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Characterization, antioxidant, and cytotoxic effects of some Egyptian wild plant extracts

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Abstract

Background: Natural products from plants are very safe as compared to synthetic ones, so the aim of this study was to assess the in vitro antioxidant and antitumor activities of the ethanolic extracts of four Egyptian wild plant species (*Varthemia candicans*, *Peganum harmala*, *Suaeda vermiculata*, and *Conyza dioscoridis*), as well as polyphenols and flavonoid contents with gas chromatography–mass spectrometry (GC-MS). The antioxidant activity of the four plant extracts was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) to determine 50% inhibition of DPPH radical scavenging activity and reducing power by phosphomolybdate assay. In addition, the chemical composition of the four sample extracts was investigated using GC-MS. The total phenolic and flavonoid levels were also determined. Then, the antitumor activity of the plant extracts against HepG2 cells was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Results: The results showed that *Varthemia candicans* extract was the highest one regarding both polyphenols and flavonoid contents. Moreover, the extract of *Suaeda vermiculata* exhibited the lowest half maximum inhibitory concentration (IC₅₀) against DPPH, thus indicating its highest effectiveness. All studied plant extracts decreased the viability of HepG2 cells, in a dose- and time-dependent manner, and the lowest IC₅₀ was for *Suaeda vermiculata*.

Conclusion: The investigated plant extracts showed potent antioxidant and antitumor activities in vitro due to their phytochemical contents.

Keywords: Egyptian plants, Antitumor activity, Phytochemicals, Antioxidant activity

1 Background

Medicinal plants contain, aside from primary metabolites, secondary metabolites, like flavonoids, alkaloids, phenolics, tannins, glycosides, and steroids, which are very important alternative medicines whether in a single form or in combination [1]. These plants are cheap and available for all people, especially the Third World, and could be used as antioxidants against free radicals that cause many human diseases [2].

The wild Egyptian plant *Conyza dioscoridis* was potentially used in the treatment of many diseases like rheumatism, intestinal distension, and cramps in folk medicine [3].

A previous study has emphasized the medicinal significance of *C. dioscoridis* plant as anti-diarrheal and diuretic [4]. Additionally, the volatile constituents of *C. dioscoridis* showed potential antimicrobial activity against many pathogenic microorganisms [5], and its aerial parts extract owned anti-inflammatory activity [6]. Moreover, the species *C. dioscoridis* has been also reported to encompass antioxidant and antihyperglycemic constituents [7]. Secondary phytochemical metabolites including hydrolyzable tannins, alkaloids, flavonoids, essential oils, terpenoids, and polyphenols have been reported in *Conyza* species [8]; these therapeutically active components have raised its medicinal significance. However, as far as we are aware, nothing has been traced concerning either plant constituents or anticancer activity. For example, the hydrolyzable tannins showed the free radical scavenging capacity because they can donate a hydrogen atom and form a stable quinone [9–11]. In

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addition, a previous study indicated the essential oils' therapeutic potential which included antimicrobial, anticancer, skin permeation enhancing, and antiviral effects [12]. In addition, plant terpenoids have been used in food and pharmaceutical industries [13].

The second plant in this work is *Varthemia candicans*, a member of the family Asteraceae, which has been reported to be a competent treatment for some diseases in folk medicine. Phytochemical constituents of *V. candicans* showed that it contains many prophylactic components such as sesquiterpenes, sesquiterpene-lactone derivatives, polymethoxylated flavonoids, and coumarins [14]. Plant sesquiterpenes are known to have diverse biological and therapeutical activities, including antineoplastic agents. Another detailed study on the active pharmaceutical constituents of *V. candicans* revealed that its aerial parts encompass a variety of flavonoids, alkaloids, phenols, and saponins, which possess medicinal and antioxidant properties [15].

Suaeda vermiculata is a halophytic member of the family Chenopodiaceae, which grows naturally in the Egyptian coastal region. Some earlier works concerned with prophylactic characteristic of wild plants showed that *S. vermiculata* has high quantities of phenolics and flavonoids with a pronounced antioxidant activity [16]. Additionally, *S. vermiculata* has been demonstrated to have hypoglycemic, hypolipidemic, and antitumor activities. Such activities were attributed to polyphenolic compounds (including flavonoids) in the aerial parts of the plant [17]. Nevertheless, triterpenoids and polyphenols in *S. vermiculata* increased the pharmacological importance of the plant due to its role in preventing cardiovascular and cancer diseases [18].

Peganum harmala is a member of the Zygophyllaceae family with a long history in traditional medicine as being widely used to treat apoplexia, asthma, jaundice, and lumbago. Recently, some alkaloidal species like β -carboline and quinazoline has been identified as principal components in *P. harmala* aerial parts. These alkaloidal species have been reported to encompass pharmacological and remedial properties, such as antimicrobial, antiparasitic, analgesic, and antitumor [19].

In this work, the volatile constituents of the ethanolic extracts of four wild Egyptian plants: *Conyza dioscoridis*, *Varthemia candicans*, *Suaeda vermiculata*, and *Peganum harmala*, were quantified by means of GC-MS. Additionally, their phenolic and flavonoid contents, as well as their antioxidant activities, were evaluated. Subsequently, the antitumor activity of the plant extracts against HepG2 cell line was evaluated.

2 Methods

2.1 Collection of plant material

The investigated four wild plant species collectively known as *Varthemia candicans* (Del.) Boiss., *Peganum*

harmala L., *Suaeda vermiculata* Forssk., and *Conyza dioscoridis* L. were collected during the summer season (August 2015) from Borg El Arab city (North West Coastal region), Alexandria, Egypt. Voucher specimens were deposited in the herbarium of the Botany Department, Faculty of Science, Tanta University (TANE) (Table 1). The collected samples were washed thoroughly with tap water then dist. water and the leaves were separated from the aerial parts. Leaf samples were oven dried at 40 °C for 5 days, ground into a fine powder using an electric mixer, and sieved through 0.2 mm sieve to uniform size particle then kept in paper pages at 4 °C for further investigation.

