OPEN ACCESS
Freely Available Online

openaccessPub

INTERNATIONAL JOURNAL OF NUTRITION
ISSN NO: 2379-7835

Research

DOI: 10.14302/issn.2379-7835.ijn-19-3144

In Vitro Assessment of Antioxidant Enzymes, Phenolic Contents and Antioxidant Capacity of the *Verdolaga* (*Portulacaceae*)

Yaaser Q. Almulaiky[1,2,*], Musab Aldhahri[3,4], Fahad A. Al-abbasi[3], Sami A. Al-Harbi[5], Mohamed H. Shiboob[6]

[1]Chemistry Department, Faculty of Sciences and Arts, University of Jeddah, Khulais, P.O. Box 355, Khulais, 21921, Saudi Arabia

[2]Chemistry Department, Faculty of Applied Science, Taiz University, Taiz, Yemen

[3]Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia

[4]Center of Nanotechnology, King Abdulaziz University, Jeddah, Saudi Arabia

[5]Department of Chemistry, University College in Al-Jamoum, Umm Al-Qura University, Makkah, Saudi Arabia

[6]Environmental Department, Faculty of Meteorology, Environment and Arid Land Agriculture King Abdulaziz University, Jeddah, Saudi Arabia

Abstract

In this study, the antioxidants and photosynthetic compounds of *Verdolaga* were examined. Compounds were extracted from distinctive segments of the *verdolaga* using various solvents such as methanol (40, 60, 80%), ethanol (40, 60, 80%), acetone (40, 60, 80%), and deionized water. The use of 80% methanol led to the highest extracted concentration of phenolic substances and flavonoids. The extracted products (Leaves, Stem strips, and Root strips) were evaluated for their radical scavenging capabilities with DPPH (IC_{50}= 22.26, 20.56, and 32.10), and ABTS (IC_{50}= 2.86, 3.70, and 5.24), reducing power (EC_{50}= 15.70, 16.39, and 21.69), and peroxide scavenging activity ($1C_{50}$= 1.717, 2.937, and 3.255), respectively. The extracted products were analyzed by a gas chromatography-mass spectrometer. Peroxidase, catalase, and polyphenol oxidase assays were completed for the crude extract of verdolaga's leave, stem strips, and root strips. As indicated by these tests, extracts of the verdolaga's roots, stems and leaves using 80% methanol yielded high antioxidant activity. The most elevated concentrations of extracted chlorophyll, lycopene, and carotenoids were from the leaves and the highest concentration of extracted tannin was noted from strips of stems. The highest measures of peroxidase and polyphenol oxidase were identified in root strips and the highest units of catalase was identified in leaves.

Corresponding author: Yaaser Q. Almulaiky, Chemistry Department, Faculty of Sciences and Arts, University of Jeddah, Khulais, P.O. Box 355, Khulais, 21921, Saudi Arabia, Tel. +966566880264. E-mail: yaseralmoliki@hotmail.com

Citation: Yaaser Q. Almulaiky, Musab Aldhahri, Fahad A. Al-abbasi, Sami A. Al-Harbi, Mohamed H. Shiboob (2020) In Vitro Assessment of Antioxidant Enzymes, Phenolic Contents and Antioxidant Capacity of the Verdolaga (Portulacaceae). International Journal of Nutrition - 4(4):36-47. https://doi.org/10.14302/issn.2379-7835.ijn-19-3144

Keywords: Verdolaga, phenolic content, antioxidant enzyme, methanol extracts

Received: Dec 24, 2019 **Accepted:** Jan 02, 2020 **Published:** Jan 06, 2020

Editor: Ishan Wadi, National Institute of Malaria Research, India.

Introduction

Reactive oxygen species (ROS) are produced in higher volumes during tissue injury. An excessive amount of ROS can denature deoxyribonucleic acid (DNA) and proteins, disrupt cell layers, and negatively affect lipids through chain reactions [1]. The production of reactive oxygen species (ROS) mediated lipid peroxidation plays a key role in cell death, including autophagy, ferroptosis, and apoptosis. Cell reinforcements, such as atoms that have the capability to neutralize radical, protect against these damages. These compounds can assist in forestalling diseases, including malignancy, hepatitis, asthma, atherosclerosis, joint inflammation, coronary illness, and diabetes [2]. Recently, plants and herbs have been placed in dietary supplements as cancer prevention agents, as natural alternatives to manufactured cell reinforcements [3]. The reports of side effects from synthetic ingredients of supplements have prompted buyers to explore options that are associated with less egregious results [4]. It has been reported that extracts from herbs are able to support cell defense mechanisms and stimulate antimicrobial activity [3,5]. The extraction of polyphenols from a plant through diverse solvents and the percent yield primarily depends on the strategy for extraction [6, 7]. The extraction strategy must result in the maximum yield of the target compounds [8]. There are a few studies that have utilized blends of ethanol, methanol, acetone and water, to extract polyphenols from plants [3–10]. *Portulaca oleracea*, (common verdolaga) is an imperative restorative plant with range of pharmacological benefits, including the ability to increase the rate of tissue repair and antimicrobial activity. It also contains vitamins A and C, omega-3 unsaturated fats, β-carotene, and α-linolenic acid [11]. The airborne segments of the plant are utilized in different cultures as a diuretic, antibiotic, antispasmodic, and antihelminthic [12]. It also eaten with other greens in the Middle East and Mediterranean, as the stems and leaves are succulent with a salty and acidic taste that is similar to spinach. Verdolaga is an almost certain competitor as a valuable cosmetic ingredient. Thus, further exploration of its unexamined uses can benefit humanity [11]. It is widespread, quickly developing and self-compatible and creates vast quantities of seeds that have long reasonability [13]. The target of this study was to explore the use of different solvents (methanol, ethanol, acetone and water) to extract phenolic substances and flavonoids, and to appraise whether extracts from the leaves, root strips, and stem strips of Verdolaga had the best cancer preventing characteristics.

