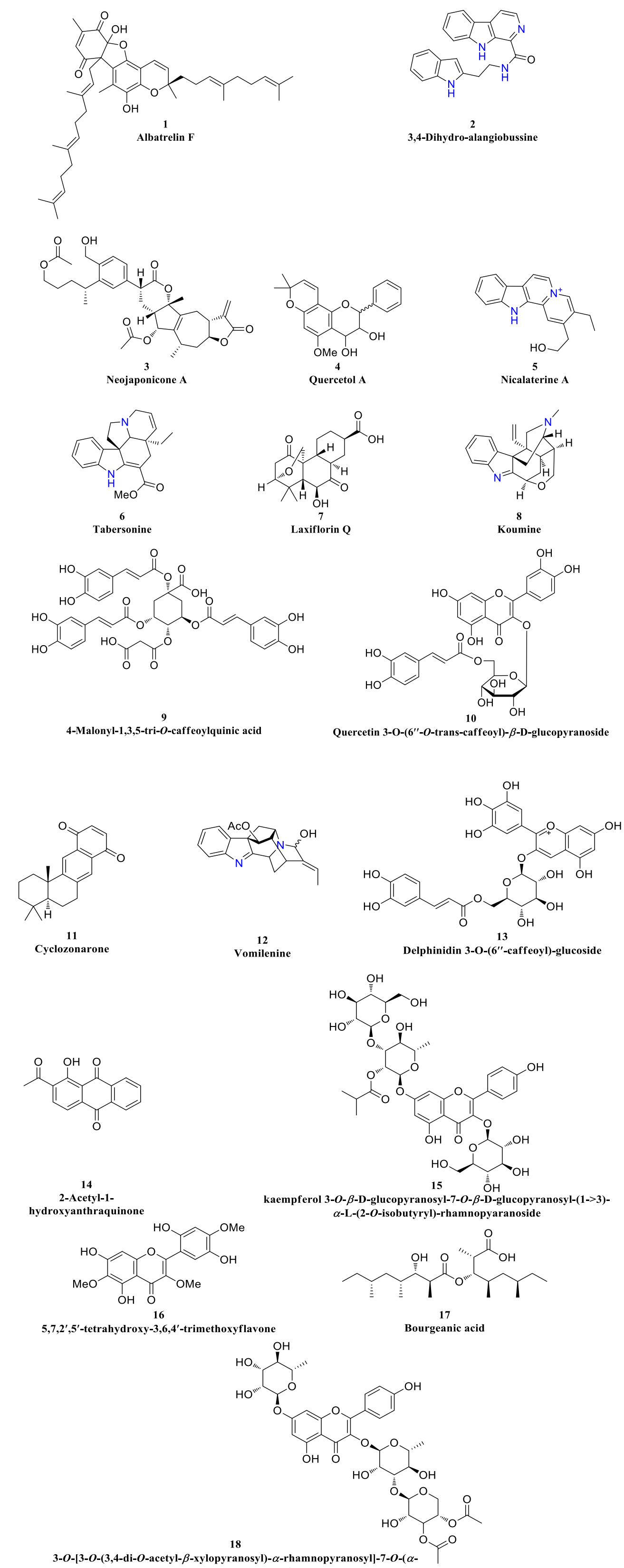

Fig. 1. Chemical structures of compounds **1**–**18**. Chemical structures of compounds **19**-**26**.

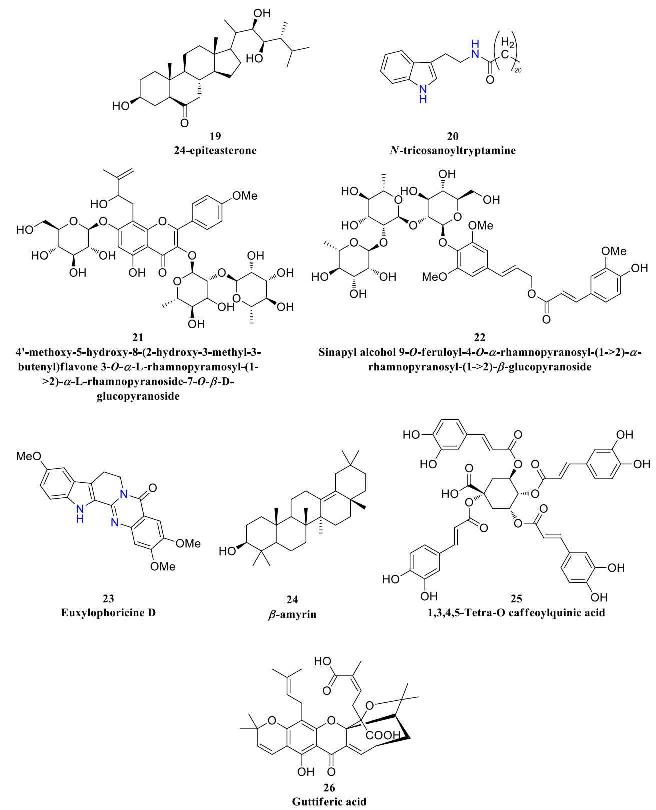

Fig. 1. Continued.

to multivariate analysis (MVA) using SPSS programming and data management software (Levesque, 2005).

3. Results and discussions

3.1. Chemical profiling and multivariate analysis

Analysis of the methanolic extract of the six samples of *A. viridis* collected from six different districts of Fayoum Depression (Table S2 and Fig. S2), using LC-HRESI-MS resulted in tracing a total of 2657 peaks in the six extracts (i.e., AV1-6) belonging to several secondary metabolites of different classes i.e., terpenes, alkaloids, phenolic compound derivatives, flavonoids, anthocyanidines and anthraquinones with different abundance. Table S1 and Fig. 1, show the highly abundant detected compounds in the examined extracts. PCA and PLS-DA models were built from the resulting HRESIMS data, allowing us to compare the chemical-profile differences between the six extracts.

The PCA is an unsupervised clustering method that acts to minimize the dimensionality of multivariate data while preserving most of the variance therein. The main components can be represented graphically as a "scores" plot. This graphic can be used to see any groupings in the data set. PCA models are constructed using all the samples in the study. Loadings are the coefficients by which the original variables must be multiplied to obtain the PC. The numerical value of a variable's loading on a PC indicates how much the variable shares with that component.

