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PRIMARY GC-MS CHEMICAL ANALYSIS OF ALCOHOLIC EXTRACT OF *EMEX SPINOSA* (L.) CAMPD. AND SCREENING OF THEIR ANTIOXIDANT, ANTIBACTERIAL, AND CYTOTOXIC CHARACTERISTICS

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ABSTRACT. The chemical constituents of the methanolic extract of *Emex spinosa* shoots were primarily characterized by gas-chromatography-mass spectrometry analysis (GC-MS), forty components were identified. The major components were ethyl 2-hydroxycyclohexane-1-carboxylate (10.11%), 3,5-dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one (38.09%), 2-propyltetrahydro-2*H*-pyran-3-ol (5.75%), oleic acid (8.02%), and methyl (*E*)-octadec-16-enoate (5.91%). The results of antioxidant activity of leaves extract presented the most potent activity (IC_{50} = 29.92 mg mL^{-1}), followed by stem extract (IC_{50} = 41.17 mg mL^{-1}), and root extract (IC_{50} = 50.14 mg mL^{-1}), comparable to that of the standard ascorbic acid (IC_{50} = 13.3 mg mL^{-1}). The antibacterial results revealed that leaves extract is the most potent (22.0 mm) antibacterial agent against the variety of the tested species. Stem and leaves extracts are more potent (20 and 20 mm, respectively) than azithromycin (13.0 mm) against *Pseudomonas aeruginosa* species, while root and leaves extracts displayed potent antibacterial activities than tetracycline (10 mm). The most potent activity was recorded for leaves extract against *Bacillus cereus* species (22.0 mm) higher than the standard antibiotics (5.0-20.0 mm). The cytotoxic activity of *E. spinosa* MeOH extract specified the EC_{50} value at 2.68 µg mL^{-1} attended by a cytotoxic effect on HepG2 cell lines with the effective dose of the plant extract at a higher concentration.

KEY WORDS: *Emex spinosa*, GC-MS, Antioxidant, Antibacterial, Cytotoxic activities

INTRODUCTION

In recent years, traditional medicinal plants acting the main topic in the research of the medicinal fields, which these plants are rich in therapeutic components that could be applied in drug development [1]. The downgraded side effects that associated with the synthetic drugs were the essential objective of the researchers, so the natural plants remain the superior source of bioactive, and pharmaceutical drugs [2, 3]. Particularly, the Polygonaceae family as an anniversary prostrate herb with small elliptic-lanceolate leaves [4] included approximately 1200 herb species assorted into about 50 genera [5], and distributed in the coastal strip of the Mediterranean Sea in Egypt. *Emex spinosa* is a sprawling weed frequently identified as devil's thorn or lesser jack, is an annual herbaceous plant of the Polygonaceae family grown in sandy soils [6, 7]. The plant decryption revealed the plain shape of the leaves, and the roots are thick and juicy and the plant form seeds after its life end at the base of the stem.

Many plants of the Polygonaceae family "tiny annual herbs" constituted diverse advantaged biological characteristics, specifically, the bioactivity estimation of *Rumex abyssinicus* as an anti-inflammatory, and wound healing agent [8], antibacterial, and antioxidant properties of *Rumex vesicarius* [9], *Rumex maritimus* as a potent antioxidant, antimicrobial and anti-diarrhoeal agents [10], bioactivity study of *Coccoloba* [11], and antimicrobial activity of medicinal plants grown in China [12]. Moreover, the extracts of five genera of the Polygonaceae family known as *Fallopia*, *Oxyria*, *Persicaria*, *Polygonum*, and *Rumex* were reported as anti-proliferative agents against HeLa, A431, and MCF7 cells [13]. Currently, several types of research studies had been focused on the identification of the chemical components of the plant extracts of the herbs related to the

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Polygonaceae family. Thus, these chemical constituents have been studied using spectroscopic analysis such as the utility of GC-MS for the characterization of the components isolated from *Rumex pictus* and *R. vesicarius* [14], *Calligonum polygonoides* [15], and *Triplaris gardneriana* [16]. Lately, the extracted constituents of the plants of this family were characterized by GC-MS spectrometry analysis such as ethyl acetate fractions of *Emex spinosa* [17], *Rumex confertus* [18], and *Calligonum* species [19]. On the other hand, the investigation studies concerning the medical importance of plant extracts were directed towards the biological aspects and characteristics of these plants. Accordingly, the different biological properties of *Emex spinosa* plant issues extracts were estimated as antioxidants, cytotoxic, and antimicrobial activities [20-22].

Consequently, bearing in mind our attention to the research of Egyptian medicinal plants [23-27], we intended herein the biological properties of these plants such as antibacterial, antioxidant, and cytotoxic activities. The current research deals with the extraction of *Emex spinosa* (L.) Campd. issues such as leaves, stems, and roots by methanol to *in vitro* assess their potency as antioxidant activities using DPPH colorimetric assay, antibacterial impacts against the varied Gram-positive, and Gram-negative species, and cytotoxic activity on human liver cancer cell line (HepG2) using MTT assay. As well as, characterization of the chemical components of the methanol extracted *E. spinosa* using GC-MS spectrometry analysis.