Materials and Methods

Chemicals

2,2′-Diphenyl-1-picrylhydrazyl (DPPH), gallic acid, Folin-Ciocalteu reagent (FCR), and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Catechin, ferric chloride, catechol, hydrogen peroxide, and guaiacol were purchased from Extrasynthese (Lyon, France). The rest of the standard solvents were purchased from Sigma-Aldrich (USA).

Verdolaga Samples

The sample used in this experiment were collected from Al-Baha city, Saudi Arabia, situated at 20.0119N 41.2607E, Oct, 2019. Leaves, as well as strips of root and stems, were obtained by manual separation, washed, and air-dried at ambient temperature. The air-dried samples were ground and placed in a dry environment until further use except samples used for determination of antioxidant enzymes, fresh sample was used.

Determination of the Antioxidant Enzymes

Crude Enzyme Extracts

Two grams each of fresh leaves, root strips and stem strips were ground in a mortar separately and then combined with 20 mM Tris-HCl buffer, which had a pH of 7.2. The homogenates were centrifuged at 13,000 rpm for 10 min at 4°C. The supernatants were stored as crude extract at -18°C for further analysis.

Class III Plant Peroxidase (EC 1.11.1.7) Assay

Miranda's method was used to estimate the level of peroxidase activity [14]. The crude extract (0.1 mL) was mixed with 40 mM guaiacol (extinction coefficient, $\xi = 26.6\ mM^{-1}\ cm^{-1}$), 8 mM H_2O_2, 50 mM acetate buffer (pH 5.5) for a total 1 mL. The level of absorbance of the mixture was recorded at 470 nm every 1 min using a spectrophotometer.

Polyphenol Oxidase Assay

The polyphenol oxidase activity was measured with catechol (extinction coefficient, $\xi = 2.2 \times 10^3$ mM^{-1} cm^{-1}), as the substrate, as stated by the method used by Siddiq and Cash [15]. The reaction contained 0.9 mL of 20 mM catechol reagent prepared in 10 mM phosphate buffer (pH 6.8) and 0.1 mL crude extract. The absorbance of the mixture, at 420 nm, is recorded for 3 min.

Catalase Assay

The activity of catalase enzymes was detected using the method in Ref [16]. 2 ml of substrate were made by mixing 25 mM H_2O_2 (extinction coefficient, $\xi = 43.6$ mM-1 cm^{-1}), in a 75 mM phosphate buffer (pH 7.0) with 0.5 mL crude extract. The absorbance at 240 nm, was registered for 1 min.

Preparation of Plant Extracts

10 gr each of dried verdolaga leaves, root strips, and stem strips were extracted by combining with different concentrations of organic solvent (80%, 60%, 40% methanol, 80%, 60%, 40% ethanol, and, 80%, 60%, 40% acetone) and 30 mL of deionized water, then shaken at 120 rpm for 24 h. The mixtures were poured through filter paper. The extracts were placed in a cooler at 4°C until they were used in further biochemical assays. The extracts were analyzed in triplicate.

Measurements of Other Contents

Determination of Total Phenolic Content

The phenolic contents were measured using FCR by the method explained by Velioglu [17]. Distilled water (800 µL) and 100 µL FCR were mixed with 100 µL of the plant extract for 5 min at ambient temperature. Then 500 µL of 20% sodium carbonate was added to the reaction mixture. After 30 min the absorbance was recorded at 750 nm. Gallic acid was used as the standard phenolic compound. The results were expressed as an equivalent mg gallic acid/g dry matter (mg GAE/g DM).

Total Flavonoid Content

The flavonoid contents of plant extracts were detected using the method described by Zhishen [18] with a slight modification: a solution of catechin was used as the standard. The reaction mixture was produced by mixing 250 µL plant extract, 1.25 mL distilled water and 75 µL $NaNO_2$ solution (5%) and permitted to stand for 6 min. Then 150 µL of $AlCl_3$ solution (10%), 0.5 mL of 1M NaOH and 275 µL of distilled water was added to the reaction mixture, and permitted to stand for 5 min. After that, the absorbance of the solutions was recorded at 510 nm. The results were calculated as mg catechin equivalent/g dry matter (mg CE /g DM).

Total Condensed Tannin Contents

The tannin contents of each portion of the verdolaga were measured using the methods in [19] with a slight modification, in which catechin was used as standard. The reaction mixture was prepared by mixing 400 µL of plant extract, 3 mL vanillin solution (4% in methanol) and 1.5 mL concentrated hydrochloric acid. The absorbance was recorded at 500 nm after 15 min of incubation at ambient temperature. The tannin contents recorded with the units of µg CE /g DM.

Determination of Carotenoids, Chlorophyll, and Lycopene

The presence of chlorophyll, carotenoids, and lycopene were confirmed by the method that was described by Wang [20]. One gram of each part of the fresh plant was cut into small pieces, mixed well and ground with 10 mL acetone and hexane (40:60). The organic supernatant was transferred into a capped tube that was placed on ice. The remaining aqueous layer was re-extracted with 10 mL of the same solvent and the organic layer was transferred to the same tube, and this process was repeated until the aqueous layer became colorless. One milliliter from the total volume of the organic extract was utilized to determine the absorbance at 450, 502, 645, and 663 nm. The following formulae were used to calculate the concentrations of carotenoids, lycopene, and chlorophylls:

$$\text{Carotenoid } (\mu g/g) = \frac{4 \times OD450 \times \text{Total Volume of organic extract}}{\text{sample weight}} \quad ....(1)$$

$$\text{Chlorophyll} (\mu g/g) = \frac{(20.2 \times OD645) + (8.2 \times OD663) \times \text{Total Volume of organic extract}}{\text{sample weight}} \quad ....(2)$$

$$\text{Lycopene } (\mu g/g) = \frac{3.12 \times OD502 \times \text{Total Volume of organic extract}}{\text{sample weight}} \quad ....(3)$$

Antioxidant Assays

DPPH Radical Scavenging Activity

The free radical scavenging activities (FRSAs) of crude methanol extracts were detected using the DPPH radical scavenging assay [21]. Each methanol extract (100 µL) was added to 900 µL of 0.1 mM freshly prepared DPPH reagent and placed in the dark at 30°C for 30 min. The absorbance was recorded at 517 nm. The scavenging activity (%) was determined by the following equation:

$$\text{DPPH radical scavenging (\%)} = \frac{\text{ODcontrol} - \text{OD sample}}{\text{ODcontrol}} \times 100 \quad ....(4)$$

The results were plotted as the % of scavenging activity versus the sample concentration. The IC_{50} value, which was the concentration that was required to produce 50% FRSA, was interpolated from the plots.