A total of 2657 peaks were annotated in the six extracts (i.e., AV1-6), as shown in both the PCA and PLS-DA (The model was further validated by a permutation test of 1000 permutations, which yielded a significance level of p 0.001. Q^2 = 0.89, R^2 = 0.9) score plots (Fig. 2), AV1, AV2, and AV6 extracts were clustered together indicating low variance between them in terms of their chemical composition. In contrast, the chemical compositions of AV3-5 extracts were significantly varied from each other and from the remaining extracts (i.e., AV1, AV2, and AV6) indicating that each extract has a unique chemical fingerprint.

Table 1 show the results of analysis of the variable importance in projection (VIP) scores (only variables with VIP values >1.5 were selected) derived from PLS-DA to determine which phytochemicals were most prominent in each extract. During dereplication, a taxonomic filter was used to narrow down the results to only those that were relevant to the investigated medicinal plant.

It can be concluded from the previous multivariate analysis along with the results of antimicrobial assays that compounds euxylophoricine D, guttiferic acid, and 2-acetyl-1-hydroxyanthraquinone (i.e., the characteristic phytochemicals in AV1, AV2, and AV6 extracts) might be associated with the antibacterial activity of *A. viridis* extracts against *S. aureus* (Table 4), particularly, AV1, AV2, and AV6 extracts.

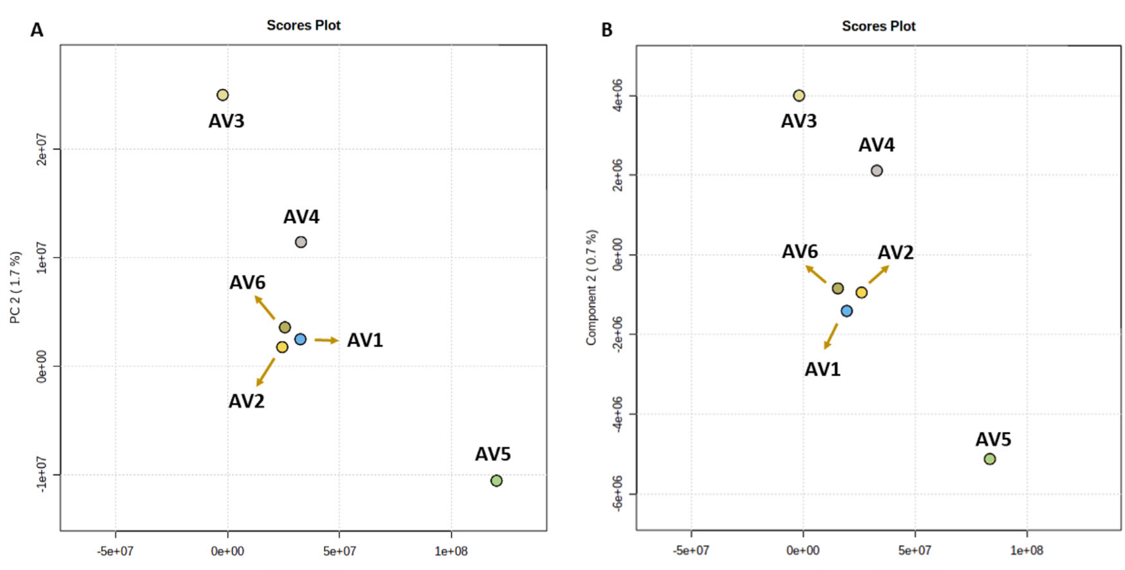

Fig. 2. PCA and PLS-DA scores plots of extracts derived from *A. viridis* (i.e., AV1-6) (A and B, respectively. The plots were generated by MetaboAnalyst 5.0. using HRMS data.

Table 1
Dereplication table of the characteristic phytochemicals from the studied *A.viridis*-derived different extracts AV1-6.

Extract	No.	Retention Time	Molecular Formula	Dereplication	VIP Score*
AV1	23	6.6	$C_{21}H_{19}N_3O_4$	Euxylophoricine D	2.25
	26	8.72	$C_{33}H_{38}O_9$	Guttiferic acid	2.04
	14	3.10	$C_{16}H_{10}O_4$	2-Acetyl-1-hydroxyanthraquinone	1.93
AV2	23	6.6	$C_{21}H_{19}N_3O_4$	Euxylophoricine D	2.21
	26	8.72	$C_{33}H_{38}O_9$	Guttiferic acid	2.16
	14	3.10	$C_{16}H_{10}O_4$	2-Acetyl-1-hydroxyanthraquinone	1.83
AV3	4	2.23	$C_{21}H_{22}O_5$	Quercetol A	2.11
	10	2.87	$C_{30}H_{26}O_{15}$	Quercetin 3-O-(6′′-O-trans-caffeoyl)-β-D-glucopyranoside	1.96
AV4	9	2.79	$C_{37}H_{32}O_{18}$	4-Malonyl-1,3,5-tri-O-caffeoylquinic acid	1.98
	17	5.43	$C_{22}H_{42}O_5$	Bourgeanic acid	1.94
AV5	16	4.71	$C_{18}H_{16}O_9$	5,7,2′,5′-tetrahydroxy-3,6,4′-trimethoxyflavone	1.92
	20	5.93	$C_{33}H_{56}N_2O$	N-tricosanoyltryptamine	1.85
	25	6.68	$C_{43}H_{36}O_{18}$	1,3,4,5-Tetra-O caffeoylquinic acid	1.74
AV6	23	6.6	$C_{21}H_{19}N_3O_4$	Euxylophoricine D	2.19
	26	8.72	$C_{33}H_{38}O_9$	Guttiferic acid	2.09
	14	3.10	$C_{16}H_{10}O_4$	2-Acetyl-1-hydroxyanthraquinone	1.98

* VIP stands for variable importance in projection.

3.2. Total phenolics contents and antioxidant activities

In Folin-Ciocalteu assay, the TPC values for the tested extracts ranged from 240.58 to 74.20 mg GAE/g plant extract. The results are in the order: AV6 > AV2 > AV5 > AV3 > AV4 > AV1 (Table 2). As indicated by many previous studies, this remarkable variation in the phenolic content may be due to the diversity in ecological conditions, which also have significant effects on the observed antioxidant activities of the tested extracts (Bibi et al., 2022; Mpofu et al., 2006).

