



## In Vitro Assessment of Antioxidant and Cytotoxic Activities of *Zygophyllum coccineum* L. Methanolic Extract



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### Abstract

*Zygophyllum coccineum* is a halophytic plant species that belongs to the Zygophyllaceae family and can survive in high-salted and coastal-land areas. The present study intends to identify *Z. coccineum* extract. GC/MS spectral analysis was used to characterize the volatile components of the plant extract and estimate their biological profiles. From the methanol extract of *Z. coccineum*, six components were discovered by GC-MS analysis, with 2-ethylhexanoic acid is the major volatile component with (82.98%) of total composition. It was found that 91b,5,5,6a-tetramethyl-octahydro-1-ox a-cyclopropane[a]inden-6-one had a high composition percentage (8.13%) in the terpene category, and 6H-[1]benzopyrano[4,3-C]isoquinoline-6,11(5H)-dione was found by composition percentage (2.90%) from the category of alkaloid. When evaluated for DPPH antioxidant activity, at 1000 mg/ml, the scavenging activities of 61.89 for *Z. coccineum* extract. However, the samples with the lowest concentration (100 mg/ml) demonstrate the weakest antioxidant activity overall. With IC50 values of 5.24, 8.80, and >100 g ml<sup>-1</sup> for HepG-2, PC3, and WI-38, respectively, the 50% methanol extract of *Z. coccineum* demonstrated an enhanced potential anticancer impact and significant cytotoxicity for the two tumor cells. As a result, the biological activity of *Z. coccineum* supports its use as a traditional therapy for a variety of diseases, including cancer treatment.

**Keywords:** *Zygophyllum coccineum*, Antioxidant activity, GCMS, tumor cells.

### 1. Introduction

*Zygophyllum* is the genus in the family Zygophyllaceae, with approximately eighty species worldwide. In Egyptian flora, seven species of *Zygophyllum* grow naturally in coastal and inland deserts [1]. Hypertension, diabetes, and even fungal infections are just some of the conditions that have been treated with their help [2]. Earlier studies had shown that these plants' genus had been the subject of several phytochemical studies, which had discovered a variety of biological components from different chemical classes, including flavonoids, triterpenes, esters, and phenolics [3,4].

The plant species *Zygophyllum coccineum*, which is part of the Zygophyllaceae family, is known for its capacity to thrive in highly salinated and coastal

environments [5]. *Z. coccineum* is the most popular and expanded *Zygophyllum* species in Egypt, where it lives in several and varied habitats and records a wide soil scope [6]. *Z. coccineum's* ability to resist salt concentration is due to its ability to generate secondary metabolites that function as antioxidants, and chemical and biomechanistic arbitrators to regulate various harmful environmental conditions and help plants adapt to the physiological, biochemical, and pathological effects of the desert environment [7,8].

The word "secondary compounds" in plants refers to a wide range of chemical substances that are not required for plant growth and development but are necessary for connections with other interactions with other microorganisms and environmental adaptations

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[9,10]. Plant secondary metabolites are distinctive sources of medications, food products, and other commercial materials, and the manufacture of these secondary metabolites has been made easier with the use of plant cell cultures [11-14]. And the major purposes of these metabolites are to defend plants against pests, diseases, insect attack, as well as other biotic and abiotic stresses [9,15,16]. Alkaloids, phenolic compounds, and terpenes are three classes of secondary metabolites found in plants. Each of these classes contains hundreds of different molecules, each of which has an ecological purpose [10,17]. The purpose of this investigation is to identify *Z. coccineum* extract. To determine the biological profiles of the volatile components of the plant extract, GC/MS spectrum analysis was utilized.

## 2. Materials and Methods

### 2.1. Plant materials and extraction methods

*Zygophyllum coccineum* was collected from the coastal desert near Port Said City, Egypt (31°17'26.08" N, 32°10'9.57" E). So, we washed it, let it air dry, and then sliced it up. Fifteen grams of the plant and 125 milliliters of methanol were put into a 250-milliliter conical flask. Using No. 1 Whatman filter paper, the mixture was filtered after being shaken for four hours at 25 °C in a horizontal water bath shaker (125 mm, Cat No 1011 126, Germany). The extracted substance was stored stably at 4 °C in a sterile bottle [18].

### 2.2. Gas chromatography-mass spectroscopic analysis (GC-MS)

By using the extract of plants on the Trace GC-TSQ mass spectrometer, the volatile plant components of the extract were effectively separated and identified by GC/MS spectrometry (Thermo Scientific, Austin, TX, USA) [19]. Based on the acquired results, the extracted components were analyzed using the mass spectroscopic databases WILEY 09 and NIST 14.