ABTS Radical Cation Decolorization Assay

Re's method was used to estimate the capacity of the extracts to scavenge ABTS radicals (ABTS•+) [22]. The ABTS•+ solution was diluted in 0.1 M sodium phosphate buffer (pH 7.4) to provide an absorbance of 0.750 at 734 nm, then 1 mL of the diluted $ABTS^{\bullet+}$ solution was added to 0.5 mL crude methanol extract. The absorbance was recorded at 1 min after mixing, and the percentage of radical scavenging was relative to a blank that containing no scavengers. The scavenging activity of test compounds was determined using the following equation:

$$\text{ABTS scavenging (\%)} = \frac{\text{ODcontrol} - \text{OD sample}}{\text{ODcontrol}} \times 100 \quad ....(5)$$

Reducing Power Assay

To evaluate the reducing power of the extracts, we used Oyaizu's method [23] with a slight modification: One mL of reaction mixture was made from combining the various concentration of 80% methanol extract, 250 µL of 0.2 M phosphate buffer (pH 6.6) and 250 µL of 1% potassium ferricyanide. The mixture was incubated at 50ºC for 20 min. After, 250 µL of 10% trichloroacetic acid was added, and the mixture was centrifuged at 3000 rpm for 10 min. The supernatant (0.5 mL) was mixed with 0.5 mL distilled water and 0.1 mL of 0.1% ferric chloride, and the absorbance was immediately recorded at 700 nm. The concentration of extract that would produce 0.5 of absorbance (EC_{50}) was interpolated from the diagram of absorbance at 700 nm.

Hydrogen Peroxide Scavenging Activity

Ruch's method was used to evaluate the H_2O_2 scavenging activities of the methanol extracts [24]. A solution of H_2O_2 (2 mM) was mixed with 50 mM sodium phosphate buffer (pH 7.4). The concentration of H_2O_2 was calculated using the molar extinction coefficient of H_2O_2 (81 mol^{-1} cm^{-1}). The 1 mL reaction mixture contained (0.5–2.5 µg/mL methanol extracts, and the volume was made up of 0.4 mL of 50 mM phosphate buffer (pH 7.4), and then 0.6 mL H_2O_2 was added). The reaction mixture was vortexed and its absorbance was recorded at 230 nm over 10 min against a blank solution containing 50 mM phosphate buffer without H_2O_2. Gallic acid (0.5–2.5 µg/mL), was used as a positive control. The scavenging activity was calculated using the following equation:

$$\text{Hydrogen peroxide scavenging activity (\%)} = \frac{\text{ODcontrol} - \text{OD sample}}{\text{ODcontrol}} \times 100 \quad ....(6)$$

Gas Chromatography-Mass Spectrometer (GC-MS) Analysis

The methanol extracts of the verdolaga were analyzed using a TRACE GC ultra-system (Thermo Fisher Scientific, Waltham, MA, USA), equipped with a 30 m X 0.25 mm X 0.25 µm Elite-5-MS capillary column (Thermo Fisher Scientific). The column temperature was increased from 40ºC to 220ºC at a rate of 4ºC/min. The injector temperature was 250ºC; injection volume, 1 µL; helium carrier gas flow rate was 20 mL/min; transfer temperature was 280ºC. MS parameters were as follows: EI mode, with an ionization voltage of 70 eV, an ion source temperature of 180ºC, and a scan range of 50-600 Da. The peaks were tentatively identified based on a library search using NIST and Wiley Registry 8 Edition.

Statistical Analysis

Data were analyzed by a one-way ANOVA and the Student's t-test. The results were expressed as mean ± SD. The results were significant when $P < 0.05$.

Results and Discussion

The capacity for antioxidant activity in plant tissues is related to the level of cell-reinforcing substances that are present, including phenolic compounds, carotenoids, tocopherol, ascorbic acid, and compounds that are able to catalyze the scavenging of free radicals (e.g., catalase, polyphenol oxidase, and peroxidase) [25]. Furthermore, phenolics and anthocyanins, as cancer prevention agents, are associated with oxidative reactions. Catalase, peroxidase, and polyphenol oxidase have been identified in verdolaga extracts (Table 1). In this study, the peroxidase enzyme activity was high in verdolaga root strips, stem strips and leaves (225 ± 0.124, 49 ± 0.86 and 71.5 ± 0.101 units per gram of tissue, respectively). The action of polyphenol oxidase, one of the terminal oxidases in the plant cell, was improved under unfavorable conditions. This enzyme, alongside peroxidase, engaged in the oxidation of phenolic components, which are cancer prevention agents that favor cell resistance [26]. Therefore, a measurement of the concentration of oxidizing phenols could be representative of cell's vulnerability. In the present investigation, polyphenol oxidase was highly active in the verdolaga's root strips, stem strips, and leaves (132 ± 0.79, 83.2 ± 0.43 and 61.7 ± 0.56 units per g tissue, respectively). Catalase activity was present in the verdolaga's leaves, root strips, and stem strips as well (60.25 ± 0.25, 23±0.22 and 15.13 ± 0.64 units per g tissue, respectively). These enzymes have been examined in other plants, including the peroxidase from the latex of *Euphorbia cotinifolia* [27]. The activity of catalase and antioxidant capacity were screened in nine medicinal plants that are customarily provided in Chinese prescriptions [28]. The partial characterization of the activity of polyphenol oxidase in the herb *Thymus longicaulis subsp. chaubardii var* has been described [29].