EXPERIMENTAL

Plant material and extraction process

The investigated *E. spinosa* plant issues were collected from Wadi Hagul, northern part of the Eastern Desert of Egypt (56°29′29.40″N 10°32′47.90″E) during the flowering season (April, 2021) and authenticated by the staff members, Department of Botany, Faculty of Science, Mansoura University, Egypt. The plant issues, i.e. leaf, stem, and root, were subsequently cleaned, air-dried, and divided into small pieces. A weighed of 10 g of each plant issue was retained in a 250 mL conical flask, then 150 mL of methanol was added. The conical flask was placed in a horizontal water bath shaker (Memmert WB14, Schwabach, Germany), for two hours at room temperature. The mixture was filtered using qualitative Whatman filter paper no. 1 (125 mm, Cat No 1001 125, Germany), and the obtained extracts were concentrated to a definite volume and stored at 4 ºC [28]. For GC-MS the obtained alcoholic extract was evaporated to dryness using rotary-evaporator at 40 °C. The viscous residue was collected in glass vials and stored in the refrigerator at 4 °C or can use it directly.

Gas chromatography-mass spectrometry analysis (GC-MS)

The chemical constitutes of the methanol extract of *E. spinosa* plant shoots were characterized by implementing the plant extract on Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG-5MS (30 m x 0.25 mm x 0.25 µm film thickness) [29]. The temperature of the column oven was firstly held at 50 ºC, raised subsequently by a rate of 5 ºC per minute to reach 250 ºC, and hold for 2 min, and then the temperature was raised to the final temperature (300 ºC) by 30 ºC per minute and hold for 2 min. the injector and MS transfer line temperature were kept at 270, and 260 ºC, respectively. Helium (He) was used as a carrier inert gas at a constant flow rate of 1 mL/min. The solvent was released after 4 min and the diluted samples of 1 µL were injected directly using Autosampler AS1300 coupled with GC in the split mode. EI mass spectroscopy was collected at 70 EV ionization voltage over a range of 50-500 for *m/z* in packed scan mode. The temperature of the ion source was fixed at 200 ºC. The chemical components of the individual extracted plant materials were interpreted by a comparison of their mass spectral data with those of WILEY 09, and NIST 14 mass spectroscopic database.

Potential biological characteristics

Antioxidant DPPH assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) colorimetric assay was *in vitro* applied to estimate the antioxidant aptitude of the readily extracted issues of *E. spinosa* such as root, stem, and leaves. The procedure was accomplished as formerly reported [30], by the determination of the reduction in violet color of DPPH solution with the calculation of the scavenging activity percentages, and inhibitive concentrations of the investigated samples. In this method, the stocks of the extracts were diluted with methanol to acquire diverse concentrations of each extracted *E. spinosa* part at different concentrations (5, 10, 20, 30, 40, and 50 mg L^{-1}). Next, the DPPH solution was prepared at a concentration of 0.135 mM, and successfully 1 mL of the DPPH solution was added to each prepared concentration. The mixture was shaken and therefore retained in dark at room temperature for 15 min. The positive control was prepared using catechol with the same concentrations of the tested samples, adding 1 mL of DPPH solution, and kept under the same previous conditions. The absorbance reads of the investigated samples were measured at $\lambda = 517$ nm by UV/Vis spectrophotometer apparatus (Spekol 11 spectrophotometer, analytic Jena AG, Jena, Germany). Therefore, the inhibitive concentrations (IC_{50}, mg L^{-1}) were calculated by applying the plotted exponential curve [31], specifying the relationship of the sample concentration versus the percentage of remaining $DPPH^{\bullet}$ radical. In addition, the percentages of radical scavenging activities were calculated from the following equation, as "A control" expresses the absorbance read of the positive control, and "A sample" expresses the read of the sample absorbance.

$$\% \text{ Inhibition} = [(\text{A control} - \text{A sample})/\text{A control}] \times 100 \quad (1)$$

(A control) expressed the absorbance of the control sample. (A sample) expressed the absorbance of each sample concentration.

Assessment of the antibacterial activity

Culture media

The nutrient agar (28.0 g) was subjected to a conical flask (2 L) and mixed with 1 L of distilled water. The contents of the conical flask were heated until boiling point for the complete dissolution of the medium. Sterilization of the mixture by autoclave was accomplished at 15 lbs pressure, 121 ºC for 15 min. The medium was left to cool to 45-50 ºC, enriched with 5-10% blood or biological fluids. The mixture was shaken and poured into sterile Petri plates.

Bacterial species

The microbial species were purchased from the Cairo Microbiological Resources Centre (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University. Gram-negative bacteria: *Escherichia coli* (ATCC_10536), *Pseudomonas aeruginosa* (ATCC_9027), *Salmonella typhimurium* (ATCC_25566) and *Klebsiella pneumoniae* (ATCC_10031). Gram-positive bacteria: *Bacillus cereus* (EMCC_1080), *Staphylococcus aureus* (ATCC_6538), *Staphylococcus haemolyticus* (ATCC_29970), *Staphylococcus xylosus* (NCCP_10937), and *Staphylococcus epidermidis* (ATCC_12228). Cephradin, Tetracycline, Azithromycin, and Ampicillin were used as standard antibiotics.