2.2 Chemicals

Cell culture grade dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), phosphate-buffered saline (PBS), fetal bovine serum (FBS), Dulbecco's modified Eagle medium (DMEM), penicillin/streptomycin, Quercetin, and 2,2-diphenyl-1-picrylhydrazyl (DPPH), all analytical grade chemicals, were purchased from Sigma-Aldrich (St Louis, USA). Folin-Ciocalteu reagent, potassium ferricyanide, ammonium molybdate, ethanol, sodium hydroxide, chloroform, anhydrous sodium sulfate, sulfuric acid, sodium carbonate, and gallic acid were purchased from Cornell Lab (Cairo, Egypt). Cisplatin (Cytoplatin-50) was purchased from Verna Industrial Estate, France.

2.3 Preparation of sample extract

Twenty-five grams of each plant material was extracted using 80% aqueous ethanol in a Soxhlet assembly for 16 h. The obtained extracts were filtered twice through Whatman No. 1 filter papers then subjected to reduced-pressure evaporation at 40 °C using a rotary evaporator. The residue remaining after evaporation was used in calculating the yield per each extract (Table 2) then stored at 4 °C for further analysis and investigations. At the time of phytochemical analysis and antitumor activity, pellets were dissolved in 10% DMSO.

2.4 Quantitative analysis of plant extracts

2.4.1 Total phenolic contents

The total phenolic content of sample extract was determined spectrophotometrically using the Folin-Ciocalteu method [20]. Briefly, 0.1 ml of the plant extract was mixed with 2.8 ml of deionized water, 2 ml of 20% sodium carbonate, and 0.1 ml of 50% Folin-Ciocalteu reagent. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 750 nm. Gallic acid (GA) was used as a standard phenol for construction of the standard graph and the total phenolics were expressed as mg gallic acid equivalents (GAE)/g d.wt.

Table 1 Voucher specimen Latin names, Arabic names, and numbers of the studied plant species





Scientific name	Arabic name	Family	Voucher No	Plant Photo
<i>Varthemia candidans</i> (Del.) Boiss.	زعتر الحمار	Asteraceae	TAN-2015-12	
<i>Peganum harmala</i> L.	الحرمل	Nitrariaceae	TAN-2015-62	
<i>Suaeda vermiculata</i> Forssk.	السويداء	Amaranthaceae	TAN-2015-162	
<i>Conyza dioscoridis</i> L.	بارنوف	Asteraceae	TAN-2015-93	

Table 2 Extraction yield of plant extract in aqueous ethanol

No.	Plant extract	Yield (%)
1	<i>Varthemia candidans</i>	1.6
2	<i>Peganum harmala</i>	4.6
3	<i>Suaeda vermiculata</i>	1.0
4	<i>Conyza dioscoridis</i>	6.6

2.4.2 Total flavonoid content

Flavonoids in the investigated samples were determined according to the aluminum chloride colorimetric method [21]. Aliquot of 0.5 ml extract was mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride hexahydrate, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water. Following incubation at room temperature for 40 min, the reaction mixture absorbance

was measured at 415 nm. Quercetin was chosen as a standard flavonoid for constructing a standard curve and flavonoids were expressed as mg quercetin equivalents (QE)/100 g d. wt.

2.4.3 DPPH radical scavenging activity

The electron-donating ability of the investigated extracts was measured by bleaching a purple solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical according to the method of Sermakkani and Thangapandian [22]. Aliquot of 0.1 ml extract (20, 40, 60, 80, 100 µg/ml) was added to 3.9 ml of 36 mg/l DPPH–methanol solution. After incubation for 1 h in the dark at room temperature, the absorbance was determined against blank at 515 nm. The percentage inhibition of free radical DPPH was calculated from $(A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}}) \times 100$, where A_{blank} is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of plant extract. The concentration of extract that caused 50% inhibition (IC_{50}) was calculated from the regression equation for the concentration of extract and percentage inhibition. Ascorbic acid was used as a positive control.

2.4.4 Total antioxidant capacity using phosphomolybdate reagent

The assay is based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of a green phosphate–Mo(V) complex at acid pH [23]. An aliquot (0.1 ml) of plant extract containing 100 µg/ml phenol was added to 1 ml of reagent solution (0.6 mol/l H_2SO_4 , 28 mmol/l Na_3PO_4 , and 4 mmol/l ammonium molybdate). The tubes were incubated in a thermal block at 95 °C for 90 min. Once the mixture had cooled to room temperature, the absorbance of each solution was measured at 695 nm against a blank. Antioxidant capacity was expressed as mg ascorbic acid equivalent per gram dry weight (mg ascorbic acid (mg/g d. wt)).

2.5 Preparation of samples for GC-MS analysis

All samples were dissolved in chloroform after removal of DMSO for GC-MS analysis.

2.6 Gas chromatography–mass spectrometry (GC-MS) analysis

The chemical composition of the studied samples was performed using a Trace GC Ultra-ISQ mass spectrometer (Thermo Scientific, USA). The column type is TG–5MS (30 m × 0.25 mm × 0.25 µm film thickness), and the oven temperature was initially held at 60 °C, then increased by 5 °C/min to 150 °C with hold 2 min, and then increased to 280 °C with the rate of 10 °C/min. The inlet and transfer line temperatures were kept at 250 °C. Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 3 min and diluted

samples of 1 µl were injected automatically using Autosampler AS3000 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 40–650 in full scan mode. The ion source temperature was set at 200 °C. All obtained components of the studied extracts were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral databases.

2.7 Cell culture

Human hepatocellular carcinoma cells (HepG2) supplied by tissue culture unit in Vacsera Institution (Cairo, Egypt) were grown in DMEM medium supplemented with 10% FBS, 2 mM glutamine, 100 µg/ml of streptomycin, and 100 U/ml of penicillin in an incubator at 37 °C and 5% CO_2 .