In order to extract phenolic and flavonoid compounds, the most ideal solvent must be selected. Table 2 shows the ability of different solvents in extracting phenolic and flavonoid substances from various parts of verdolaga. After the extraction process, methanol (40, 60, 80%), ethanol (40, 60, 80%), acetone (40, 60, 80%) and water were examined to assess for the most ideal solvent to be utilized in this investigation. Among four solvents, the methanol (80%) yielded the highest concentration of elevated absolute phenolic compounds (15.7, 12.8 and 9.1mg GAE/g DM for leaves, stem strips, and root strips, respectively). Flavonoid contents were also of higher concentration in 80% methanol (5.5, 4.1 and 2.6 CE/g DM for Leaves, stem strips, and root strips, respectively). Methanol was the most appropriate solvent to extract polyphenolic compounds from plant tissues, because of its capacity to repress the activity of polyphenol oxidase, which is an enzyme that causes the oxidation of polyphenols and its ease of dissipation, in contrast to water [9]. Methanol extracts have been utilized in the investigation of antioxidant activities and flavonoids compounds in the wood pulp and pericarp of *Caesalpinia decapetala* [30] and *Lantana camara* [31]. Tannins are phenolic plant secondary compounds and are present throughout the plant kingdom. They exist in structures called condensed tannins (CTs) [32]. Jones and Mangan wrote that CTs can attach to proteins at close proximities (pH 3.5–7.5) to form CT–protein structures, which separate at a pH of 3.5 [33]. In most cases CTs are available in the leaves

Table 1. The antioxidant enzyme activities of Verdolagasamples.

portulaca oleracea	Units of Peroxidase/g Tissue	Units of Polyphenol Oxidase/g Tissue	Units of Catalase/g Tissue
Leaves	71.5 ± 0.101	61.7 ± 0.56	60.25 ± 0.25
Stem strips	49 ± 0.86	83.2 ± 0.43	15.13 ± 0.64
Root strips	225 ± 0.124	132 ± 0.79	23 ± 0.22

Values are presented as means± SE (n=3)

Table 2. Solvent effect on phenolic contents and flavonoids of Verdolaga

Solvent	phenolic contents GAE/g DM			Flavonoids CE/ g DM		
	Leaves	Stem strips	Root strips	Leaves	Stem strips	Root strips
Methanol 80%	15.7±1.05	12.8±0.88	9.1±0.53	5.5±0.22	4.1±0.75	2.6±0.64
Methanol 60%	13.5±1.11	10.6±0.54	8.4±0.64	3.5±0.16	3.3±0.43	2.3±0.34
Methanol 40%	10.2 ± 1.02	8.7 ± 0.37	7.3 ± 0.78	2.9 ± 0.26	2.8 ± 0.23	2.5 ± 0.72
Ethanol 80%	12.3 ± 1.04	10.5 ± 0.21	8.2 ± 0.45	3.2 ± 0.24	3.2 ± 0.11	2.9 ± 0.24
Ethanol 60%	10.1 ± 1.05	8.9 ± 0.85	7.4 ± 0.32	2.5 ± 0.15	2.3 ± 0.16	1.9 ± 0.26
Ethanol 40%	7.8 ± 0.75	6.9 ± 0.71	6.6 ± 0.12	1.3 ± 0.19	1.8 ± 0.10	1.6 ± 0.28
Acetone 80%	13 ± 0.82	11.1 ± 0.92	8.9 ± 0.12	4.4 ± 0.49	3.1 ± 0.14	3.4 ± 0.23
Acetone 60%	12.2 ± 0.53	9.0 ± 0.11	8.1 ± 0.015	3.8 ± 0.73	2.6 ± 0.19	2.8 ± 0.63
Acetone 40%	10.6 ± 0.77	7.4 ± 0.78	7.3 ± 0.013	2.6 ± 0.34	2.3 ± 0.16	2.1 ± 0.38
water	9.5 ± 0.014	7.8 ± 0.026	5.4 ± 0.01	1.8 ± 0.52	1.5 ± 0.13	2.5 ± 0.43

GAE, gallic acid equivalent, CE, catechin equivalent. Values are presented as means± SE (n=3)

Table 3. The total concentration of antioxidant in Verdolagasamples

	Leaves	Stem strips	Root strips
Chlorophyll (µg/g DM)	531.78 ± 0.69	83.75 ± 0.35	2.75 ± 0.10
Lycopene (µg/ g DM)	28.63 ± 0.03	4.84 ± 0.05	3.32 ± 0.13
Total carotenoids (µg/ g DM)	271.9 ± 0.41	48.8 ± 0.26	9.41 ± 0.17
Tannin contents (µg CE/g DM)	497.8 ± 0.34	533.9 ± 0.39	368 ± 0.12

Values are presented as means ± SD (n=3)

Table 4. The antioxidant effects of gallic acid equivalents in Verdolagaon the reduction of DPPH, ABTS radicals, and reducing power.

portulaca oleracea	DPPH		ABTS		reducing power	
	IC_{50} (µg GAE)	R^2	IC_{50} (µg GAE)	R^2	EC_{50} (µg GAE)	R^2
Leaves	25.26	0.952	2.86	0.938	15.70	0.969
Stem strips	20.56	0.965	3.70	0.994	16.39	0.969
Root strips	32.10	0.966	5.24	0.974	21.69	0.963

IC_{50} is the inhibition concentration, which is the concentration that is required to produce 50% free radical scavenging activity. EC_{50} is the efficient concentration, which is the concentration of extract that would produce 0.5 units of absorbance. R^2 is the correlation coefficient between the phenolic contents and the DPPH scavenging activities, ABTS scavenging activities, and reducing power.