Microbial testing

The antimicrobial activity of the extracted plant issues was estimated by agar well diffusion assay [32] using inoculums containing 10^6 bacterial cells/mL to spread on nutrient agar plates. The sterilized filter paper discs (Whatman no.1, 6 mm in diameter) were immersed overnight in the

extracted issues of the plant until saturation, and another set of filter paper discs was immersed in methanol as a control. The discs were placed on the surface of agar plates seeded with definite bacterial microorganisms. The plates were incubated at 37 ºC for 18-24 hours, and the inhibition zone diameters (mm) were measured [33].

Cytotoxicity assay

Tumor cell lines

HePG-2 (Hepatocellular carcinoma) was selected as a human tumor cell line purchased from ATCC *via* a holding company for biological products and vaccines (VACSERA), Cairo, Egypt.

Preparation of MTT solution

The MTT solution was prepared by mixing a solution of MTT in water (10 mg/mL), ethanol (20 mg/mL), and buffered salt solutions and media (5 mg/mL). The mixture was mixed by vortex or sonication, then filtered, and stored at -20 °C.

Procedure of MTT assay [34]

The cytotoxic activity of the extracted *E. spinosa* was evaluated by MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide) colorimetric assay utilizing an altered process termed by Terblanche *et al*. [35]. For the determination of the IC_{50} of each particular drug, the adherent HepG2 cells were seeded onto 96-well plates at an initial density (3×10^3 cells/well suspended in 100 µL of complete medium). The plant extracted issues were prepared to stimulate the cells with five concentrations (31.3, 62.5, 125, 500, and 1000 µg mL^{-1}) in culture media. The experimental technique was performed in duplicates, then plates were incubated for 24 hours in a 5% CO_2 at 37 ºC for settle down and adherence. Next, a serial dilution of the drug was applied to the cells for 48 hours after adherence. The medium was removed by aspiration and a weighed MTT (0.5 mg mL^{-1}) was dissolved in a culture fresh medium and practically applied onto cells and the plates were incubated at 37 ºC and 5% CO_2 for 4 hours. Ultimately SDS (100 µL) was added to each well. The reduction in cell growth was measured at (λ_{max} = 570 nm) (BioTek, Elx800, US) and the results were expressed as a percentage of control. The IC_{50} values of the drug, which expressed the concentration that affects roughly 50% of the death of tumor cells, were assessed through a straight linear regression, type sigmoidal, analyzed using Origin 8.0® software (Origin Lab Corporation). The IC_{50} values were calculated by the fit line, i.e. Y = a*X + b, in which IC_{50} = (0.57-b)/a. The percentage of inhibition in cell growth was calculated from the following equation (Eq. 2), in which A is the absorbance read of the control, and the tested sample:

$$\% \text{ Inhibition} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100 \quad (2)$$

The relative cell viability percentage was subsequently calculated from the following equation (Eq. 3), in which A expressed the control, and sample absorbance at λ_{max} = 570 nm.

$$\% \text{ Cell viability} = \frac{\text{A treated sample} - \text{A blank}}{\text{A control} - \text{A blank}} \times 100 \quad (3)$$

RESULTS AND DISCUSSION

Primary GC-MS chemical analysis

The chemical constitutes of the methanol extract of *E. spinosa* were established by gas-chromatography mass spectrometry "GC-MS" (Table 1). The results confirmed that forty components were characterized based on a comparison of the different mass spectra with the spectroscopic database of WILEY 09, and NIST. In general, 3,5-dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one is situated as the major component with 38.09% composition which was identified after 9.07 min. This result is agreeing with the GC-MS analysis of *Rheum ribes* rhizomes belonging to the Polygonaceae family [36]. Consequently, other constitutes were characterized with high percent of composition such as ethyl 2-hydroxycyclohexane-1-carboxylate (10.11%), 3-hydroxydodecanoic acid (1.83%), melezitose (2.64%), (*E*)-octadec-11-enal (2.88%), 5-heptyldihydrofuran-2(3*H*)-one (3.28%), nonyl propionate (3.29%), 2-propyltetrahydro-2*H*-pyran-3-ol (5.75%), methyl (*E*)-octadec-16-enoate (5.91%), and oleic acid (8.02%) with total composition percent 43.71%. The identified components were classified under numerous categories of naturally occurring components, in which the class of fatty acids, and/or their ester derivatives have the majority of these components. Thus, the class of aromatics included mainly six components, for instance, di-2-benzothiazole disulfane (0.40%), and (*E*)-1-(butan-2-ylidene)-2-(2,4-dinitrophenyl)hydrazine (0.71%), in addition to the class of carbohydrates, which also included six components, i.e. D-fructose, diethyl mercaptal, pentaacetate (1.56%), l-gala-l-ido-octose (0.69%), and melibiose (0.57%) with relatively low composition percentages.