One of the main causes of several serious diseases including cardiovascular, cancer and inflammation is oxidative stress, a phenomenon that happens due to the over-production of free radicals inside the cells of the human body (Tziveleka et al., 2021). Antioxidants including those obtained from natural sources can serve as scavenger agents for free radicals, which resulted in reducing the destructive effects of this phenomenon (Ghareeb et al., 2018; Sobeh et al., 2018). Herein, the antioxidant activities of the tested extracts have been evaluated using more than one assay to obtain a credible profile of antiradical potential such as (DPPH, ABTS and TAC). In DPPH assay, the IC_{50} values for the investigated extracts ranged from 20.52 to 75.98 μg/mL in comparison with standard (ascorbic acid) with IC_{50} equal to 7.04 μg/mL. The results are in the order: AV6 > AV2 > AV5 > AV3 > AV4 > AV1 (Table 2). Moreover, all examined extracts displayed comparable activity using ABTS test expressed as mmol Trolox® equivalent /100 g extract. The values are in the order: AV6 > AV5 > AV4 > AV3 > AV2 and AV1 (Table 2). In phosphomolybdenum assay, the sample (AV6) showed high total antioxidant capacity (TAC) with 400.66 mg AAE / g extract, followed by AV2, AV5, AV3, AV4 and AV1 samples, respectively (Table 2). To conclude, the obtained results of the three antioxidant assays agreed with each other and the high activity of AV 6, 2 and 5 are possibly due to their high phenolic contents.

Based on LC-MS/MS data (Table S1), extracts AV1-6 contain variable amounts of bioactive phenolic ingredients which have been reported for their potent antioxidant activities including flavonoids, phenolic acids, anthraquinones, anthocyanidines, and xanthones (Moazzen et al., 2022; Rice-Evans et al., 1996). Moreover, differences in antioxidant activity observed among the six extracts could be attributed to differences in amounts of phenolic compounds in them. In addition, antioxidant activities are often affected by the presence or absence of structural features in phenolic compounds such as hydroxyl (OH), methoxy (OCH_3) and carboxylic (COOH) groups. In this context phenolic compounds with heavy hydroxylation patterns have been known to show potent antioxidant activities due to their strong abilities to scavenge free radicals (Chen et al., 2020; San Miguel-Chávez, 2017). Moreover, it has been reported that there is a direct positive correlation between phenolic content and their antioxidant activities in which the antioxidant activities vary according to the diversity of phenolic contents (Irakli et al., 2018; Piluzza and Bullitta, 2011).

Previous studies indicate that production of secondary metabolites and phytochemical content of plants are affected by numerous factors like environmental and ecological conditions, climatic factors, seasonal variation, geographical location, and stages of growth (Dandjlessa et al., 2022; Pant et al., 2021). The diversity in chemical composition and in the production of secondary metabolites leads to

Table 2
Total phenolic content (TPC), DPPH free radical antioxidant activity, total antioxidant capacity (TAC) and ABTS free radical scavenging antioxidant activity of the methanolic extracts of *A. viridis* collected from six sites in Fayoum Depression.

Sample code	Total phenolic content (mg GAE/g plant extract)[a,b]	DPPH free radical scavenging activity IC50 (μg/ml)[a,c]	ABTS assay (mmol Trolox® equivalent/ 100 g extract)[a,5]	Total antioxidant capacity (mg AAE / g extract)[a,d]
AV1	74.2 ± 0.30	75.98 ± 0.11	229.68 ± 1.05	170.33 ± 1.52
AV2	196.83 ± 0.25	26.23 ± 0.26	229.68 ± 1.05	373.66 ± 1.52
AV3	152.47 ± 0.54	40.39 ± 0.12	626.37 ± 0.78	202.0 ± 2
AV4	105.27 ± 0.42	59.32 ± 0.11	323.44 ± 0.42	192.0 ± 2
AV5	170.06 ± 0.68	35.15 ± 0.06	688.78 ± 0.69	241.0 ± 2.64
AV6	240.58 ± 0.56	20.63 ± 0.06	1180.55 ± 0.56	400.66 ± 1.15
Ascorbic acid	–	7.04 ± 0.07	–	–
p-value	<0.001*	<0.001*	<0.001*	<0.001*

[a] Results are (means ± S.D.) (n = 3)
[b] GAE: Gallic acid equivalent
[c] IC_{50}: The amount of extract needed to scavenge 50 % of DPPH radicals
[d] AAE: Ascorbic acid equivalent, *significance difference with p-value <0.05.

a great variation in the biological activities (Kowalska et al., 2022). Results shown in Table 2 show differences in values of phenolic content as well as antioxidant activities among the six tested extracts, which indicates possible roles of ecological factors in this diversity (Liu et al., 2016; Zargoosh et al., 2019). *Amaranthus* species are known for their antioxidant and free radical scavenging activities (Bang et al., 2021; Roy et al., 2021). Sharma et al. reported that the methanol extract of *A. viridis* leaves showed free radical scavenging activity against DPPH radical with IC_{50} value of 28.92 μg/mL (Sharma et al., 2012). The methanol extract of *A. viridis* leaves growing in Malaysia exhibited DPPH antioxidant activity with IC_{50} value of 115.74 μg/mL and TPC value of 85.83 (mg GAE/g DW) (Salvamani et al., 2016b). In another study, the methanol extract of *A. viridis* leaves growing in Pakistan displayed DPPH antioxidant activity with IC_{50} value of 14.25 μg/mL (Muhammad Javid Iqbal, 2012). These results agree with our current findings. On the other hand, Jaiswal et al. reported that the 80 % ethanol extract of *A. viridis* leaves growing in India showed ABTS free radical scavenging activity value equal to 121.07 catechin equivalent mg/dm^3 (Jaiswal et al., 2017). While the 75 % ethanol extract of *A. viridis* leaves growing in Thailand exhibited ABTS antioxidant activity with inhibition percent equal to 72.15 % (Maneetong, 2019).