## 2.3. Biological Procedures

### 2.3.1. Procedure of the antioxidant activity

Estimating the plant extract's antioxidant activity using the DPPH assay with ascorbic acid as a standard was the focus of a study by Kitts *et al.* [20]. Methanol was serially diluted to provide a range of concentrations for each sample. Each sample was

diluted with the same 0.135 mM DPPH solution throughout the serial dilution process. The samples were then kept at 25 °C in the dark for 30 minutes. The absorbance of the samples' colour intensity was evaluated below 517 nm. The following antioxidant activity was found using graphically calculated IC<sub>50</sub> values:

$$\% \text{ Of DPPH scavenging} = [1 - (A_{\text{sample}} / A_{\text{control}})] \times 100$$

The IC<sub>50</sub> analysis indicates how much antioxidant was needed to lower the starting concentration of DPPH• solution by 50%. The antioxidant activity of the studied samples is inversely associated with the IC<sub>50</sub> values [21].

### 2.3.2. Cytotoxicity assay

The types of human cancers, hepatocellular carcinoma (HePG-2), human prostate cancer (PC3), and WI-38 (fibroblasts generated from lung tissue), were selected to serve as human tumor cell lines. MTT dissolved in water at a concentration of 10 mg/mL, ethanol at a concentration of 20 mg/mL, buffered salt solutions, and media at a concentration of 5 mg/mL were all combined to produce the MTT solution. Before being cleansed and stored at a temperature of -20 °C, the mixture was either sonicated or given a vortex.

To determine the IC<sub>50</sub> for each sample, we evaluated the *Z. coccineum* extract for its cytotoxic activities using an enhanced MTT colorimetric technique developed by Opoku *et al.* [22]. At first, 3 × 10<sup>3</sup> cells/well were seeded into 96-well plates while suspended in 100 L of complete media. There were seven sample concentrations used to activate the cells in the culture medium. Each plate was incubated for 24 hours at 37 °C and 5% carbon dioxide to allow for settling and adherence. After 2 days of adhesion, the cells were treated with repeated dilutions of the samples.

Once the old medium was removed, new media were added to the culture, and a weighted MTT solution (0.5 mg/mL) was added to the cells. After that, we stored the plates in a 37 °C and 5% CO<sub>2</sub> environment for four hours. Finally, 100 liters of Sodium Diacetate (SDS) were poured into each well. Cell growth inhibition was measured at (max. = 570 nm), and the findings were reported as a percentage

of the control (BioTek, Elx800, US). The IC<sub>50</sub> values for the drugs under study were calculated using sigmoidal straight linear regression and analysis in Origin 8.0®. (Origin Lab Corporation). The IC<sub>50</sub> values were determined by fitting a line to the data:  $Y = a \cdot X + b$ , with  $IC_{50} = (0.57 - b) / a$ . The percentage of growth inhibition was determined using the following equation, where A is the absorbance value of the control and test samples.

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

After that, we used the following equation, where A is the absorbance of the control and B is the absorbance of the sample at  $\lambda_{\text{max.}} = 570 \text{ nm}$ , to determine the relative cell viability %.

$$\% \text{ Cell viability} = (A_{\text{samples}} - A_{\text{blank}}) / (A_{\text{control}} - A_{\text{blank}}) \times 100$$