2.8 Overall cell activity—MTT assay

The MTT assay based on the method of Mitry et al. [24] measures the metabolism of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-biphenyl tetrazolium bromide to form an insoluble formazan precipitate by mitochondrial dehydrogenases only present in viable cells. The samples were tested at a concentration of 100 µg/ml phenolic content in 1% DMSO to establish its cytotoxic activity on HepG2 liver carcinoma cell line. To determine the half-maximal inhibitory concentration (IC_{50}), the samples were tested in serial dilutions (5, 10, 20, 50, and 100 µg gallic acid equivalent/ml) in triplicate. The results of IC_{50} were compared to cisplatin (2, 4, 8, 16, and 24 µg/ml) as a reference antitumor drug according to Qin and Ng [25].

The culture medium was removed from the 96-well microplates after 48 h of the drug (extract or cisplatin) treatment, cells were washed gently twice with ice-cold PBS, and 200 µl of 0.5 mg/ml of MTT solution was added per well. The microplate was incubated at 37 °C for 4 h in a cell culture incubator. After incubation, 180 µl medium/MTT was removed and 100 µl of acidified isopropanol was added per well. In order to dissolve the formazan produced, the microplate was incubated with shaking (at the highest speed) for 15 min at room temperature. Using a microplate reader, the absorbance of each well was measured at 630 nm.

2.9 Statistical analysis

Replicate means ± standard deviations were computed by Student's paired two-tailed t test using Graph Pad software. Dose–response in proliferation rate experiments was evaluated by analysis of variance (ANOVA) and $P < 0.05$ was considered statistically significant.

3 Results

3.1 Total phenolic and flavonoid contents of extracts

Data represented in Table 3 summarize the total phenolic and flavonoid content in the ethanolic extracts of four Egyptian wild plants (*Varthemia candidans*, *Peganum harmala*, *Suaeda vermiculata*, and *Conyza dioscoridis*). The total phenolic content of *V. candidans* showed a mean value of 119.0 mg GAE/100 g d.wt., which was the uppermost value among the studied plants. Meanwhile, the total phenolic content of *P. harmala*, *S. vermiculata*, and *C. dioscoridis* recorded mean values of 43.0, 83.0, and 70.0 mg GAE/100 g d.wt., respectively. Thus, the higher content of phenolics subsequent to *V. candidans* has been recorded in *S. vermiculata*, while the lower content has been obtained in *P. harmala* extract. Different phenolic compounds have different responses in the Folin–Ciocalteu method.

Our results showed that *V. candidans* possessed the highest flavonoid content compared to other tested plants (96.2 mg QE/100 g d. wt.). This result was in conformity with those recorded in phenol content results (Table 1). The extracts of *P. harmala*, *S. vermiculata*, and *C. dioscoridis* showed mean values of 8.8, 16.5, and 70.4 mg QE/100 g d. wt., respectively. On the other hand, the lowest flavonoid content was recorded in *P. harmala* extract. This result runs parallel with phenol content results (Table 1).

3.2 DPPH radical scavenging and total antioxidant activities

The radical scavenging activity (RSA) expressed as 50% DPPH radical scavenging and the total antioxidant capacity (TAC) as measured through phosphomolybdate assay of the four studied plant extracts are represented in Fig. 1. The results showed that *S. vermiculata* possessed the highest RSA as it resulted in the lowest 50% DPPH activity followed by *C. dioscoridis* and *V. candidans*, whereas the lowest RSA was observed in *P. harmala* extract, as compared with the reference antioxidant ascorbic acid (Fig. 1a). The IC_{50} values of the aforementioned plant extracts were 2.8, 15.8, 22.1, and 128.8 μ g/ml for *S. vermiculata*, *C. dioscoridis*, *V. candidans*, and *P. harmala*, respectively; meanwhile, the IC_{50} value of the standard ascorbic acid was 15.0 μ g/ml. Thus, the proceeding data confirmed that the extract of

S. vermiculata has a high bleaching activity towards the free radical DPPH more than other plant extracts and the standard ascorbic acid.

In order to validate the obtained data, the total antioxidant capacity (as μ g ascorbic acid equivalent/g d.wt) using phosphomolybdate assay was determined (Fig. 1b). The present study demonstrated that *V. candidans* exhibited the highest antioxidant capacity for phosphomolybdate reduction compared to other plant extracts.

The order of decreasing antioxidant activity in the ethanolic extracts of plants was *V. candidans* > *S. vermiculata* > *C. dioscoridis* > *P. harmala* with values of 368.2, 216.9, 165.0, and 92.9 μ g ascorbic acid equivalent/g d.wt, respectively. This order is similar to the phenolic contents of the extracts that showed the extent of antioxidant activity of the extract is in accordance with the amount of phenolics present in that extract.