3.3. Cytotoxic effects of the examined extracts

The rise of cancer worldwide is of great concern to healthcare providers (Febriyanti et al., 2021; Zhu et al., 2016). According to the Global Cancer Observatory (GLOBOCAN), the most common malignancies in Egypt (5-year prevalence of all ages) are breast (61,160) and liver (28,977) with a total of 278,165 confirmed case, for all cancer diseases while the highest incidence numbers for specific cancer cases in Egypt in 2020 were: liver (27,895), breast (22,038), bladder (10,655), non-Hodgkin lymphoma (7305), lung (6538), leukemia (5231), and prostate (4767); with a total of 134,632 confirmed case, for all cancer types ("Global Cancer Observatory," n.d.). Many studies have been done by previous research on the medicinal value of *Amaranthus viridis* as anticancer extract to enhance the cytotoxic effect of chemotherapy and decrease its toxicity. Here, we used paclitaxel and DMSO as the positive and negative control respectively. The *in vitro* ability of the six examined extracts AV1-6, were evaluated against two human tumor cancerous cell lines i.e., human liver cancer cell line or hepatocellular carcinoma cell line (HepG2) and human metastatic breast cancer cell line (MDA-MB-231), the examined cancer cell lines were obtained frozen in liquid nitrogen (−180 °C) from VACSERA (the Egyptian Company for Production of Vaccines, Sera and Drugs), where they were originally obtained from the American Type Culture Collection (ATCC). Paclitaxel was used as a positive control against MDA-MB-23 and HepG2 in rang of concentration (0−15) μg/mL where it had IC_{50} values of (14.2 and 13.3) μg/mL, respectively (Fig. S1). Fig. 3 showed the effect of the examined extract against the tested cancer cell lines. While the organic extracts of AV2, 3 and 4 displayed cytotoxic ability against MDA-MB-23 and HepG2 with IC_{50} values of (49.5 ± 0.39, 62.0 ± 0.54 and 81.0 ± 0.22) and (74.0 ± 0.4, 70.0 ± 0.7 and 74.0 ± 0.26) μg/mL, respectively, the organic extracts of AV1, 5 and 6 showed no cytotoxicity against the examined cancer cell lines. After analyzing the chemical composition of the different examined extracts of AV which showed that the three extracts AV2, 3 and 4 have high abundance of Guttiferic acid **(26)** a

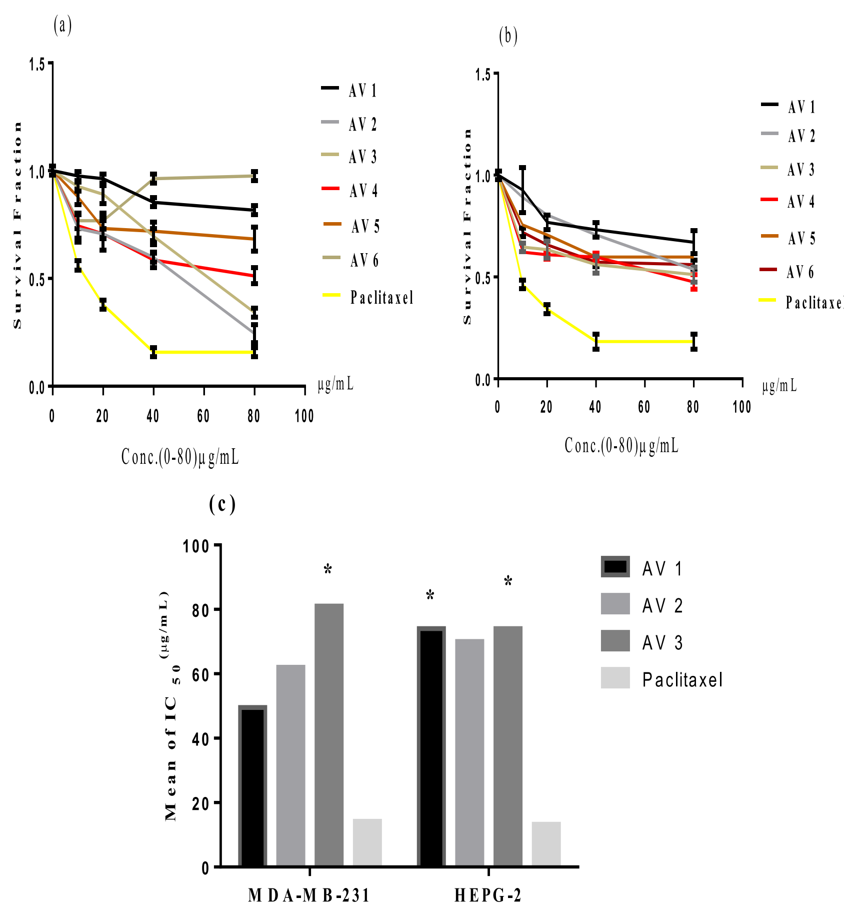

Fig. 3. a). represents the effect of different extracts of AV against MDA-MB-231 cell line, at different concentration 0,10,20,40, and 80 μg/mL. b). represents the effect of different extracts of AV against HEPG-2 cell line, at different concentration 0,10,20,40, and 80 μg/mL. c). comparison between the mean of IC_{50} of positive control (paclitaxel) and AV 1, 2 and 3 extracts, significant difference (* p-value $\leq$ 0.05).

xanthone derivative as active ingredient, which might play an important role for cytotoxicity of the abovementioned extracts towards the examined cancer cell lines.

Xanthone derivatives have also been observed in other studies to show antioxidant, anti-inflammatory, and anti-tyrosinase effects. Recent updates on these biological effects of xanthone derivatives are already available (Feng et al., 2020; Gunter et al., 2020; Rosa et al., 2021). Shagufta and Ahmed, revealed that cyclooxygenase, protein kinase, and topoisomerase are the protein receptors that xanthone derivatives can connect with, confirming their anticancer effect (Shagufta and Ahmad, 2016). Moreover, the observed anticancer activity of AV2, 3 and 4 may be attributed to high abundance of alkaloids i.e., 3,4-dihydro-alangiobussine **(2)**, tabersonine **(6)**, vomilenine **(12)**, *N*-tricosanoyltryptamine **(20)** and euxylophoricine D **(23)**. Alkaloids has a large range of bioactivities, such as, antibacterial, myocardial ischemia-reperfusion injury protection, antidiabetic, anti-inflammatory, antiulcer, blood vessels expansion, platelet aggregation inhibition, neuroprotective, and hepatoprotective effects (Han et al., 2011; Kulkarni and Dhir, 2010; Yu et al., 2005). In both *in vitro* and *in vivo* studies alkaloids have shown synergistic or enhancing effects when combined with chemotherapeutic drugs (Makhov et al., 2012; Zhang et al., 2011). Furthermore, the anticancer ability of extracts 2 and 4 may be attributed to the presence of quercetol A **(4)**, a polyphenol derivative. Polyphenolics derivatives can serve as anticancer agent through different mode of actions, such as altering signaling pathways (Bhamre et al., 2010; Höpfner et al., 2008), causing arresting in cell cycle events, and inducing apoptosis, additionally, polyphenols control the actions of enzymes necessary for the growth of malignant cells or prolifration (Höpfner et al., 2008; Qing et al., 2012). Recent research has linked natural polyphenols to a variety of anti-cancer effects, including antiangiogenic, antimetastasis, DNA interaction (Abbas et al., 2017; Spatafora and Tringali, 2012). Similar finding to our results were reported by House et al. who examined anticancer effects of methanolic extract of *A. viridis* against MDA-MB-231 with IC_{50} values 101 μg/mL (House et al., 2020). Meanwhile, Jin et al. found that *A. viridis* organic extract displayed greater anticancer effect against HT-29 and HepG2 cancer cells (Jin et al., 2013).