Also, three esters of the oxygenated hydrocarbon class are not related to the derivatives of fatty acids were interpreted. In addition, eleven esters of fatty acids were characterized as the major class of compounds, and three fatty acids, for example, 3-hydroxydodecanoic acid (1.83%), oleic acid (8.02%), and 2-bromotetradecanoic acid (0.42%) were established after retention time at 6.57, 15.27, and 16.21 min, respectively. The free fatty acids were recently reported and isolated from *Polygonum orientale* [37], and *Polygonum aviculare* [38] that are related to the Polygonaceae family. The curan-17-oic acid, 19,20-dihydroxy-, methyl ester, (19*S*)- was classified as a type of indole alkaloid was found with a composition percentage of 0.92% after 12.21 min of the scan. The oxygenated hydrocarbons included also six components namely, (*E*)-octadec-11-enal, ethyl 2-hydroxycyclohexane-1-carboxylate, 5-heptyl-dihydro-furan-2(3*H*)-one, 3,5-dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one, 2-propyltetrahydro-2*H*-pyran-3-ol, l-gala-l-ido-octonic lactone, and 2-((tetrahydro-2*H*-pyran-2-yl)oxy)-9-oxabicyclo[3.3.1]-nonan-3-yl acetate. On the other hand, two components of steroids were identified as estra-1,3,5(10)-trien-17β-ol, and ethyl *iso*-allocholate with very low composition percentages after retention times at 19.79, and 28.32 min, respectively. The GC-MS analysis of ethyl acetate fractions of *E. spinosa* [17], and volatile constituents of *Rumex confertus* [18] was recently reported.

In this work, the oxygenated hydrocarbons displayed the majority of the components with 63.84% of the total identified extracted components, followed by fatty acids, and lipids (25.73%). Particularly, 3,5-dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one (38.09%), ethyl 2-hydroxycyclohexane-1-carboxylate (10.11%), and 2-propyltetrahydro-2*H*-pyran-3-ol (5.75%) are the major constituents of the oxygenated hydrocarbons, while oleic acid (8.02%), and methyl (*E*)-octadec-16-enoate (5.91%) are the major components of the fatty acids and lipids. The identified carbohydrates are located in the third order with six constituents not exceeding 2.64% for melezitose (Figure 1).

Table 1. The identified components of the MeOH extract of *E. spinosa* aerial parts using GC-MS analysis.

Entry	Chemical name	Classification	Retention time (min)	Molecular weight	Molecular formula	Composition %
Oxygenated hydrocarbons						
1	(*E*)-Octadec-11-enal	Oxygenated hydrocarbon	4.15	266.47	$C_{18}H_{34}O$	2.88
2	Omuralide	Lactone	4.21	213.23	$C_{10}H_{15}NO_4$	0.13
3	Ethyl 2-hydroxycyclohexane-1-carboxylate	Oxygenated hydrocarbon	4.96	172.22	$C_9H_{16}O_3$	10.11
4	5-Heptyldihydrofuran-2(3*H*)-one	Oxygenated hydrocarbon	7.27	184.28	$C_{11}H_{20}O_2$	3.28
5	(3-Acetoxy-4,5-dihydroisoxazol-5-yl)methyl acetate	Ester	7.42	201.18	$C_8H_{11}NO_5$	1.32
6	Methyl (*Z*)-2-(dimethylamino)-*N*-((methylcarbamoyl)oxy)-2-oxoethanimidothioate	Ester	7.88	219.26	$C_7H_{13}N_3O_3S$	0.15
7	Thymidin	Nitrogen base	8.40	242.23	$C_{10}H_{14}N_2O_5$	0.35
8	Methyl 6-oxoheptanoate	Ester	8.49	158.20	$C_8H_{14}O_3$	0.36
9	3,5-Dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one	Oxygenated hydrocarbon	9.07	144.13	$C_6H_8O_4$	38.09
10	2-Propyltetrahydro-2*H*-pyran-3-ol	Oxygenated hydrocarbon	9.44	144.21	$C_8H_{16}O_2$	5.75
11	Deoxyspergualin	Polyamine spermidine	10.23	387.53	$C_{17}H_{37}N_7O_3$	0.13
12	l-Gala-l-ido-octonic lactone	Oxygenated hydrocarbon	11.50	238.19	$C_8H_{14}O_8$	0.38
13	2-((Tetrahydro-2*H*-pyran-2-yl)oxy)-9-oxabicyclo[3.3.1]nonan-3-yl acetate	Oxygenated hydrocarbon (ester)	12.10	284.35	$C_{15}H_{24}O_5$	0.60
14	Dasycarpidan-1-methanol,acetate	Ester	21.44	326.44	$C_{20}H_{26}N_2O_2$	0.31
Aromatics						
15	N^2,N^4-Diisopropyl-6-(methylsulfonyl)-1,3,5-triazine-2,4-diamine	Hetryl amine	4.37	273.36	$C_{10}H_{19}N_5O_2S$	0.27
16	(*E*)-1-(Butan-2-ylidene)-2-(2,4-dinitrophenyl)hydrazine	Aromatic hydrocarbon	5.80	252.23	$C_{10}H_{12}N_4O_4$	0.71
17	Di-2-benzothiazole disulfane	Aromatic heterocycle	29.65	332.47	$C_{14}H_8N_2S_4$	0.40
Carbohydrates						
18	D-Fructose, diethyl mercaptal, pentaacetate	Carbohydrate	6.17	496.59	$C_{20}H_{32}O_{10}S_2$	1.56
19	l-Gala-l-ido-octose	Carbohydrate	6.84	240.21	$C_8H_{16}O_8$	0.69
20	Melibiose	Carbohydrate	8.78	342.30	$C_{12}H_{22}O_{11}$	0.57
21	Desulphosinigrin	Glycoside	11.15	279.31	$C_{10}H_{17}NO_6S$	0.21
22	d-Gala-l-ido-octonic amide	Glycosyl amide (carbohydrate)	11.40	255.22	$C_8H_{17}NO_8$	1.04
23	Melezitose	Trisaccharide sugar	14.62	504.44	$C_{18}H_{32}O_{16}$	2.64
Fatty acids, and lipids						
24	Methyl octadeca-8,11-diynoate	Ester of fatty acid	4.45	290.45	$C_{19}H_{30}O_2$	0.26
25	3-Hydroxydodecanoic acid	Fatty acid	6.57	216.32	$C_{12}H_{24}O_3$	1.83