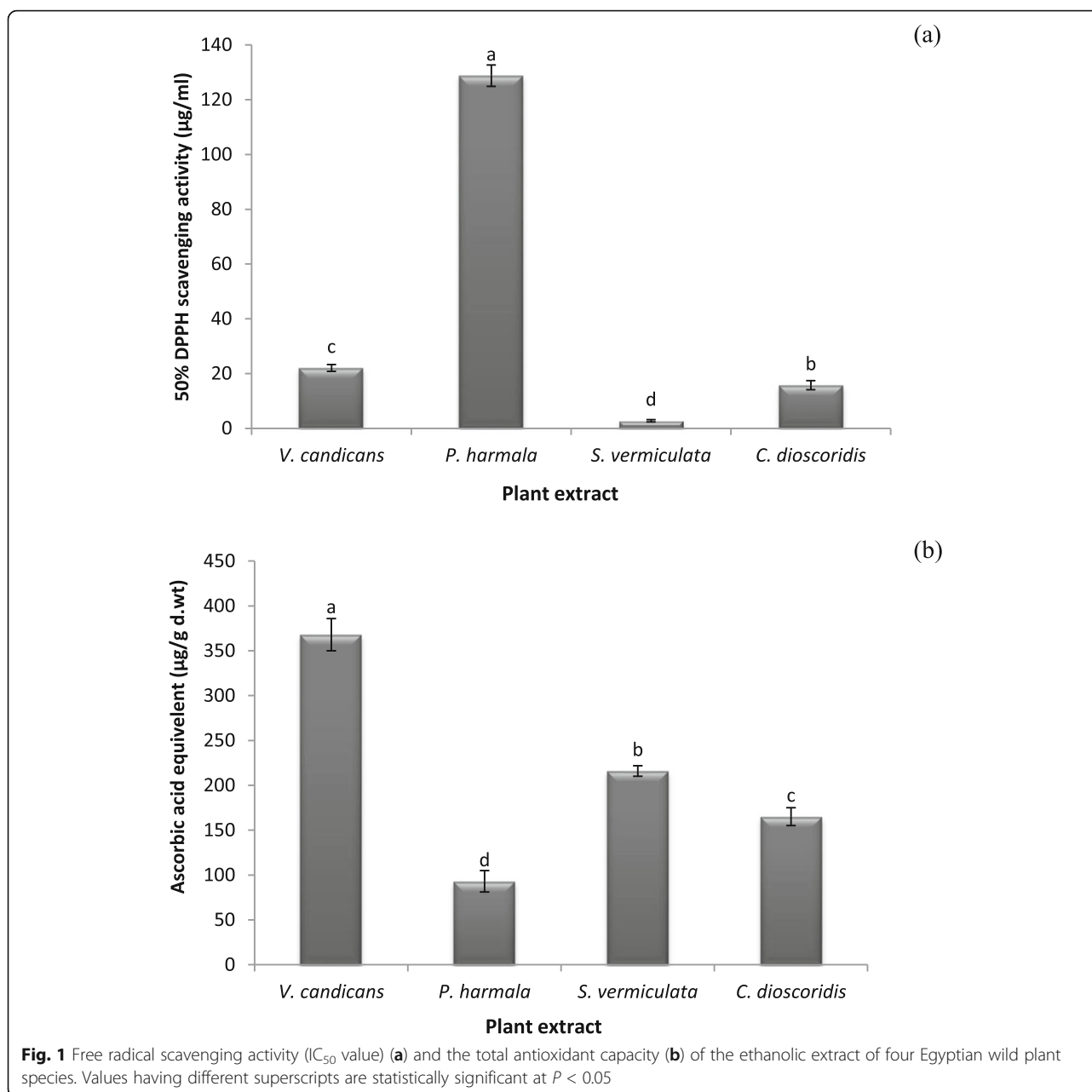
3.3 Identification of experimental sample components using the GC-MS technique

The GC-MS analysis of the ethanolic extract of *V. candidans* revealed the identity of 24 compounds (Table 4). Panaxatriol, 9,12-octadecadienoic acid (Z,Z)-, cis-9-octadecenoic acid, 1-heptatriacotanol, hexadecatrienoic acid, methyl ester, and 3 α ,5 α -cyclo-ergosta-7,9(11),22-triene-6 α -ol are the main identified compounds representing 25.88, 25.65, 7.60, 5.21, 3.80, and 3.25%, respectively. At the main time, the results of GC-MS revealed that other active phytochemical compounds are dominated in the ethanolic extract of *V. candidans* belonging to fatty acids (2,5-octadecadienoic acid, methyl ester, 7, 10,13-eicosatrienoic acid, methyl ester, 8,11,14-docosatrienoic acid, methyl ester, 9-octadecenoic acid (Z)-, 9-hexadecenyl ester, (Z)- and oleic acid,3-(octadecyloxy) propyl ester), fatty alcohols (13-heptadecyn-1-ol and 1-heptatriacotanol), sterols (cholestan-3-ol, 2-methylene-, (3 α ,5 α)-, stigmasteran-3,5-diene, stigmasteran-5-en-3-ol, (3 α)- and 3 α ,5 α -cyclo-ergosta-7,9(11),22-triene-6 α -ol), terpenoids (fischeroside C), carotenoids (lycopene), and siloxane derivatives (trisiloxane, 1,1,1,5,5,5-hexamethyl-3-(4-methylpentyl)-3-[(trimethylsilyloxy)-,1,1,3,3,5,5,7,7, 9,9,11,11-dodecamethyl-hexasiloxane and 1,1,3,3,5,5,7,7, 9,9-decamethyl-pentasiloxane).

The results of the GC-MS analysis of *P. harmala* ethanolic extract showed the identity of 14 compounds

Table 3 Total phenolics and flavonoids in the ethanolic extracts of four Egyptian wild plant species. Data are expressed as mean \pm SD

No.	Plant species	Phenolics (mg/100 g d. wt.)	Flavonoids (mg/100 g d. wt.)
1	<i>V. candidans</i>	119 \pm 7.6	96.2 \pm 1.4
2	<i>P. harmala</i>	43 \pm 1.5	8.8 \pm 0.2
3	<i>S. vermiculata</i>	83 \pm 1.5	16.5 \pm 6.0
4	<i>C. dioscoridis</i>	70 \pm 7.2	70.4 \pm 2.0



belonging to different chemical classes (Table 5). The most prominent compounds are represented in hexadecatrienoic acid, methyl ester (63.13%), Cis-9-octadecenoic acid (11.43%), and 6-ethoxyquinaldine (6.00%). Moreover, other phytochemical constituents were identified in the ethanolic extract of *P. harmala* belonging to quinolines (5-Cyano-1,2,3,4-tetrahydro-2-methylisoquinoline and 4-Acetamido-1,2,3,4-tetrahydroisoquinoline), carotenoids (rhodopin), corticosteroids (betamethasone valerate), fatty acids (hexadecanoic acid, ethyl ester, 8,11-octadecadienoic acid, methyl ester, 2,5-octadecadienoic acid, methyl ester, 6-octadecenoic acid, (Z)-, 5,8,11,14,17-eicosapentaenoic acid, methyl ester and 4,7,10,13,16,19-docosaheptaenoic

acid, methyl ester), and fatty acid derivatives (9-octadecenamamide, (Z)-).