Paclitaxel is a cytotoxic drug used as a standard chemotherapy in the treatment of different types of tumours including ovarian, breast, and non-small-cell lung (Eniu et al., 2005). Herein, while paclitaxel has a more potent cytotoxic activity, the extracts have promising effects as seen in their IC_{50s}. These results can be explained due to the presence of flavonoid, quercetin, xanthone, polyphenolic compounds which have been found to possess cytotoxic effects against cancer cells. These finding can be the foundation for finding a natural remedy that can be developed further for the treatment and management of cancer.

3.4. Antimicrobial properties

One of the major health concerns in the world is the quick rise in antibiotic resistance. Owing to, various types of bacteria and fungi involved in the spread of human serious infections such as *S. aureus, P. aeruginosa, E. coli* and *A. niger* (Peleg and Hooper, 2010; Sedighi et al., 2015), Numerous studies have looked at antibacterial properties of various medicinal plant extracts in several areas of the world (Banadkoki et al., 2018; Govindasamy and Srinivasan, 2012). Therefore, it is essential to investigate how plant bioactive compounds and their antimicrobial activities are influenced by ecological habitats. The six *A. viridis* extracts were assessed for their potential antibacterial and antifungal effects that cause human diseases.

3.4.1. Antibacterial activity

Millions of chemical compounds have been synthesized, and thousands of them have been tested for their ability to combat microbes. Previous studies on comparative analysis of chloroform, ethanol, and methanol extracts of *A. viridis* leaves and stems confirmed their antibacterial efficacy against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*E. coli, K. pneumoniae*, etc.) bacteria (Malik* et al., 2016). In another study, the antibacterial activity of *A. viridis* was tested against pathogenic and clinically significant bacteria and it showed maximum antibacterial activity against *K. pneumonia,* while leaf extracts of *A. viridis* showed approximately equal zone of inhibition against *S. aureus* and *E. coli* (Maiyo et al., 2010). Table 3 shows that all plant extract of AV1-6 have moderate or weak antibacterial activity on *E. coli* compared to control (ciprofloxacin). Furthermore, all extracts of *A. viridis* (AV1-6) displayed no antibacterial activity against pathogenic strain *P. aeruginosa* which might act as more resistant to plant extracts of *A. viridis*. There were statistically significant differences with *p-value* <0.001 for antibacterial activity of methanol extracts *A. viridis* (AV1 and AV6) against different bacterial strains (*S. typhi, S. aureus, and E. coli*), compared to control (ciprofloxacin). In contrast, AV5 showed low antibacterial activity against *S. aureus*, while AV3 showed no activity against the same organism.

Interestingly, our results indicated that the highest antibacterial potency was observed for AV2 against *S. typhi*. According to Iqbal et al. and Walter et al., *A. viridis* leaves extract had higher anti-microbial activity against *S. aureus* and *E. coli* than stem extract (Muhammad Javid Iqbal, 2012; Walter et al., 2011). Previous reports have shown higher antibacterial activity of ethyl acetate extract of *A. viridis* leaf against all tested microorganisms compared to ethyl acetate extract of *A. viridis* root (Malik* et al., 2016; Ragasa et al., 2015). Also, the highest inhibitory effects were observed in case of leaf extract of *A. viridis* against *P. syringae* and *E. carotovora*, respectively (Akbar et al., 2020). In another study, better antimicrobial activity of *A. viridis* leaf and seed were found against *Staphylococcus aureus* and *Escherichia coli*, and the fungi: *Fusarium solani* and *Rhizopus oligosporus*. Thus, our results support earlier findings that *A. viridis* leaf can be a potential source of antimicrobial agents (Lipkin et al., 2005; Muhammad Javid Iqbal, 2012).

Table 4 showed the MIC values of the examined extracts AV1-6 against clinically important bacterial strains. Results indicate that there were statistical significant differences with *p*-value <0.001

Table 3
Comparisons of antibacterial activity of each plant extracts *A. viridis* against bacterial strains.

Sample code	*S. typhi*	*S. aureus*	*E. coli*	*P. aeruginosa*	*p*-value
AV1	71.54 ± 1.4	77.22 ± 1.1	18.84 ± 0.11	0 ± 0	<0.001*
AV2	82.25 ± 0.25	41.17 ± 1.04	24.68 ± 1.3	0 ± 0	<0.001*
AV3	76.03 ± 0.17	0 ± 0	31.28 ± 0.78	0 ± 0	<0.001*
AV4	49.5 ± 0.5	40.15 ± 0.5	20.87 ± 0.32	0 ± 0	<0.001*
AV5	71.13 ± 1.1	8.25 ± 0.23	26.44 ± 0.45	0 ± 0	<0.001*
AV6	47.76 ± 1.1	76.10 ± 0.49	14.78 ± 0.61	0 ± 0	<0.001*
Cip	0.39 ± 0.001	–	1.01 ± 0.01	–	<0.001*

Results are (means ± S.D.) (n = 3), *Significance difference of antibacterial activity of each plant extracts (AV1-AV6) against bacterial strains with *p*-value <0.05. Positive control (Cip: Ciprofloxacin).

Table 4
Comparisons of Minimum Inhibitory Concentrations (MIC) of each plant extract *A.viridis* against bacterial strains.

	Minimum Inhibitory Concentration (MIC, μg/mL)			
Sample code	*S. typhi*	*S. aureus*	*E. coli*	*p*-value
AV1	24.50 ± 0.50	12.27 ± 0.25	125 ± 0.50	<0.001*
AV2	49.5 ± 0.5	24.5 ± 0.50	74.73 ± 0.64	<0.001*
AV3	24.5 ± 0.50	0 ± 0	40 ± 0.50	<0.001*
AV4	49.5 ± 0.5	125 ± 0.5	99.87 ± 0.32	<0.001*
AV5	34.5 ± 0.5	0 ± 0	125 ± 0.50	<0.001*
AV6	74.73 ± 0.64	12.3 ± 0.20	99.87 ± 0.32	<0.001*
Cip	0.39 ± 0.001	–	1.01 ± 0.01	<0.001*

Results are (means ± S.D.) (n = 3), Significance difference lowest concentration of antimicrobial agent (MIC) in each plant extracts (AV1-AV6) against bacterial strains with *p*-value <0.05. Positive control agent (Cip: Ciprofloxacin).

between the six extracts of *A.viridis* AV1-AV6 against the bacterial strains (*S. typhi, S. areues,* and *E.coli*).