26	3-(Icosanoyloxy)propane-1,2-diyl diacetate	Ester of fatty acid	6.71	470.69	$C_{27}H_{50}O_6$	1.66
27	Nonyl propionate	Ester of fatty acid	8.14	200.32	$C_{12}H_{24}O_2$	3.29
28	*tert*-Butyl palmitate	Ester of fatty acid	12.15	312.54	$C_{20}H_{40}O_2$	0.41
29	Oleic acid	Fatty acid	15.27	282.47	$C_{18}H_{34}O_2$	8.02
30	2-Bromotetradecanoic acid	Fatty acid	16.21	307.27	$C_{14}H_{27}BrO_2$	0.42
31	2,3-Dihydroxypropyl palmitate	Ester of fatty acid	17.12	330.51	$C_{19}H_{38}O_4$	1.42
32	Octadecanoic acid,2,3-dihydroxypropyl ester	Ester of fatty acid	21.66	358.56	$C_{21}H_{42}O_4$	0.21
33	3-Hydroxypropane-1,2-diyl dipalmitate	Ester of fatty acid	21.73	568.92	$C_{35}H_{68}O_5$	1.35
34	Methyl 11-((2R,3S)-3-pentyloxiran-2-yl)undecanoate	Ester of fatty acid	22.12	312.49	$C_{19}H_{36}O_3$	0.35
35	2-Hydroxypropane-1,3-diyl (9*E*,9'*E*)-*bis*(octadec-9-enoate)	Ester of fatty acid	27.57	621.00	$C_{39}H_{72}O_5$	0.24
36	Methyl (*E*)-octadec-16-enoate	Ester of fatty acid	29.16	296.50	$C_{19}H_{36}O_2$	5.91
37	Petroselinic acid, TMS derivative	Ester of fatty acid	31.45	354.65	$C_{21}H_{42}O_2Si$	0.36
Alkaloids						
38	Curan-17-oic acid, 19,20-dihydroxy-, methyl ester, (19*S*)-	Indole alkaloid	12.21	358.44	$C_{20}H_{26}N_2O_4$	0.92
Steroids						
39	Estra-1,3,5(10)-trien-17β-ol	Steroid	19.79	256.39	$C_{18}H_{24}O$	0.19
40	Ethyl iso-allocholate	Steroidal ester	28.32	436.63	$C_{26}H_{44}O_5$	1.2
						Σ = 99.97

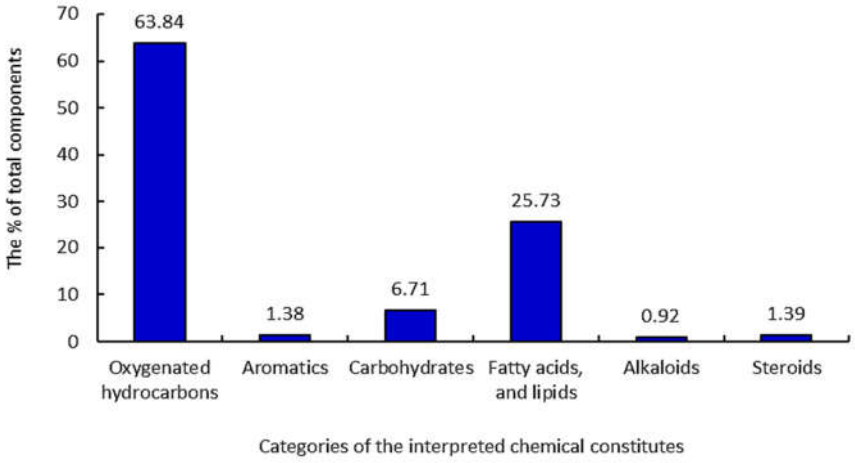

Figure 1. A comparison of the identified categories of the extracted *E. spinosa* aerial parts.