As for *C. dioscoridis* ethanolic extract, 13 compounds were identified by the GC-MS technique (Table 6). The most abundantly identified compounds were the fatty acids 9,12-octadecadienoic acid (Z,Z); 9,12,15-octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z); and 9-octadecenoic acid, eicosyl ester, (9Z) (41.54, 7.86, and 5.40%, respectively) and the phytosterol stigmast-5-en-3-ol, (3á) (7.84%). Moreover, the results showed the existence of other fatty acids (6,9-octadecadienoic acid, methyl ester, 9-octadecenoic acid (Z), 4,7,10-hexadecatrienoic acid, methyl ester, 10,13-eicosadienoic acid, methyl ester,

Table 4 Phytochemical components of *Varthemia candidans* ethanolic extract as identified by GC-MS analysis

Peak no.	RT	Compound name	Area %	Molecular formula	Class	Reported bioactivity	References
1	16.66	2,5-Octadecadienoic acid, methyl ester	3.01	C ₁₉ H ₃₀ O ₂	Fatty acid	No activity reported	
2	16.83	Megastigma-4,6(E),8(Z)-triene	1.90	C ₁₃ H ₂₀	Alkene	No activity reported	
3	17.26	9,12-Octadecadienoic acid (Z,Z)-	25.65	C ₁₈ H ₃₂ O ₂	Fatty acid	-Antitumor -Anti-inflammatory, hypocholesterolemic, hepatoprotective, nematocide, 5-alpha reductase inhibitor, antihistaminic, antiacne, antieczemic, antiandrogenic, antiarthritic, anticoronary, and insect repellent	[26]
4	18.13	7,10,13-Eicosatrienoic acid, methyl ester	0.63	C ₂₁ H ₃₆ O ₂	Fatty acid	No activity reported	
5	18.66	13-Heptadecyn-1-ol	1.66	C ₁₇ H ₃₂ O	Fatty alcohol	Anti-inflammatory and antifungal	[27]
6	18.81	Stigmast-5-en-3-ol, (3á)-	0.43	C ₂₉ H ₅₀ O	Phytosterol	Antidiabetic and anti-hypercholesterolemic	[28]
7	19.21	1-Heptatriacotanol	5.21	C ₃₇ H ₇₆ O	Fatty alcohol	-Anti-hypercholesterolemic -Antimicrobial	[29] [30]
8	19.41	Cholestan-3-ol, 2-methylene-, (3á, 5â)-	0.90	C ₂₈ H ₄₈ O	Sterol	Antimicrobial anti-inflammatory, anticancer, diuretic, anti-asthma, and antiarthritic	[26]
9	21.23	Hexadecatrienoic acid, methyl ester	3.80	C ₁₇ H ₂₈ O ₂	Fatty acid	-Cardio-protective -Antimicrobial, antioxidant, and anti-inflammatory	[26] [31]
10	21.29	Cis-9-octadecenoic acid	7.60	C ₁₈ H ₃₄ O ₂	Fatty acid	Cancer preventive flavor, hypocholesterolemic, 5-alpha reductase inhibitor, antiandrogenic, perfumery insectifuge, anti-inflammatory, anemiagenic, and dermatitigenic choleretic	[26]
11	22.02	8,11,14-Docosatrienoic acid, methyl ester	2.71	C ₂₃ H ₄₀ O ₂	Fatty acid	No activity reported	
12	23.61	ç-Isosparteine	1.28	C ₁₅ H ₂₆ N ₂	Alkaloid	Antimicrobial	[32]
13	24.53	Fischeroside C	2.29	C ₂₈ H ₄₀ O ₁₂	Diterpene	Antiviral	[33]
14	26.54	Lycopene	0.62	C ₄₀ H ₅₆	Carotenoid	Anti-inflammatory, antioxidant, anti-allergic, and anticancer -Anti-autophagic and antiapoptotic substance	[34] [35]
15	27.38	Stigmastan-3,5-diene	2.02	C ₂₉ H ₄₈	Sterol	Antimicrobial	[36]
16	28.17	1-Methyl-3-(3,4-dimethoxyphenyl)-6,7-dimethoxyisochromene	2.90	C ₂₀ H ₂₂ O ₅	Benzopyran derivative	Anti-hypertension, anti-cardiac ischemia, arrhythmia treatment, smooth muscle relaxation, antidiabetic, cardio-protective and anti-angiogenic	[37]
17	29.45	Panaxatriol	25.88	C ₃₀ H ₅₂ O ₄	Triterpene sapogenin	Protect against ischemia and reperfusion injuries	[38]
18	29.82	9,12,15-Octadecatrienoic acid, 2-[[trimethylsilyloxy]-1-[[[(trimethylsilyloxy)methyl]ethyl]ester, (Z,Z,Z)-	1.67	C ₂₇ H ₅₄ O ₄ Si ₂	Fatty acid	Antimicrobial, anticancer, hepatoprotective, anti-arthritis, anti-asthma, and diuretic	[26]
19	29.98	9-Octadecenoic acid (Z)-, 9-hexadecenyl ester, (Z)-	0.92	C ₃₄ H ₆₄ O ₂	Fatty acid	No activity reported	
20	31.52	3â,5â-Cyclo-ergosta-7,9(11),22-triene-6á-ol	3.25	C ₂₈ H ₄₂ O	Steroid	Antitumor and anti-angiogenic	[39]
21	31.98	Trisiloxane, 1,1,1,5,5,5-hexamethyl-3-(4-methylpentyl)-3-[[trimethylsilyloxy]-	0.38	C ₁₅ H ₄₀ O ₃ Si ₄	Siloxane derivative	No activity reported	
22	32.37	1,1,3,3,5,5,7,7,9,9,11,11-Dodecamethyl-hexasiloxane	0.83	C ₁₂ H ₃₈ O ₅ Si ₆	Siloxane derivative	Antimicrobial, antiseptic, hair conditioning agent, skin-conditioning agent-emollient and solvent	[26]
23	32.79	1,1,3,3,5,5,7,7,9,9-Decamethyl-pentasiloxane	2.87	C ₁₀ H ₃₂ O ₄ Si ₅	Siloxane derivative	No activity reported	
24	33.26	Oleic acid,3-(octadecyloxy)propyl ester	1.59	C ₃₉ H ₇₆ O ₃	Fatty acid	Anti-fungal	[40]