In the current study, results obtained exhibited a high level of MIC for three plant extracts (AV1, AV4 and AV6) against *E. coli, S. aureus* and *S. typhi* compared to control agent (ciprofloxacin). However, the results showed no detection for MIC values of two plants extracts (AV3 and AV5) against *S. aureus*. In addition to, results revealed that only two plant extracts (AV1and AV4) have the highest MIC values against *S. aureus* and *E. coli* compared to the remaining plant extracts (AV2, AV3, AV4 and AV5) and control respectively. Our results were consistent with a previous study investigated that the betacyanin fraction from *Amaranthus dubius* showed a high inhibitory effect against nine Gram-positive and five Gram-negative bacterial strains (Yong et al., 2017). As in previous studies, this study confirmed that *E. coli* was most sensitive microbe tested, showing the largest inhibition zones for *A. viridis* leaves and minimum for seeds extracts (Ahmed et al., 2013; Lipkin et al., 2005). The present results confirm earlier studies that shown that family of *Amarathaceae* have antibacterial activities (Ahmed et al., 2013; Cai et al., 2005).

3.4.2. Antifungal activity

In this study, the antifungal activities of each plant extracts (AV1-AV6) against fungal strains are shown in Table 5. The trend for antifungal activity was similar to that for antibacterial activity, with the exception that the efficacy against fungal strains was not as effective as for bacterial strains. The results revealed that there was a statistically significant difference with *p*-value <0.001 in antifungal activity in each methanol extracts *A. viridis* AV1-AV6 against three different fungal strains (*A. flavus, A. niger* and *C. albicans*). AV6 showed the highest inhibitory effects against both *A. flavus* and *A. nigr* compared to control (Fluconazole). AV2 and AV5 showed the least antifungal activity against pathogenic yeast *C. albicans*. AV6 showed no antifungal activity on two fungal strains *C. albicans* and *R. solani*. Similarly, AV2 did not show any antifungal activity against A. flavus and *A. niger*. As shown in Table 5, phytopathgenic fungus *R.solani* was more resistant to antifungal effects of any of the six examined extracts of *A. viridis* (AV1-6).

Table 5
Antifungal activity of *A. viridis* against fungal strains.

Sample code	*A. flavus*	*A. niger*	*C. albicans*	*R. solani*	*P*-value
AV 1	23.68 ± 1.3	50.58 ± 0.59	16.26 ± 0.58	0 ± 0	<0.001*
AV2	0 ± 0	0 ± 0	10.84 ± 0.21	0 ± 0	<0.001*
AV3	26.22 ± 0.54	0 ± 0	16.73 ± 0.30	0 ± 0	<0.001*
AV4	0 ± 0	0 ± 0	15.25 ± 0.54	0 ± 0	<0.001*
AV5	0 ± 0	0 ± 0	10.24 ± 0.49	0 ± 0	<0.001*
AV6	57.08 ± 0.60	53.71 ± 0.55	0 ± 0	0 ± 0	<0.001*
Flu	-	98.00 ± 0.12	99.50 ± 0.23	-	––

Results are (means ± S.D.) (n = 3), *Significance difference of antifungal activity of each plant extracts (AV1-AV6) against fungal strains with *p*-value <0.05. Positive control agent (Flu: Fluconazole).

Previous studies have exhibited that the methanolic and ethanolic extracts of *Amaranthus* sp. were screened for antifungal against tested mycotoxigenic fungi such as (*Penicillium verrucosum, Penicillium expansum, Fusarium graminearum, Aspergillus ochraceus, Aspergillus niger*) were used. The most effective extracts were *A.deflexus, A. hybridus* ethanol flower extract, *A.retroflexus*, ethanolic root extract *A. retroflexus*, methanol leaves and stem extract showed activity against all examined strains (Terzieva et al., 2019). In addition, hexanic, ethylacetate and ethanolic extracts of *Amaranthus viridis* showed antifungal activity against *Fusarim solani* (Carminate et al., 2012).

3.4.3. Antibiofilm assay

Since biofilm-forming bacteria are 10 to 1000-fold more resistant to routinely used antibiotics, it is extremely difficult to produce antimicrobials specifically to treat biofilms. Bacterial biofilm plays a crucial role in the persistence of bacterial illnesses. Traditional outcomes of in vitro antimicrobial susceptibility testing, including the MIC determination, may not be suitable to direct treatment for biofilm-associated infections (Pletzer and Hancock, 2016). Accordingly, we used a microtiter biofilm plate assay to assess the six pure extracts' biofilm inhibitory activities against four clinical biofilm-forming pathogenic microorganisms. There are numerous substances that can damage a bacterial cellular membrane, cytoplasm, and protein such as terpenes and carvacrol are two active components of herbal plants, have a substantial impact on many bacterial sites and can help to completely eradicate bacterial illness (Kapoor et al., 2017). The obtained results from Table 6, showed that there were statistically significant differences with *p*-value <0.001 in antibiofilm effect (biofilm inhibitory) of each plant extracts AV1-6 against four pathogenic bacterial strains (*S. aureus, E. coli, P. auerginosa* and *B. subtilis*).

The results indicated that the highest antibiofilm was noticeable from plant extract AV5 against *B. subtilis*. Whereas results revealed no biofilm inhibitory effect of plant extract AV1 against all tested bacterial strains (*S. aureus, E. coli, P. auerginosa* and *B. subtilis*). Likewise, the results showed the plant extract AV2 with no biofilm inhibitory values were detected against three bacterial strains (*S. aureus, E. coli*, and *B. subtilis*). As well, the results exhibited that plant extract AV2 has least antibiofilm activity against *P. aeruginosa*. Our findings agreed with a recent study that investigated the chemical profile, phytotoxic and antibiofilm properties of six different species of eucalyptus (Eos) that grow in Tunisia (Polito et al., 2022). According to those outcomes, both Gram-positive (*Staphylococcus aureus* and *Listeria monocytogenes*) and Gram-negative (*Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Escherichia coli*) bacterial strains were significantly inhibited in their ability to form bacterial biofilms and to function metabolically.

Plant extracts are well known with its highly content of terpenes and phenolics compounds, which are reported by different researchers as antimicrobial compounds (Manganyi and Ateba, 2020; Tian et al., 2022). The antimicrobial ability of the examined extracts may be attributed for one or more or the combination of the detected secondary metabolities which detected using LC-HRES-MS technique, as shown in Fig. 2, characteristic phytochemicals compounds were most

Table 6
Comparisons of biofilm inhibition (antibiofilm) effect of each plant *extracts A. viridis* against pathogenic bacterial strains.