### 3. Results and Discussion

#### 3.1. GC-MS Spectroscopy

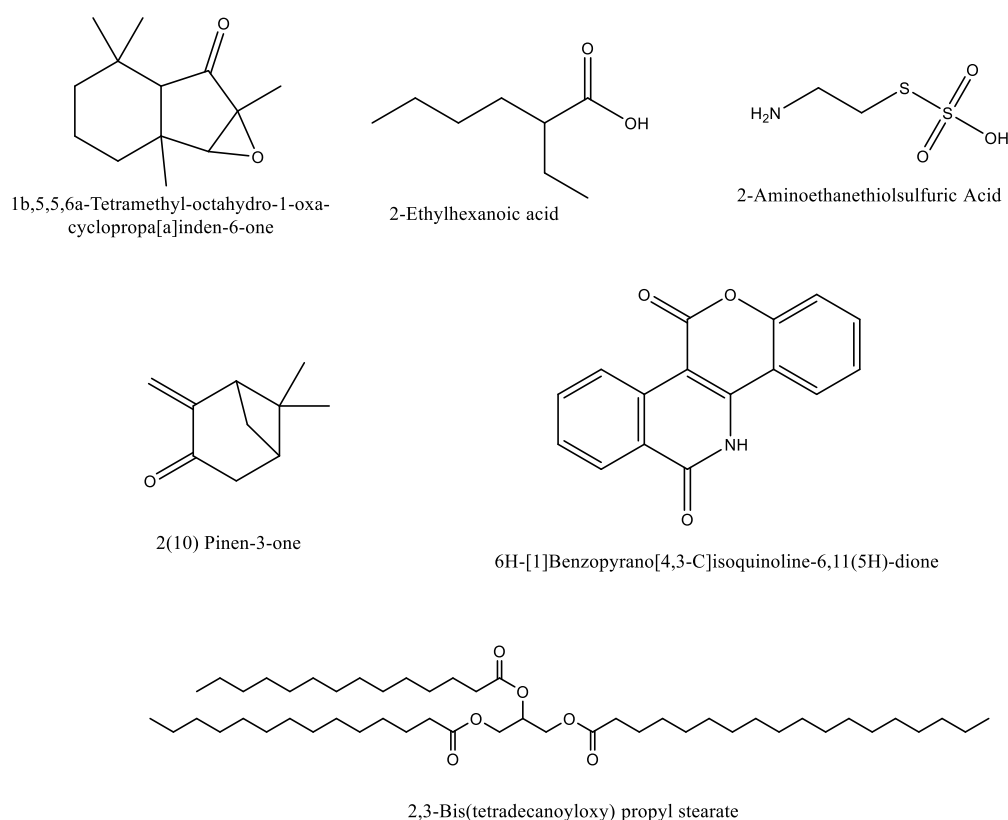
Gas-Chromatography Mass Spectroscopy "GC-MS" analysis was utilized for the characterization of the volatile components of *Zygophyllum coccineum* extracted by methanol. The relative abundance of the analyzed volatile components is depicted in Figure 1, with each residence time referring to the distinct major abundant components. The results of GC-MS analysis, as mentioned in Table 1, demonstrated that six components were recorded on the identified scale at diverse retention time with varied composition

percentages. Thus, 2-Ethylhexanoic acid is the major volatile component, with (82.98%) of the total composition recorded after 8.91 min. Particularly, 1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one (8.13%); 2,3-Bis(tetradecanoyloxy) propyl stearate (3.42%); 6H-[1] benzopyrano[4,3-C]isoquinoline-6,11(5H)-dione (2.90%); 2-aminoethanethiolsulfuric acid (1.29%), and 2(10) Pinen-3-one (1.26%). It was found that 91b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one had a high composition percentage (8.13%) in the terpene category, and 6H-[1]benzopyrano[4,3-C]isoquinoline-6,11(5H)-dione had a high composition percentage (2.90%) in the alkaloid.

As verified from Table 1, The volatile components of *Z. coccineum* extracted with methanol were classified into three groups. As a result, the compounds are related to terpenes, alkaloids, fatty acids, and their derivatives. The major class is documented for fatty acids and derivatives, with (86.4%) of the total area. Terpenes revealed a percentage of 9.39%, while the other class revealed a percentage of 4.19% for alkaloids. Alkaloids, fatty acids, and terpenes all have two components. Hernández-Aparicio *et al.* [23] found that tomato plants release variable amounts of the chemical 2-Ethylhexanoic acid. The phytochemical 2(10) Pinen-3-one was reported to be extracted from *Artemisia taurica*, according to Khodakov and Kotikov [24].

**Table 1.** Chemical constituents identified by GC/MS technique from methanol extract of *Zygophyllum coccineum*.

No.	Chemical name	Classification	RT (min.)	Molecular Weight	Molecular Formula	Composition%
Terpenes						
1	1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	Monoterpenes	4.40	208	C <sub>13</sub> H <sub>20</sub> O <sub>2</sub>	8.13
2	2(10) Pinen-3-one	Monoterpenes	15.50	150	C <sub>10</sub> H <sub>14</sub> O	1.26
Alkaloid						
3	6H-[1]Benzopyrano[4,3-C]isoquinoline-6,11(5H)-dione	Alkaloid	17.72	263	C <sub>16</sub> H <sub>9</sub> NO <sub>3</sub>	2.90
4	2-Aminoethanethiolsulfuric Acid	Alkaloid	12.03	157	C <sub>2</sub> H <sub>7</sub> NO <sub>3</sub> S <sub>2</sub>	1.29
Fatty acid and derivatives						
5	2-Ethylhexanoic acid	Fatty acid	8.91	144	C <sub>8</sub> H <sub>16</sub> O <sub>2</sub>	82.98
6	2,3-Bis(tetradecanoyloxy) propyl stearate	Fatty acid derivative	59.76	778	C <sub>49</sub> H <sub>94</sub> O <sub>6</sub>	3.42



**Figure 1.** Main chemical compounds identified by GC-MS from the MeOH extract of *Zygophyllum coccineum* aerial parts.

### 3.2. Biological Evaluation

#### 3.2.1. DPPH Antioxidant Activity

The potential antioxidant scavenging activity of the *Z. coccineum* extract by DPPH• free radical assay because of their methodological simplicity and stability, DPPH radical scavenging tests are frequently employed to explore the biological potential of natural extracts. The results of the samples that were put to the test were compared to those of catechol, which proved that the plant extract was more effective at capturing DPPH free radicals (Table 2). The results concur with phytochemical findings because the sample's ability to capture free radicals in the solution is improved by the presence of alkaloids and terpenes.