and stems of plants while in a few studies, CTs have been found only in the petals of flowers, including white and red clover [34]. The concentration of CTs in *P. oleracea* are shown in Table 3. The highest concentration of CTs identified in stem strips (533.9 µg CE/g DM) compared to leaves and root strips (497.8 and 368 µg CE/g DM, respectively). Methanol has been shown to be excellent in extracting CT, demonstrated by a process involving *Limonium delicatulum* (48.38 mg/g DM) [35]. Chlorophyll enables the conversion of light energy into plant substance. Chlorophyll, lycopene, and other carotenoids were found in fresh leaves, stem strips, and root strips (Table 3). Chlorophyll (531.78 µg/g DM), total carotenoids (271.9 µg/g DM), and lycopene (28.63 µg/g DM) were higher in leaves ($P < 0.05$) than in stem strips and root strips. Singlet oxygen quenching via carotenoids occurs through physical or chemical quenching which has been discussed in a few studies [36, 37]. The viability of physical quenching surpasses that of chemical quenching and includes the exchange of electrons from $^1O_2$ to the carotenoid, leading to the formation of a ground-state oxygen and an energized, triplet-state carotenoid. Carotenoids are situated in chromoplasts, as they color vegetables and other organic products. Along with chlorophyll, they are engaged in two photosystems. Vechetel and Ruppel [38] detailed that carotenes provided the most critical photosynthetic colors, and they protected chlorophyll and thylakoid films from peroxidation. The scavenging of a stable DPPH radical is a widely used method to estimate antioxidant activity [39]. The phenolic substance in three segments of verdolaga demonstrated a low level of scavenging of the DPPH radical (Table 4 and Fig. 1a, b, and c in the Supplementary Material). The IC_{50} of methanol extraction products from leaves, stem strips, and root strips were 25.26, 20.56 and 32.10 µg GAE/mL, respectively. The correlation coefficient (R^2) between the phenolic contents and DPPH scavenging activities for leaves, and stem strips and root strips were 0.952, 0.965, and 0.966, respectively, which indicated a strong correlation. The IC_{50} values measured for Yemeni guava segments ranged from 19 to 22 µg /mL [40]. ABTS is a basic and frequently utilized technique to assess the activity of cancer prevention agents [41, 42]. The phenolic substances in verdolaga demonstrated their reliance on the convergence of the ABTS radical, which might contribute to its reducing capacity (Table 4). Each of these concentrates presented a linear variety of inhibition power with the additional concentration of extract (Fig. 2a, b, and c in the Supplementary Material). The IC_{50} value for leaves, stem strips, and root strips were found to be 2.86, 3.70 and 5.24 µg GAE/mL, respectively. Compounds with reducing power demonstrate that they are electron providers and can reduce the oxidized intermediates of lipid peroxidation products. Thus, they are essential and are auxiliary antioxidants [43]. It was evident that methanol became more powerful in extracting compounds from verdolaga (Fig.3a, b, and c in the Supplementary Material). The presence of reducers nearby causes the transformation of the Fe^{3+}/ferricyanide complex to become the ferrous structure. The development of Perl's Prussian blue was recognized at 700 nm and shows a higher reducing power. The reducing power of root strips was observed to be higher than that of stem strips and leaves (EC_{50} 21.69, 16.39, and 15.70 µg GAE, respectively) (Table 4). The R^2 between the phenolic group for the leaves, strips of stems and roots, and the formation of the ferrous complexes were observed to be 0.969, 0.969, and 0963, respectively, demonstrating a strong relationship. It was noted that the reducing properties were because of the proximity of reductones. Reductones are able to act as a cancer prevention agent by breaking the free radical chain, by providing a hydrogen molecule [44]. The EC_{50} values for the medicinal plant, *Coleus forskohlii*, was found to be 96.15 and 14.6 µg phenolic concentrations/mL for its stem and leaves, respectively [45]. The scavenging capacities of methanol extraction products from verdolaga on H_2O_2 are in Figure 1 and Table 5. They are compared to gallic acid. The verdolaga extracts were intense in scavenging H_2O_2 in a sum subordinate way. To exhibit 50% scavenging activity on H_2O_2, 1.717, 2.937, and 3.255 µg gallic acid equivalent was required to equal the strength of leaves, stem strips, and root strips, respectively. On the other hand, the elimination of H_2O_2 was 50% with 1.296 µg gallic acid. Because H_2O_2 has no unpaired electrons, it does not qualify as a radical. However, it tends to be lethal to a cell because it might deliver hydroxyl radicals to the cells [46]. Therefore, the disposal of H_2O_2 is beneficial for the antioxidant defense

Table 5. The antioxidant effect of the gallic acid equivalent of Verdolagaon Hydrogen peroxide hydrolysis scavenging.

portulaca oleracea	H_2O_2 hydrolysis scavenging %	
	IC_{50} (µg GAE)	R^2
Leaves	1.717	0.993
Stem strips	2.937	0.973
Root strips	3.255	0.969
Gallic acid	1.296	0.992

IC_{50} is the inhibition concentration as µg gallic acid equivalent of the test sample that eliminate 50% of hydrogen peroxide.