RT retention time (min)

Table 5 Phytochemical components of *Peganum harmala* ethanolic extract as identified by GC-MS analysis

Peak no.	RT	Compound name	Area %	Molecular formula	Class	Reported bioactivity	References
1	12.21	5-Cyano-1,2,3,4-tetrahydro-2-methylisoquinoline	2.74	C ₁₁ H ₁₂ N ₂	Quinoline derivative	No activity reported	
2	13.57	6-Ethoxyquinaldine	6.00	C ₁₂ H ₁₃ NO	Quinoline derivative	No activity reported	
3	15.17	Hexadecanoic acid, ethyl ester	1.44	C ₁₈ H ₃₆ O ₂	Fatty acid	Anti-inflammatory	[41]
4	15.58	4-Acetamido-1,2,3,4-tetrahydroisoquinoline	1.73	C ₁₁ H ₁₄ N ₂ O	Quinoline derivative	No activity reported	
5	16.57	8,11-Octadecadienoic acid, methyl ester	1.55	C ₁₉ H ₃₄ O ₂	Fatty acid	No activity reported	
6	16.66	2,5-Octadecadienoic acid, methyl ester	1.83	C ₁₉ H ₃₀ O ₂	Fatty acid	No activity reported	
7	17.57	6-Octadecenoic acid, (Z)-	2.09	C ₁₈ H ₃₄ O ₂	Fatty acid	Anticancer and insect repellent	[26]
8	19.58	5,8,11,14,17-Eicosapentaenoic acid, methyl ester	2.49	C ₂₁ H ₃₂ O ₂	Fatty acid	-Antimicrobial -Anti-osteoporosis -Cardio-protective	[42] [43] [44]
9	21.24	Hexadecatrienoic acid, methyl ester	63.13	C ₁₇ H ₂₈ O ₂	Fatty acid	-Cardio-protective -Antimicrobial, antioxidant, and anti-inflammatory	[26] [31]
10	21.30	Cis-9-octadecenoic acid	11.43	C ₁₈ H ₃₄ O ₂	Fatty acid	-Anticancer, anemiagenic, insect repellent, antiandrogenic, dermatitogenic, anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematocidal, antihistaminic, antieczemic, antiacne, alpha reductase inhibitor, antiandrogenic, antiarthritic, and anticoronary	[26]
11	21.39	Rhodopin	3.73	C ₄₀ H ₅₈ O	Carotenoid	Antioxidant	[26]
12	21.72	4,7,10,13,16,19-Docosahexaenoic acid, methyl ester	3.68	C ₂₃ H ₃₄ O ₂	Fatty acid	Dietary DHA is essential for brain functioning and it has a positive effect on diseases such as hypertension, arthritis, atherosclerosis, depression, diabetes mellitus, myocardial infarction, thrombosis, heart disease, and some cancers	[45]
13	22.62	Betamethasone valerate	1.85	C ₂₇ H ₃₇ FO ₆	Corticosteroid	Treatment of psoriasis, eczema, and other dermatoses	[46]
14	24.51	9-Octadecenamide, (Z)-	2.37	C ₁₈ H ₃₅ NO	Fatty acid derivative	Anti-inflammatory and antibacterial	[47]

RT retention time (min)

ethyl linoleate and 9-octadecenoic acid (Z)), vitamin A derivative (vitamin A aldehyde), and luteolin derivative (luteolin 6,8-di-C-glucoside) in the ethanolic extract of *C. dioscoridis* (Table 6).

The GC-MS analysis of *S. vermiculata* ethanolic extract predominantly revealed the presence of 10 phytochemical compounds belonging to different classes (Table 7). The main constituents of this extract were hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl (25.66%); stearic acid, 3-(octadecyloxy)-propyl ester (21.37%); fucoxanthin (14.43%); astaxanthin (13.89%); and 2-(4-ethoxy-3-methoxy-phenyl)-3-nitro-2H-chromene (10.90%). Furthermore, *S. vermiculata* extract comprised other two fatty acid derivatives (octadecanoic acid, 2-(hexadecyloxy)ethyl ester and hexadecanoic acid, 2-[(trimethylsilyloxy)-1,3-propanediyl ester) and two carotenoid

derivatives (rhodoviolascins and canthaxanthin), in addition to the isoprenylated flavonoid (cyclomulberrin).

These data revealed that the plant extracts are a good source of many bioactive compounds like 9,12-octadecadienoic acid (Z,Z), hexadecatrienoic acid, methyl ester, stearic acid, astaxanthin, and Stigmast-5-en-3-ol, (3á).

3.4 In vitro anticancer activity

To evaluate the anticancer activity of the ethanolic extracts of the investigated plants, the extracts were initially tested for cytotoxicity against HepG2 cells as in vitro model of hepatocellular carcinoma cells. The cytotoxic activity was determined by the MTT assay.