Sample code	*S. aureus*	*E. coli*	*P. aeruginosa*	*B. subtilis*	*P*-value
AV 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	–
AV2	0 ± 0	0 ± 0	5.74 ± 0.51	0 ± 0	<0.001*
AV3	0 ± 0	8.87 ± 0.27	0 ± 0	41.18 ± 0.17	<0.001*
AV4	0 ± 0	23.15 ± 0.22	0 ± 0	0 ± 0	<0.001*
AV5	0 ± 0	11.61 ± 0.66	0 ± 0	59.36 ± 0.68	<0.001*
AV6	0 ± 0	0.49 ± 0.05	17.2 ± 0.97	26.44 ± 0.49	<0.001*

Results are (means ± S.D.) (n = 3), *Significance difference of antibiofilm activity of each plant extracts (AV1-AV6) against bacterial strains with *p*-value <0.05.

prominent in each extract such as euxylophoricine D, guttiferic acid and 2-acetyl-1-hydroxyanthraquinone in AV1, AV2, and AV6 plant extracts which might be play important role in antibacterial potency of *A. viridis* extracts against S. aureus and S. typhi. Also, obtained results as clear in table S1, xanthone derivatives was the highest abundance in AV4 compared to other extracts (AV1, AV2 and AV3).

Narasimhan et al., report that chemical xanthone derivatives is active constituent have antibacterial activity and antifungal activity (Narasimhan et al., 2017). Furthermore, alkaloids have a large range of bioactivities, such as, antibacterial activity (Meng et al., 2022; Yan et al., 2021), obtained results from table S1, showed that alkaloids were high abundance in four extract AV1, AV2, AV4 and AV6. Our results are consistent with an earlier study that discovered 561 antibacterial phytochemicals, including 77 alkaloids, as novel antimicrobials. This study also discovered alternative herbal chemotherapeutic agents to combat antibiotic-resistant microorganisms (Setzer et al., 2016). Besides, in prior study, *A. viridis* L. leaf and seed extracts showed phytochemical profiling with antioxidant and antibacterial screening (Ahmed et al., 2013). Despite of gram-negative bacteria are thought to be more resistant because their outer membrane serves as a barrier to numerous environmental elements, including antibiotics. Nonetheless, according to present findings, certain components of *A. virids* extracts have high antibacterial and antibiofilm action. Therefore, important therapeutic ingredients have been introduced under the name *A. virids*.

3.5. *Environmental impact of the study area on secondary metabolites profile of Amaranthus viridis*

Despite the scarcity of available meteorological data concerning Fayoum Depression, the maximal recorded levels of temperature in 2016 were 47.62 °C for bare land, 41.73 °C for urbanized areas and 39.12 °C for vegetation. (The latest recorded temperature maxima during months of sampling ranged from 35 to 45 °C according to meteorological station of Itsa). As a result, thermal values of different land covers are increasing (El-Zeiny and Effat, 2017). A possible impact is that introduced plant species like *Amaranthus viridis* have shown prodigious growth and have become more invasive in Fayoum Depression as it can germinate successfully in warmer climate (Khan et al., 2022).

According to Egyptian Meteorological Authority ("Egyptian Meteorological Authority," n.d.), Fayoum climate is arid with scarce rainfall, which does not exceed 7.2 mm annually. Fayoum climatic monotony with prevailing arid conditions may influence the activities of plant extracts (AV1 to AV6) but with different efficiency. Along with climatic conditions, the soil factor is considered as the backbone of environmental conditions. Both can influence production of bioactive compounds in plants (Vázquez-León et al., 2017). Troublesome weeds like *Amaranthus viridis* can acclimatize to wide range of variable soil characteristics (Khan et al., 2021).

Although the samples were collected from a narrow area in Egypt, Fayoum Depression has unique natural features in addition with those anthropogenically made. Fayoum like Egypt has both fresh and saline watercourses. Bahr Youssof and Qarun Lake resemble the River Nile and Mediterranean Sea respectively (Elgamal et al., 2020). The districts near Qarun Lake were affected by salinity than others were. Urban sprawl and other human activities were also unequal in constituting land use through the six mentioned districts, so changes in land temperature and emissivity as well as soil quality could be expected.

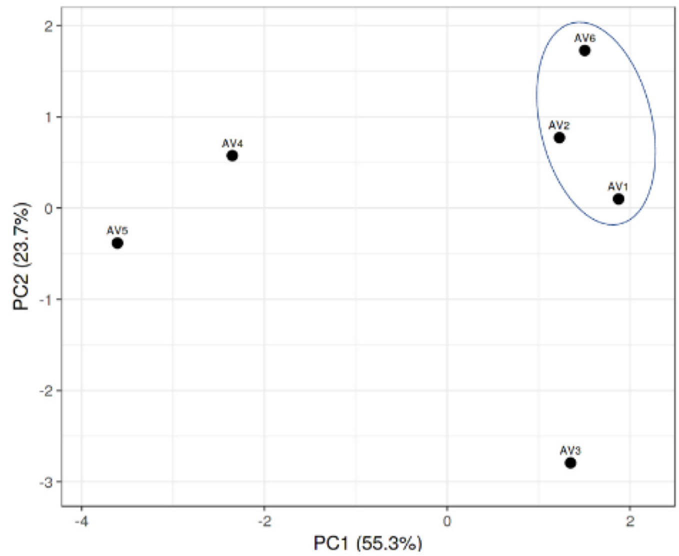

Fig. 4. PCA score plot of *A. viridis* different soils (i.e., AV1-AV6). The plot was generated from the determined soil parameter (Table 7).

In this study, soil physicochemical data was plotted (Fig. 4) using data listed in Table 7. As shown in Fig. 4, *A. viridis* soil parameters distinguished AV1, AV2, and AV6 (i.e., clustered together with no significance difference between them) from AV3, AV4, and AV5 indicating significant differences in soil physicochemical parameters exist between these sites. Similar clustering was observed for plant chemical composition where AV1, AV2, and AV6 clustered together compared to AV3, AV4, and AV5 indicating a possible relationship between soil quality and plant chemical composition (Fig. 2).

Plants often use phytoconstituents to deal with variable abiotic stress such as high temperatures, high salinities, and drought. Functionally significant secondary metabolites have demonstrated distinct biological efficacy and therapeutic potential while facing many sicknesses like oxidative stress, cancer and microbial infection (Mohammed et al., 2023).