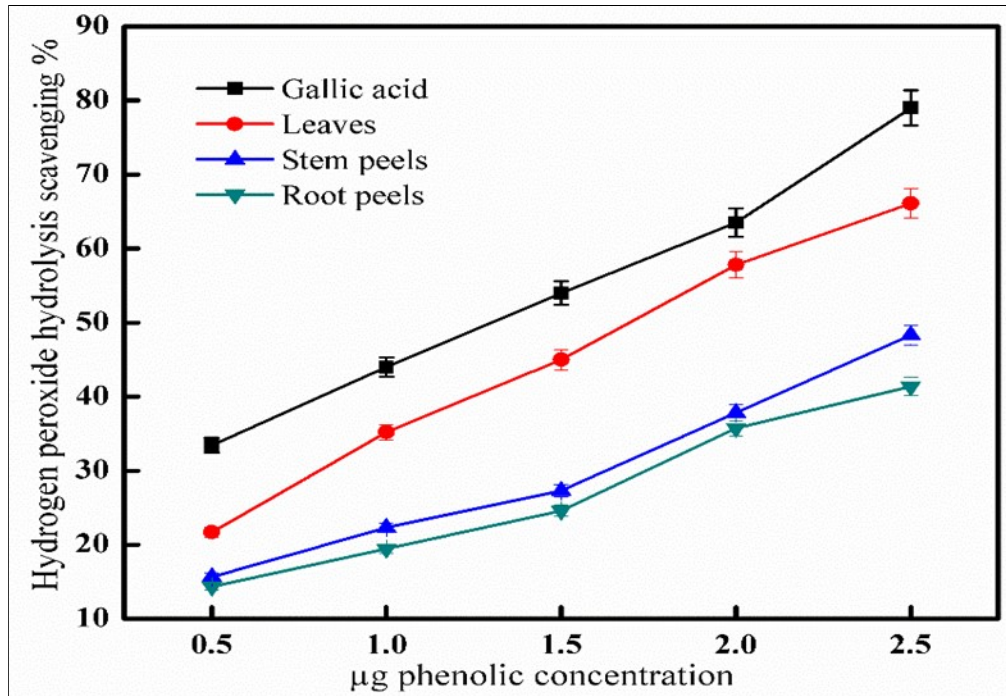
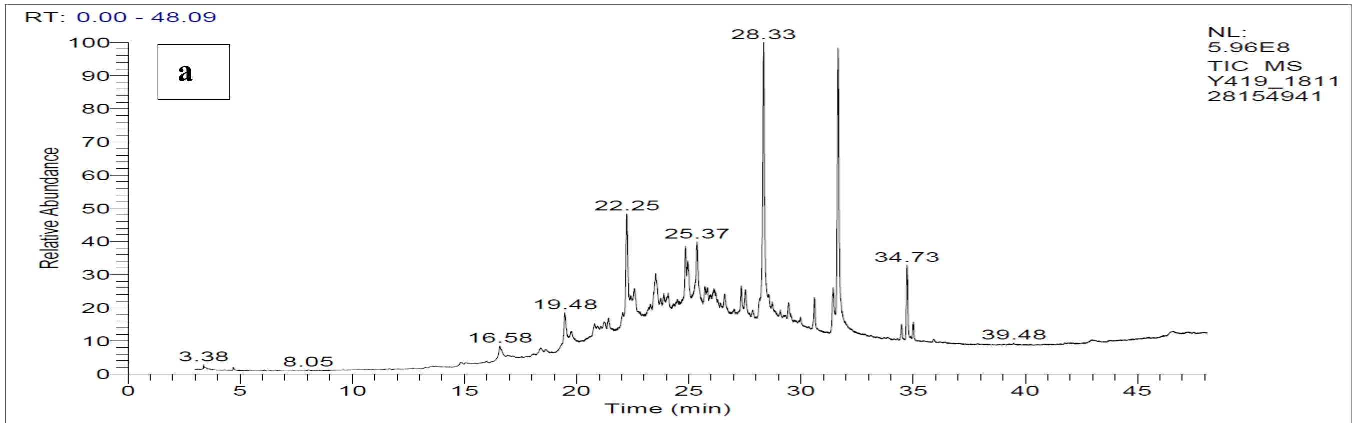
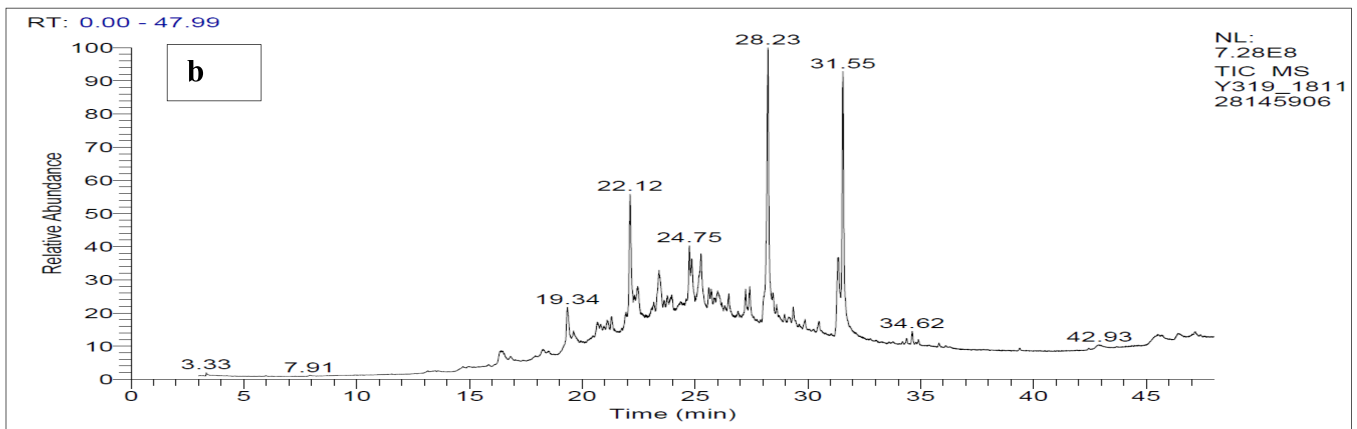


Figure 1. Hydrogen peroxide scavenging activity of leaves, strips of roots and stems. All experiments were carried out in triplicates and values are presented as mean ± SE.