HepG2 cells were treated with different concentrations of plant extracts as polyphenol content (5–100 µg/ml)

Table 6 Phytochemical components of *Conzya dioscoridis* ethanolic extract as identified by GC-MS analysis

Peak no.	RT	Compound name	Area %	Molecular formula	Class	Reported bioactivity	References
1	21.12	9,12-Octadecadienoic acid(Z,Z)-	41.54	C ₁₈ H ₃₂ O ₂	Fatty acid	Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematocide, insecticide, antihistaminic, antieczemic, antiacne, 5- α reductase inhibitor, antiandrogenic, antiarthritic, anti-coronary, and antimicrobial	[48]
2	22.96	6,9-Octadecadienoic acid, methyl ester	0.93	C ₁₉ H ₃₄ O ₂	Fatty acid	No activity reported	
3	23.79	9-Octadecenoic acid (Z)-	1.89	C ₁₈ H ₃₄ O ₂	Fatty acid	-Anticancer, anemiagenic, insect repellent, antiandrogenic, dermatitigenic, anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematocide, antihistaminic, antieczemic, antiacne, alpha reductase inhibitor, antiandrogenic, antiarthritic, and anticoronary	[26]
4	24.20	Vitamin A aldehyde	1.46	C ₂₀ H ₂₈ O	Vitamin A	Cosmetics, anti-aging, protect from UV, and antioxidant	[49]
5	24.95	4,7,10-Hexadecatrienoic acid, methyl ester	2.61	C ₁₇ H ₂₈ O ₂	Fatty acid	Cardio-protective	[26]
6	25.94	10,13-Eicosadienoic acid, methyl ester	2.16	C ₂₁ H ₃₈ O ₂	Fatty acid	No activity reported	
7	26.58	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	7.86	C ₂₁ H ₃₆ O ₄	Fatty acid	Antipyretic, anticonvulsant, antiseptic, and analgesic	[50]
8	26.67	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	14.89	C ₂₁ H ₄₀ O ₄	Fatty acid	No activity reported	
9	26.79	9-Octadecenoic acid, eicosyl ester, (9Z)-	5.40	C ₃₈ H ₇₄ O ₂	Fatty acid	No activity reported	
10	27.68	Ethyl linoleate	3.95	C ₂₀ H ₃₆ O ₂	Fatty acid	No activity reported	
11	29.87	Luteolin 6,8-di-C-glucoside	2.65	C ₂₇ H ₃₀ O ₁₆	Flavonoid derivative	Anti-ulcerogenic and antioxidative	[51]
12	33.43	9-Octadecenoic acid (Z)-	1.96	C ₁₈ H ₃₄ O ₂	Fatty acid	-Anticancer, anemiagenic, insect repellent, antiandrogenic, dermatitigenic, anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematocide, antihistaminic, antieczemic, antiacne, alpha reductase inhibitor, antiandrogenic, antiarthritic, and anticoronary	[26]
13	34.99	Stigmast-5-en-3-ol, (3 α)-	7.84	C ₂₉ H ₅₀ O	Phytosterol	Antidiabetic and anti-hypercholesterolemic	[27]

RT retention time (min)

and compared with cisplatin as the reference drug (IC_{50} = 9.4 μ g/ml). The results demonstrated that the investigated plant extracts decreased the viability of HepG2 cells, in a dose- and time-dependent manner. The cytotoxic activities are expressed as IC_{50} values (Fig. 2). The lower IC_{50} represents the higher potency of a compound to inhibit the growth of cells and cause toxicity leading to cell death.

Cytotoxic activity of *S. vermiculata* against human HepG2 cells showed the lowest IC_{50} value (25.9 μ g/ml) (Table 7). Meanwhile, other plant species showed the order of *P. harmala*, *C. dioscoridis*, and *V. candidans* with IC_{50} values of 56.7, 59.5, and 89.5 μ g/ml, respectively. Subsequently, the ethanolic extract of *S. vermiculata* showed potential anticancer activity against HepG2 cells compared with the other plant extracts.

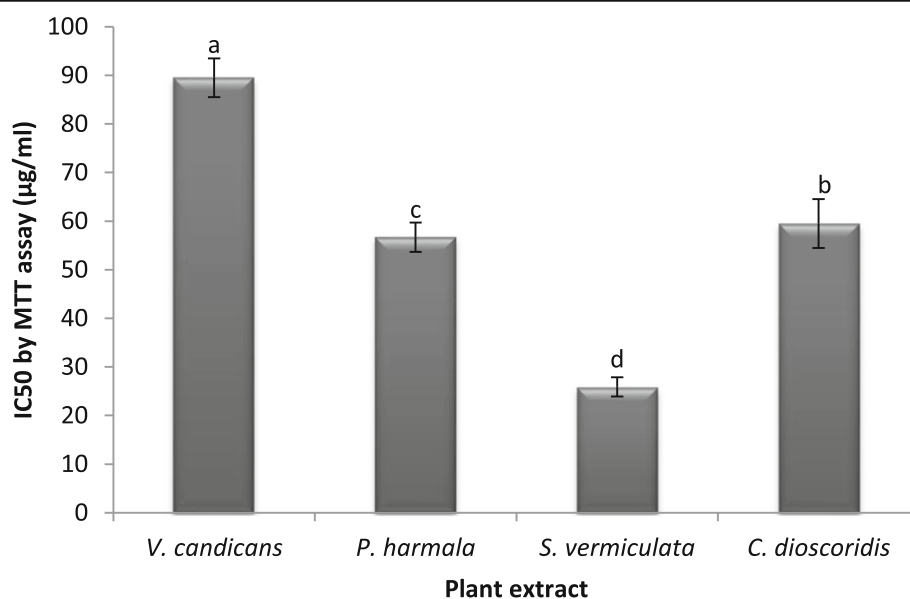
4 Discussion

Plants are a valuable gift of nature for mankind. They are the source of a diversity of phytochemicals, and they are capable of synthesizing a variety of secondary metabolites. The therapeutic actions of plants unique to particular plant species or groups are consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct. It has been reported that about 85–90% of the world's population consumes traditional herbal medicines [56]. In recent decades, studies on phytochemical constituents of medicinal plants and their pharmacological activities have received wide attention. A recent study had reported the presence of medicinal phytochemical constituents like phenolics, flavonoids, and alkaloids in methanolic extracts of *V. candidans* and *S. vermiculata* [9]. Based on this study, we determined two important phytochemical

Table 7 Phytochemical components of *Suaeda vermiculata* ethanolic extract as identified by GC-MS analysis