Biological evaluation

Antioxidant activity

The potential antioxidant capacity was evaluated for the methanol extracted issues of *E. spinosa* by a colorimetric DPPH• free radical assay. Thus, six concentrations in a serial dilution of the extracted root, stem, and leaf of *E. spinosa* were prepared in the range of 5-50 mg L^{-1}. Ascorbic acid was used as an effective antioxidant standard. The results in Table 2 demonstrated that the extracted issues of *E. spinosa* presented moderate to weak antioxidant potentials compared to the results of ascorbic acid. Therefore, leaf extract is most potent antioxidant agent (IC_{50} = 29.92 mg L^{-1}), followed by stem extract (IC_{50} = 41.17 mg L^{-1}), and root extract (IC_{50} = 50.14 mg L^{-1}).

The stem extract has the most potent scavenging activity percent (9.07±0.53%) at the lower concentration (5.0 mg L^{-1}), while leaves extract displayed the most potent activities for the other concentrations (10.0-50.0 mg L^{-1}). Particularly, the radical scavenging activity percentages increased by increasing the sample concentration in a proportional relationship. Thus, the most potent radical scavenging activity percentage was recorded for leaves extract at 50.0 mg L^{-1} with 71.71±3.77%. The antioxidant activities revealed the potent activity of leaves extract more than the other extracted issues (Table 2), these results are conceding with previous investigation [39]. The comparison of the results with the reference standard indicated that leaves extract is the most potent antioxidant agent at 10.0 mg L^{-1} with radical scavenging activity percentage of 29.07±1.71%, relative to ascorbic acid (45.19±1.22%). Also, the most potent radical scavenging activity percentage at 20.0 mg L^{-1} was recorded for leaves at 45.47±2.39% with good activity relative to ascorbic acid (64.97±2.14%) at the same concentration.

Table 2. Scavenging activity percentage of DPPH• and the IC_{50} values by methanol extracted issues of *E. spinosa*.

Samples	Conc. (mg L^{-1})	Scavenging activity (%) (average ± SD) [a]		
		Root	Stem	Leaves
Emex spinosa	5.0	5.6±0.33	9.07±0.53	7.14±0.42
	10.0	15.45±0.91	23.48±1.38	29.07±1.71
	20.0	23.69±1.25	36.36±1.91	45.47±2.39
	30.0	30.99±1.63	44.86±2.36	54.23±2.85
	40.0	43.907±2.31	49.04±2.58	60.96±3.21
	50.0	47.09±2.48	53.039±2.79	71.71±3.77
	IC_{50} (mg/mL)	50.14	41.17	29.92
	$LSD_{0.05}$ [b]	9.35***		
Ascorbic acid	Conc. (mg L^{-1})	Scavenging activity (%)		
	1.0	2.73±0.31		
	2.5	10.99±0.51		
	5.0	38.20±0.98		
	10.0	45.19±1.22		
	15.0	56.23±2.03		
	20.0	64.97±2.14		
	IC_{50} (mg/mL)	13.30		
	$LSD_{0.05}$ [b]	0.65***		

[a] Values are average (n = 3) ± standard deviation. [b] $LSD_{0.05}$ expressed the calculated least of the smallest significance between two means as each test was run on those two means (calculated by Factorial ANOVA).

The mechanism of the reactions involved in the assessment of the antioxidant activity is mainly dependent on many factors since the potent antioxidant agent provided the most stability of the formed free radicals that are responsible for trapping or scavenging the free radicals in

DPPH$^{\bullet}$ solution. The phenolic and flavonoids contents also control the process hence these components are the main sources of stable free radicals [40]. The structure-activity relationships of the identified chemical components of the extracted *E. spinosa* specified that 3,5-dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one (38.09%) has potent antioxidant characters as this component was isolated, and characterized from *Bryophyllum pinnatum* [41], *Cyamopsis tetragonoloba* seed [42], *Costus afer* [43], and *Cocus nucifera* [44] with potent antioxidant potency. Also, oxygenated hydrocarbons such as ethyl 2-hydroxycyclohexane-1-carboxylate, and 2-propyltetrahydro-2*H*-pyran-3-ol, and fatty acids or lipids such as oleic acid, and methyl (*E*)-octadec-16-enoate are effective components with reactive oxygen species increased the antioxidant potency of the investigated extracts.

Antibacterial activity

The results (Table 3) specified that most of the extracted issues of the plant revealed broad-spectrum antibacterial activities against a variety of bacterial species. Particularly, leaves extract virtually is the most potent antibacterial agent among the other ones of extracted *E. spinosa* issues. In accordance, leaf extract revealed broad spectrum against *E. coli* (inhibition zone diameter = 20.3 mm), *P. aeruginosa* (20.0 mm), *S. typhimurium* (20.0 mm), *S. epidermidis* (12.1 mm), *B. cereus* (22.3 mm), *S. aureus* (15.3 mm), *S. haemolyticus* (14.0 mm), and *S. xylosus* (15.2 mm) bacterial species. The activity of stem extract against *K. pneumoniae* (13.2 mm) referred to the higher potency to inhibit the growth of the bacterial species. On the other hand, the results indicated that the tested extracts are more applicable for potent results against the Gram-negative bacterial species (10.0-22.3 mm). Furthermore, leaf extract demonstrated antibacterial activity with an inhibition zone diameter of 20.3 mm more potent than cephradin (10.3 mm), and comparable to the results of ampicillin (20.0 mm), azithromycin (20.0 mm), and tetracycline (20.4 mm). All the extracted issues presented a superior spectrum to the tested antibiotics with inhibition zone diameters ranging from 10.4 to 20.1 mm against *P. aeruginosa*, and *S. typhimurium* species. In particular, by studying the role of the main chemical components of the extracted *E. spinosa* as antibacterial agents, we found that terpenes, oxygenated hydrocarbons, and carbohydrates presented higher antibacterial potential. Moreover, fatty acids and lipids in the essential oils of some extracted plants display improved antimicrobial characteristics [21, 26, 43, 44].