The potency of AV6 is significant among other extracts followed by AV2. This performance could be attributed to presence of compounds: euxylophoricine D and guttiferic acid, and 2-acetyl-1-hydroxyanthraquinone. These compounds are often produced by plants to cope with the stressful environmental conditions (Dehghanian et al., 2022; Kleinwächter and Selmar, 2015). From this

Table 7
Physiochemical analysis of soil samples.

Sample code	pH	ECe dS/m/25 °C	CaCO3 (%)	O.M. (%)	Na mg/kg	K mg/kg	Ca mg/kg	Mg mg/kg	Pb mg/kg
AV 1	7.58 ± 0.25	2.33 ± 0.35	5.28 ± 0.60	1.25 ± 0.23	9.03 ± 0.77	1.28 ± 0.27	9.30 ± 0.91	7.31 ± 0.54	8.90 ± 0.88
AV2	7.58 ± 0.26	3.82 ± 0.71	9.31 ± 0.13	1.21 ± 0.11	17.2 ± 0.28	0.56 ± 0.07	9.66 ± 0.53	8.66 ± 0.21	9.19 ± 0.17
AV3	7.57 ± 0.22	3.50 ± 0.86	5.22 ± 0.37	0.81 ± 0.17	16.52 ± 2.04	0.88 ± 0.19	13.31 ± 1.84	7.40 ± 1.36	42.3 ± 4.57
AV4	7.69 ± 0.26	6.32 ± 0.99	15.82 ± 0.29	1.48 ± 0.19	34.63 ± 2.28	0.92 ± 0.30	17.81 ± 2.31	13.1 ± 1.56	15.60 ± 0.86
AV5	7.68 ± 0.22	9.93 ± 1.98	10.18 ± 0.60	1.95 ± 0.23	24.79 ± 2.35	0.78 ± 0.11	16.83 ± 0.31	16.86 ± 0.57	15.84 ± 0.81
AV6	7.52 ± 0.26	3.50 ± 0.53	14.05 ± 0.44	1.27 ± 0.19	22.78 ± 1.35	0.77 ± 0.16	7.83 ± 0.72	7.05 ± 1.07	6.75 ± 0.56
p-value	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

Results are (means ± S.D.) (n = 3) *significance differences with p-value <0.05.

perspective, Youssef Al-Seddik and Atsa districts, which were the habitats of AV6 and AV2 respectively, are assumed as edges. According to El-Ghonamey et al. the western border of Youssef Al-Seddik districts is characterized by alkaline soil with high percentage of $CaCO_3$, salinity and sand. Youssef Al-Seddik is a desert extension anthropogenically affected by reclamation and urbanization (EL GHONAMEY et al., 2018). It can be visually observed (Fig. S2) that a great proportion of the district is in the vicinity of high saline Qaroun Lake (El-Zeiny and Effat, 2017). Poor soil, lack of organic matter and increasing salinity are holistically affecting secondary metabolite production in plant in this area (Pant et al., 2021)

El-Zeiny and Effat, stated that urban sprawl in Atsa has doubled during the last decade (2003–2013) and the vegetation area has slightly retreated during the period (2013 to 2016) (El-Zeiny and Effat, 2017). Increasing temperature, aridity and $CaCO_3$ content are the ultimate impact of urbanization while road density and buildings absorb solar radiation and alter the passages of runoff (Kabano et al., 2022; Solins and Cadenasso, 2020). While facing environmental challenges, plants possess adaptive physiological features in the form of active constituents (Ramakrishna and Ravishankar, 2011). Ibshaway was the habitat from which AV1 was collected. In this district, the increase in urban and water bodies was on the expense of the agricultural land loss in 2003–2013 (El-Zeiny and Effat, 2017). Youssef Al-Seddik, Atsa and Ibshaway districts were clustered together because they had common receding in agricultural areas brought on by anthropogenic interference that reduced the quality of the soil. The similarity in chemical composition for AV1, AV2 and AV6 may arise from the stress of poor soil properties.

4. Conclusions

The present study has shown correlations between plant habitat conditions and phytochemical composition of *Amaranthus viridis* aerial parts. In general, all tested extracts were promising in the total phenolics contents (TPC), total antioxidant capacity (TAC) and antibacterial ability. On one hand euxylophoricine D, guttiferic acid, and 2-acetyl-1-hydroxyanthraquinone played a crucial role in antibacterial potency of *A. viridis* extracts against *S. aureus* and *S. typhi*. On the other hand, guttiferic acid, alkaloids and quercitol content for AV2, 3 and 4 extracts support their cytotoxic property and anticancer activity as well. Metabolomic profiling and multivariate analysis have shown that samples AV1, AV2 and AV6 are similar in chemical composition. The finding emphasizes the proximity of Ibshaway, Youssef Al-Seddik and Atsa in land features and in soil characteristics of the collection points, besides they are tangible to each other. To the best of our knowledge, this research confirms the hypothesis of phytochemical ingredients fluctuating ratios as a responsive feature toward environmental adaptation. Further study is required on possible use of this plant as possible source of botanical drugs which can use existing biomass and can help in managing its fast growth in Egypt. Additionally, it would be better if the upcoming study encompasses better understanding of the role of ecological conditions on biosynthetic production of compounds at molecular level.

Supplementary Materials: The following supporting information can be downloaded, Table S1: Detected compounds in the examined extracts.; Figure S1: Effect of paclitaxel against MDA-MB-231 and HEPG-2.; Table S2: Latitudes and Longitudes for sampling sites using GPS; Figure S2: Fayoum depression map; Figure S3: TICs of the studied extracts AV1-AV6 (A-F, respectively).

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Declaration of competing interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

Mai Sayed Fouad: Conceptualization, Methodology, Investigation, Resources, Writing – original draft, Writing – review & editing. **Mosad A. Ghareeb:** Conceptualization, Methodology, Investigation, Resources, Writing – original draft. **Ahmed A. Hamed:** Methodology, Investigation, Resources, Writing – original draft. **Esraa A. Aidy:** Methodology, Investigation, Resources, Writing – original draft. **Jioji Tabudravu:** Methodology, Resources, Writing – original draft, Writing – review & editing. **Ahmed M. Sayed:** Methodology, Resources, Writing – original draft, Writing – review & editing. **Mohamed A. Tammam:** Conceptualization, Methodology, Resources, Writing – original draft, Writing – review & editing. **Mai Ali Mwaheb:** Conceptualization, Methodology, Investigation, Resources, Writing – original draft, Writing – review & editing.

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Supplementary materials

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