Table 6. GC-MS analysis of compounds in the methanol extract from strips of verdolaga

	Compound Name	Retention Time (min.)	Peak area (%)
Stem	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl) oxy] methyl]ethyl ester, (Z,Z,Z)-	4.26	0.01
	2,5-Octadecadiynoic acid, methyl ester	4.40	0.0
	Gibberellic acid	5.28	0.0
	Hexanoic acid, 3,5,5-trimethyl, 1,2,3-propanetriyl ester	14.71	0.37
	Cyclopropanedodecanoic acid, 2-octyl, methyl ester	8.70	0.00
	Cholestan-3-ol, 2-methylene, (3á,5à)	30.25	0.09
	Bis[di(trimethylsiloxy)phenylsiloxy]trimethylsil oxyphenylsiloxane	39.38	0.13
	D-Homo-24-nor-17-oxachola 1,20,22-triene 3,7,16-dione, 14,15:21, 23-diepoxy4,4,8trimethyl, (5à,13à,14á,15á,17aà)	47.17	0.57
Root	2,5-Octadecadiynoic acid, methyl ester	4.22	0.01
	Cyclotetrasiloxane, octamethy-	5.26	0.01
	9,10-Secocholesta 5,7,10 (19) triene1,3diol, 25 [(trimethylsilyl)oxy], (3á,5Z,7E)	7.17	0.01
	2,5-Octadecadiynoic acid, methyl ester	5.73	0.00
	Eucalyptol	6.01	0.03
	Hexadecanoic acid, 2-hydroxy1(hydroxymethyl) ethyl ester	42.92	0.64
Leaves	Pterin 6-carboxylic acid	5.01	0.00
	1-Monolinoleoylglycerol trimethylsilyl ether	5.33	0.02
	9,10-Secocholesta 5,7,10 (19) triene1,3diol, 25 [(trimethylsilyl)oxy], (3á,5Z,7E)	7.25	0.00
	Cyclopropanedodecanoic acid, 2-octyl, Methyl ester	7.39	0.01
	3,6,9,12-Tetraoxatetradecan 1ol, 14 [4 (1,1,3,3-tetramethylbutyl) phenoxy]	9.18	0.01
	Cholest-22-ene-21-ol, 3,5-dehydro6methoxy, pivalate	47.95	0.02





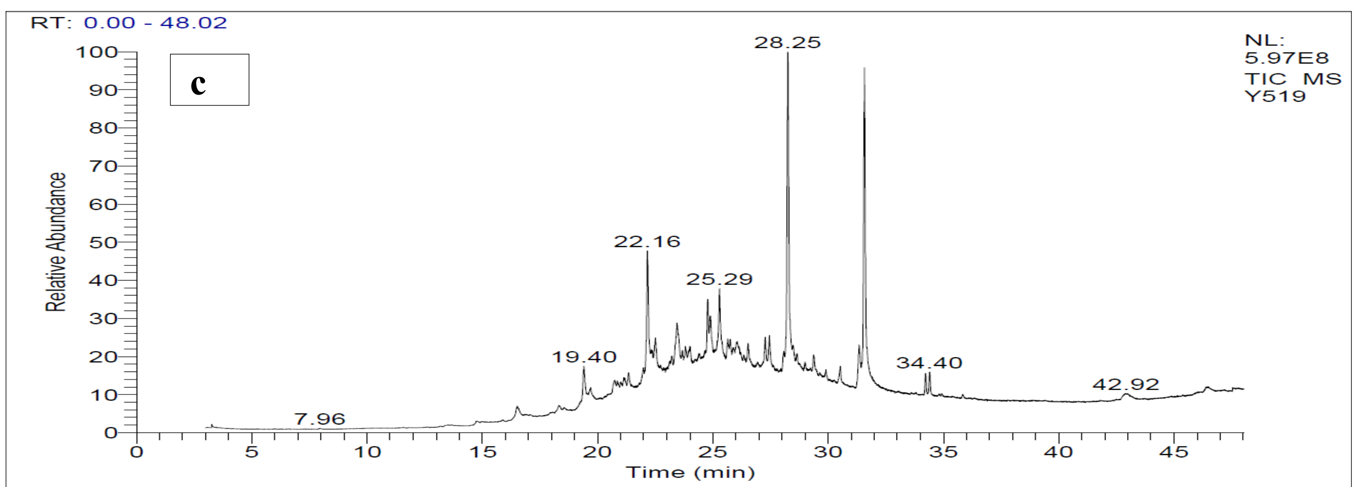


Figure 2. GC-MS chromatograms of leaves (a), stem strips (b), and root strips (c) crude methanol extract.

system in the cell. The GC-MS chromatograms for strips of stem, root, and leaves are shown in Figure 2. The analytes from stem strips, root strips, and leaves, their maintenance time, and peak area (%) are shown in Tables 6. The greater part of component derivatives contained hydroxyl groups, which could possess antioxidant potential.

Conclusion

This study revealed that *Verdolaga* contained a high concentration of phenolic, flavonoids, CTs, and compounds with powerful antioxidant activities. This plant contained a high concentration of free radical scavengers, which are useful in postponing the aging process, the development of malignancy, and the progression of other pathophysiological illnesses. This paper reveals the enzymes in *Verdolaga* responsible for combating and avoiding diseases. The GC-MS investigation of the methanol extracts of *verdolaga* enabled the quantification of cell-protecting agents. The applications of this investigation support the development of nutritional supplements and nutraceutical products using *verdolaga* extracts.

Acknowledgements

We would like to acknowledge the Department of Biochemistry at the University of Jeddah for its support.