Table 3. The antimicrobial activity of methanol extract of *E. spinosa* issues compared to standard antibiotics.

Bacterial species	*E. spinosa* extracted issues (10 mg L^{-1})			Standard antibiotic (10 mg L^{-1})			
	Root	Stem	Leaf	Ampicillin	Azithromycin	Cephradin	Tetracycline
Gram-negative bacteria							
E. coli	10.3±0.31	10.2±0.03	20.3±0.23	20.0±0.08	20.0±0.14	10.3±0.07	20.4±0.15
P. aeruginosa	14.0±0.24	20.1±0.16	20.0±0.20	-	13.4±0.07	-	-
S. typhimurium	20.0±0.27	10.4±0.05	20.0±0.18	-	-	-	10.2±0.02
K. pneumoniae	10.2±0.02	13.2±0.22	10.4±0.4	5.5±0.1	13.0±0.03	10.2±0.02	20.0±0.09
Gram-positive bacteria							
B. cereus	11.3±0.04	11.2±0.11	22.3±0.17	5.2±0.01	20.2±0.08	20.0±0.06	10.2±0.04
S. aureus	14.1±0.11	10.2±0.03	15.3±0.11	30.1±0.21	20.0±0.06	20.1±0.11	20.1±0.21
S. epidermidis	12.3±0.05	12.0±0.09	12.1±0.02	10.3±0.2	23.0±0.09	13.2±0.05	20.3±0.17
S. haemolyticus	10.2±0.06	10.0±0.02	14.0±0.09	20.0±0.24	23.1±0.22	25.2±0.31	23.3±0.10
S. xylosus	10.0±0.02	10.0±0.23	15.2±0.12	25.2±0.27	20.3±0.13	20.4±0.14	20.2±0.08
$LSD_{0.05}$	0.0000***	0.0000***	0.0000***	0.0000***	0.0000***	0.0000***	0.0000***

Inhibition zone (mm).

In addition to the similar impacts of all extracts against *S. epidermidis* species (12.3 mm) with higher potency than ampicillin (10.3 mm) but their results remain less potent than the other antibiotics. Leaf extract is a more potent antibacterial agent (22.3 mm) than the investigated antibiotics (5.2 and 20.2 mm) against *B. cereus species.* The extracted issues revealed good potency against the other bacterial species of the Gram-positive bacteria type, for instance, *S. aureus*, *S. haemolyticus*, and *S. xylosus* species (10.0 and 15.3 mm) nevertheless with lower potency than the investigated antibiotics.

The factors that control the mechanism of antibacterial activity are the bacterial status such as susceptibility and resistance, tolerance, persistence, biofilm, the extract concentration, and host response [45]. In addition, the chemical constituents of the investigated extracts such as the major components of the extracted *E. spinosa* affected the antibacterial activities against a variety of bacterial species, for example, 3,5-dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one (DDMP) characterized from *Annona muricata* fruits [46], oleic acid characterized from *Helichrysum pedunculatum* [47], and ethyl 2-hydroxycyclohexane-1-carboxylate [48].

Potential cytotoxic activity

MTT assay

The methanol extract of shoot of *E. spinosa* was estimated as a cytotoxic agent using 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) assessment under individual conditions. The method is fitting to a growth curve of cell numbers. The MTT reagent as a dependable indicator is so sensitive to the light that engaged the run of the experiments in the dark. The hepatocellular carcinoma (HepG2) cancer cell line was selected to assess the cytotoxic potency of the extracted *E. spinosa*. The method was applied for determining the cell metabolic activity based on the aptitude of nicotinamide adenine dinucleotide phosphate (NADPH)-dependent cellular oxidoreductase enzymes to reduce the MTT tetrazolium dye to its formazan "insoluble" that has a purple color. The number of viable cells should increase with growth, decrease with cytotoxic treatments, and remain the same (or plateau) with cytostatic treatments. The IC_{50} values expressed the concentration that implied 50% of the cell growth inhibition calculated by the plotted curve of the percentages of cell survival versus drug concentration (µM). Thus, the potency of cytotoxicity will rise by the decrease in the extract concentration and IC_{50} values. The MTT solution may affect the results of cytotoxicity, so we have run a control sample "blank" that was an empty well containing MTT solution without any seeded cell lines. The control sample is a benefit for calculating the cell viability percent, as it produces 100% viability of healthy cells. The experiments were run using serial dilutions with five concentrations of the individual extract (31.3, 62.5, 125, 500, and 1000 µg mL^{-1}).