Peak no.	RT	Compound name	Area %	Molecular formula	Class	Reported bioactivity	References
1	5.03	Octadecanoic acid, 2-(hexadecyloxy)ethyl ester	1.64	C ₃₆ H ₇₂ O ₃	Fatty acid derivative	No activity reported	
2	5.08	Rhodoviolascin	1.59	C ₄₂ H ₆₀ O ₂	Carotenoid derivative	Antioxidant	[52]
3	16.94	2-(4-Ethoxy-3-methoxy-phenyl)-3-nitro-2H-chromene	10.90	C ₁₈ H ₁₇ NO ₅	Chromene derivative	No activity reported	
4	18.34	Canthaxanthin	1.83	C ₄₀ H ₅₂ O ₂	Carotenoid derivative	Antioxidant	[26]
5	19.51	Cyclomulberrin	3.82	C ₂₅ H ₂₄ O ₆	Isoprenylated flavonoid	No activity reported	
6	21.50	Stearic acid, 3-(octadecyloxy)propyl ester	21.37	C ₃₉ H ₇₈ O ₃	Fatty acid derivative	5- α -Reductase inhibitor, cosmetic, flavor, hypocholesterolemic, lubricant, perfumery, propeic and suppository	[53]
7	22.48	Fucoxanthin	14.43	C ₄₂ H ₅₈ O ₆	Carotenoid	Anticancer, anti-angiogenic, anti-proliferative, and apoptosis inducer	[54]
8	24.25	Hexadecanoic acid, 2-[(trimethylsilyl)oxy]-1,3-propanediyl ester	4.88	C ₃₈ H ₇₆ O ₅ Si	Fatty acid derivative	No activity reported	
9	26.27	Astaxanthin	13.89	C ₄₀ H ₅₂ O ₄	Carotenoid	Antioxidant, anticancer, antidiabetic, anti-hypertension, anti-atherosclerosis, and anti-ulcer	[55]
10	27.18	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	25.66	C ₁₂ H ₃₈ O ₅ Si ₆	Siloxane derivative	Antimicrobial, antiseptic, hair conditioning agent, skin conditioning agent-emollient; solvent	[26]

RT retention time (min)

**Fig. 2** Antitumor activity (IC₅₀ value) of the ethanolic extract of four Egyptian wild plant species against HepG2 cell line. Values having different superscripts are statistically significant at $P < 0.05$

classes, total phenolics and total flavonoids, in the ethanolic extracts of four Egyptian plant species (*V. candicans*, *P. harmala*, *S. vermiculata*, and *C. dioscoridis*). Furthermore, we assessed their phenols and flavonoids as well as antioxidant activities, in addition to their anti-tumor activities against the hepatocellular cell line HepG2.

Natural antioxidants are very effective to fight against oxidative stress. Medicines derived from plant products are safer than their synthetic counterparts. Recently, the exploration of natural antioxidant compounds has gained considerable attention [57]. The antioxidant response varies remarkably depending on the chemical structure of phenolic compounds [58]. Phenolic compounds of plants fall into several categories. Chief among these are the flavonoids which have potent antioxidant activities [59]. Flavonoids are naturally occurring in plants which have positive effects on human health. They have a wide range of biological activities like antibacterial, antiviral, anti-inflammatory, anticancer, and anti-allergic activities, in addition to their potent scavenger activity against free radicals implicated in many diseases [59]. These results are in accordance with those obtained by [60]. As reported previously, the potential of flavonoids to act as antioxidant depends on their molecular structure. The position of the OH group and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities [61].

Antioxidants fight against free radicals. The latter are known to play a definite role in a wide variety of pathological manifestations. Antioxidants exert their action either by protecting the antioxidant defense mechanisms or scavenging the reactive oxygen species [62]. The electron donation ability of natural products can be measured by DPPH assay. The assay is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolorizes the DPPH solution. The degree of color change is proportional to the concentration and potency of the antioxidants. A decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the tested compound [63]. Our results suggest that the plant extracts contain active constituents that are capable of donating hydrogen to a free radical to scavenge the potential damage. Phenolic contents and flavonoids may be the major contributors for the antioxidant activity as the IC_{50} values of radical scavenging activity of the investigated ethanolic extracts. It has been reported that many flavonoids and related polyphenols contribute to the phosphomolybdate scavenging activity of medicinal plants [64]. A previous study by [65] indicated the close relationship between total phenolic content and antioxidative activity of plants.

Gas chromatography–mass spectroscopy (GC-MS) is a valuable tool for the reliable identification of phytochemicals. This technique has been used in defining many active components with valuable therapeutical activities in the extracts of the studied plant species (Tables 4, 5, 6, and 7).

The anticancer activity of the ethanolic extracts of the investigated plants is based on MTT conversion into formazan with water-insoluble crystals via dehydrogenases in the mitochondria of alive cells with a dose-dependent manner [66]. The cytotoxic effect of *S. vermiculata* may be attributed to the presence of astaxanthin which is considered to be a major component, in addition to stearic acid. Our results were in agreement with those obtained by Khan et al. [67], who reported that stearic acid, as an ester derivative, inhibits the growth of human breast cancer cells. The anticancer activity of astaxanthin was reported in many studies [68, 69]. The anticancer activity of the study plants may be attributed to the presence of high content of fatty acids and fatty acid esters.

5 Conclusion

In conclusion, the present study showed that the four plant extracts significantly varied in their contents of polyphenols, flavonoids, antioxidant capacity, and antitumor activity. The highest contents of polyphenols, flavonoids and antioxidant capacity were recorded in *V. candicans* extract. However, the highest DPPH scavenging and antitumor activities were reported in the extract of *S. vermiculata*. The antioxidant and antitumor activities of the extracts are mainly dependent on their active constituents. Moreover, further studies are required to investigate the mode of action and the in vivo anticancer activities of the studied extracts.

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Authors' contributions

Conceptualization and paper idea: TKD, TAD, and KMS. Experiments and methodology: TKD, TAD, and KMS. Formal analysis and investigation: TKD and TAD. Writing the original draft: TKD, TAD, and KMS. Writing the review and editing: TKD, TAD, and KMS. Resources: TKD, TAD, and KMS. Journal correspondence and paper submission: KMS. The authors read and approved the final manuscript.

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