Supplementary Figure

References

1. A. Yoon, T. Hughley, R.H. Williams, *The FASEB*. 2019, *33,* l440.
2. S. Anelise, R. Carla, S. Matheus, A. Claudia, C. Maria, V. Zefa, *Antioxidants.* 2014, *4,* 745.
3. B. Guerrero, M.G Granda-Albuja, M. Guevara, G.A. Iturralde, T. Jaramillo-Vivanco, F. Giampieri, J.M. Alvarez-Suarez, *Nat. prod. Res.* 2019, 1.
4. A.E. Hayouni, M. Abedrabba, M. Bouix, M. Hamdi, *Food Chem.* 2007*, 3,* 1126.
5. H. Dorman, S. G. Deans, *J. App. Micro.* 2000, *2,* 308.
6. J.M. Vieira, R.A. Mantovani, M.F. Raposo, M.A. Coimbra, A.A. Vicente, R.L. Cunha, *Carbo. ploy.* ,2019 *213,* 1217.
7. A.H. Goli, M. Barzegar, M.A. Sahari, *Food Chem.* 2005, *3,* 521.
8. Y. Zuo, H. Chen, Y. Deng, *Talanta,* 2002, *2,* 307.
9. L. Yao, Y. Jiang, N. Datta, R. Singanusong, X. Liu, J. Duan, K. Raymont, A. Lisle, Y. Xu. *Food Chem.* 2004*, 2,* 253.
10. S.C. Opie, A. Robertson, M.N. Clifford, *J. Agric. Food Chem.* 1990, *50,* 561.
11. M.H. Masoodi, B. Ahmad, S.R. Mir, B.A. Zargar, N. N. Tabasum, *J. Pharm. Res*. 2011, *9,* 3044.
12. L. Xiang, D. Xing, W. Wang, R. Wang, Y. Ding, L. Du, *Phytochemistry.* 2005, *21,* 2595.
13. L. Liu, P. Howe, Y.F. Zhou, Z-O. Xu, C. Hocart, R. Zhang, *J. Chromatogr.* 2000, *1,* 207.
14. M.V. Miranda, H.M. Fernandez Lahor, O. Cascone, *App. Biochem. Biotechnol.* 1995, *2,* 147.
15. M. Siddiq, J.N. Cash, *J. Food Proc. Preserv.* 2000, *24*, 353.
16. H. Aebi, In Methods of Enzymatic Analysis (Second Edition), 1974, 2, 673.
17. Y.S. Velioglu, G. Mazza, L. Gao, B.D. Oomah, *J. Agric. Food Chem*. 1998, 10*,* 4113.
18. J. Zhishen, T. Mengcheng, W. Jianming, *Food Chem.* 1999, *4,* 555.
19. R.B. Broadhurst, W.T. jones, *J. Sci. Food Agric.* 1978*, 3,* 788.
20. Z.F. Wang, T.J. Ying, B.L. Bao, X.D. Huang, *J. Zhejiang Univ. Sc.* 2005, *6,* 502.
21. C. Ao, A. Li, A.A. Elzaawely, T.D. Xuan, S. Tawata, *Food control.* 2008, *10,* 940.
22. R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, *Free Radical Bio. Med*. 1999, *10,* 1231.
23. M. Oyaizu, *Jap. J. Nutr. Diet.* 1986*, 6,* 307.
24. R.J. Ruch, S.J. Cheng, J.E. Klaunig, *Carcinogenesis.* 1989, *6,* 1003.
25. G. Bartosz, *Acta Physiologiae Plantarum.* 1997, *1,* 47.
26. M.G. Polovnikova, O.L.Voskresenskaya, *Russ. J. Plant Phys.* 2008, *5,* 699.
27. R. Kumar, K.A. Singh, V.K. Singh, M.V. Jagannadham, *Process Biochem.* 2011, *6,* 1357.

28. S.E. Lee, H.J. Hwang, J.S. Ha, H.S. Jeong, J.H. Kim, *Life Science.* 2003, *2,* 167.
29. S. Dogan, M. Dogan, *Food chem.* 2004, Mar *1*, 69.
30. C.R. Pawar, S.J. Surana, *J. Young pharm.* 2010, *1*, 45.
31. B. Mahdi-Pour, S.L. Jothy, L.Y. Latha, Y. Chen, S. Sasidharan, *Asian Pac J Trop Biomed.* 2012, *12,* 960.
32. E. Haslam, Plant Polyphenols. Vegetable Tannins Revisited. Cambridge University Press, Cambridge, UK. 1989, 154.
33. W.T. Jones, J.L. Mangan, *J. Sci. Food Agric.* 1977, *2,* 126.
34. T.N. Barry, *British J Nutr.* 1985, *1,* 211.
35. F. Medini, H. Fellah, R. Ksouri, C. Abdelly, *J. Taibah Univ. sci.* 2014, *3,* 216.
36. G.W. Burton, K.U. Ingold, *Science.* 1984, *224,* 569.
37. H. Sies, W. Stahl, Am. J. Clinic Nutr. 1995, *6,* 1315S.
38. B.W. Vechetel, H.G. Ruppel, *Plant Cell Phys.* 1992, *1,* 41.
39. A. Von Gadow, E. Joubert, C.F. Hansmann, *Food chem.* 1997, *1,* 73.
40. Y. Almulaiky, M. Zeyadi, R. Saleh, O. Baothman, W. Al-shawafi, H. Al-Talhi, *Biocata. Agric. Biotechnol.* 2018, 16, 90.
41. Y.Z. Cai, Q. Luo, M. Sun, H. Corke, *Life Science.* 2004, *17,* 2157.
42. R.Y. Gan, L. Kuang, X.R. Xu, Y. Zhang, E.Q. Xia, F. Song, H. Li. *Molecules.* 2010, *9,* 5988.
43. S. Chanda, R. Dave, *African J. Micro. Res*. 2009, *13,* 981.
44. K. Shimada, K. Fujikawa, K. Yahara, T. Nakamura, *J. Agric. Food Chem.* 1992, *6,* 945.
45. Y.Q. Almulaiky, A. Kuerban, F. Aqlan, S.A. Alzahrani, M.N. Baeshen, M. Afifi, A. Alfarga, W. Al-Shawafi, M. Alkhaled, *J. Pharm. Res.* International. 2017, *3,* 1.
46. B. Halliwell, *Am. J. Med.* 1991, *3*, 14.