Table 4. Cytotoxic activity of the extracted *E. spinosa* on HepG2 cancer cell line.

Sample	Conc. (µg mL^{-1})	R_1 [a]	R_2 [a]	IC_{50} (µg mL^{-1}) [b]
E. spinosa	1000	0.22	0.218	501.4
	500	0.92	0.96	
	125	1.6	1.6	
	62.5	1.608	1.683	
	31.3	1.63	1.64	
	0	1.3	1.3	

[a] R_1, R_2 specified the doublet of sample absorbance at a definite concentration. [b] IC_{50} values specified the sample inhibitive concentration causes approximately 50% of the death of cancer cells.

Table 4 indicated the absorbance reads for each sample concentration along with the calculated inhibitive concentration value. The results showed that the extracted shoot of this plant has moderate cytotoxic activity with an IC_{50} value of 352.4 µg mL^{-1}. According to Soumya *et al.*

[44], 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (Oxygenated hydrocarbon) has cytotoxic efficacy against a variety of poly cystic ovaries desiase (PCOD). It is worth mentioning that the mechanism of cytotoxicity is commonly dependent on the nature of the chemical components of the extract, sample concentration, and the nature of the cancer cell line [49]. Additionally, the nature of the particles of the extract such as surface morphology, size, and aggregation of the particles might control the results of cytotoxicity.

The EC_{50} value of the extracted E. spinosa

The cytotoxic effects stated by the dose-response curve of the extracted *Polygonum bistorta* herb belonging to the Polygonaceae family were recently reported [50]. The dose-response relationship or dose-response curve designated the level of the response of the extracted samples as a function of exposure to a stimulus or stressor after a definite exposure time. The increase in exposure levels is attendant by increased or decreased risk of the outcome. Figure 2 indicated the fitted dose-response curve for viability assessment in HepG2 cell lines. The potency of the *E. spinosa* extract defines the dose necessary for a specified response. The dose-response curve in Figure 2a was normalized in the X-axis direction by its EC_{50} value (Figure 2b). The value of EC_{50} of the methanol extract of *E. spinosa* was initially calculated by plotting the read of the sample absorbance against the log of doses at different concentrations of the serial dilution (Figure 5). The low concentrations of the extract are not enough to produce a response, while the high doses produce a maximal response, and the vertical point of the curve resembles an EC_{50} value. The data analysis identified that the higher concentration (dose = 500 µg mL^{-1}) as calculated for EC_{50} value (2.68 µg mL^{-1}) has a cytotoxic effect on HepG2 cell lines.

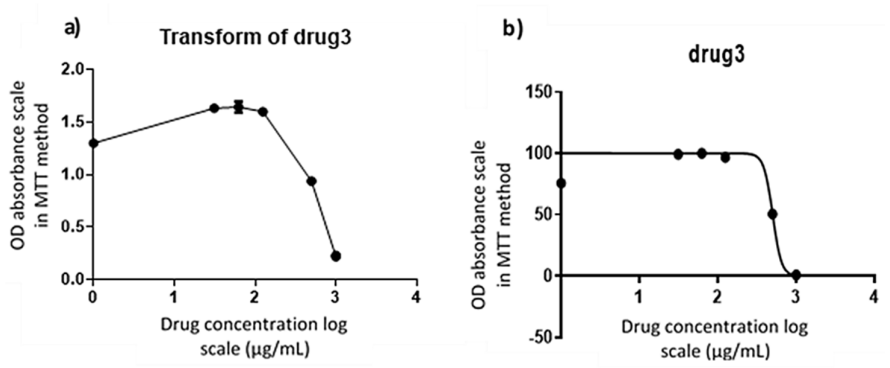

Figure 2. Dose response curve. (a) Transform of *E. spinosa* extract. (b) Normalization of transform of *E. spinosa* extract. (OD) specified the absorbance value stately at a certain concentration of the studied plant extract.

CONCLUSION

In brief, the chemical components of the extracted *E. spinosa* plant were characterized by GC-MS spectroscopy, in which 40 components were identified including oxygenated hydrocarbons (63.84%), fatty acids, and lipids (25.73%) with the major categories, while 3,5-dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one (38.09%), and ethyl 2-hydroxycyclohexane-1-carboxylate (10.11%), are the major components. The antioxidant DPPH$^{\bullet}$ free radical results revealed that leaves extract is the most potent antioxidant agent (IC_{50} = 29.92 mg mL^{-1}). In addition, leaf extract displayed the most potent antibacterial activities, in some cases, potent more than the antibiotic

standards. The cytotoxic activity of the extracted shoots of the *E. spinosa* plant indicated the EC_{50} value at 2.68 µg mL^{-1} attended by a cytotoxic effect on HepG2 cell lines with the effective dose at a higher concentration of the investigated extract. The remarkable percentage of oxygenated hydrocarbons, fatty acids, and lipids characterized from the extracted *E. spinosa*, and the considerable biological results supported the opportunity for future research on this plant for drug discovery from a natural source.

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