Based on the research results, the extract of *Z. coccineum* showed antioxidant effects in an amount of the drug ( $P \leq 0.05$ ), which was comparable with catechol as a reference standard (Table 2). At 1000 mg/ml, the scavenging activities of 61.89 for *Z.*

*coccineum* extract. However, among the examined samples, the lowest dose (100 mg/ml) exhibits the least antioxidant activity. Based on  $IC_{50}$  values, the most potent antioxidant capacity was recorded for the extracted *Z. coccineum* with an  $IC_{50}$  value of 71.23 mg/ml relative to the result of catechol ( $IC_{50}=15.23$  mg/mL). Previous studies on *Z. coccineum* have demonstrated antioxidant and antibacterial capabilities, supporting this in Gibbons and Oriowo, [25]; El-Shora et al. [26]; Abd El-Raheim et al. [27].

Free radicals can be neutralized by bioactive chemicals such as phenolics, flavonoids, terpenes, or oxygenated hydrocarbons because they contain functional active groups like the OH group [28, 29]. Fatty acid and terpenoid chemicals isolated from a variety of plants, such as *Deverra tortuosa* [30], *Salvia officinalis* [31], *Cleome amblyocarpa* [32], *Launaea* species [33], *Persicaria lapathifolia* [34], *Symphotrichum squamatum* [35] and *Coriandrum sativum* [36] have been found to play a significant role as potent antioxidant. The primary component

consists mostly of 2-ethylhexanoic acid, a family of fatty acids, and its derivatives. The antioxidant outcomes are consistent with past studies that

claimed that an increase in the amount of free hydroxy groups produced antioxidant properties that were approaching [37,38].

**Table 2.** The antioxidant results (% scavenging activity and IC<sub>50</sub>) by MeOH extract of *Zygophyllum coccineum*.

Treatment	Concentration (mg/ml)	Scavenging activity (%)	IC <sub>50</sub> (mg/ml)
<i>Zygophyllum coccineum</i>	1000	61.89±1.43 <sup>A</sup>	71.23
	800	58.43±1.56 <sup>B</sup>	
	600	43.87±1.02 <sup>C</sup>	
	400	31.97±1.21 <sup>D</sup>	
	200	30.49±1.88 <sup>D</sup>	
	100	20.09±1.21 <sup>E</sup>	
	LSD <sub>0.05</sub>	2.55***	
Catechol	500	84.35±2.34 <sup>A</sup>	15.23
	400	71.67±1.69 <sup>B</sup>	
	300	65.00±1.02 <sup>C</sup>	
	200	56.33±0.98 <sup>D</sup>	
	100	32.47±0.04 <sup>E</sup>	
	LSD <sub>0.05</sub>	3.74***	

Values are average (n = 3) ± standard deviation. LSD<sub>0.05</sub> represented the estimated least of the smallest significance between two means as each test was run on those two means (calculated by Factorial ANOVA).

### 3.2.2. Cytotoxic Activity

Cancer is one of the leading causes of mortality in the world. Numerous clinically effective anti-cancer agents have been obtained from plant-derived compounds [39]. The use of medicinal plants that are abundant in polyphenolic compounds may contribute to a lower incidence of cancer, according to growing research. The discovery of plant-based drugs primarily led to the creation of anticancer therapies and continues to support fresh leads in clinical trials [40]. The results presented here support the use of *Z. coccineum* extracts with varying medicinal characteristics in drug chemistry due to their enhanced biological potency and efficacy. In this study, the cytotoxic potential of the extracted plants was measured using an MTT assay. Several cancer cell lines and normal cell lines were used for in vitro analyses of the substance (Table 3). The drug doxorubicin was used as a standard against which to measure the effectiveness of the other drugs evaluated in this study against the various tumor cells.

This method was used to measure how well nicotinamide adenine dinucleotide phosphate (NADPH)-dependent oxidoreductase enzymes in cells changed the purple MTT tetrazolium dye into its insoluble formazan metabolite. Growth should result

in an increase in cell viability, which should decrease with anticancer therapies and remain constant (or plateau) with cytostatic ones. The control sample is useful for estimating the cell viability percentage since it yields healthy cells with 100% vitality.

Moreover, the percentages of inhibition were calculated at seven concentrations (1.56, 3.125, 6.25, 12.5, 25, 50, and 100 µg/mL) against all tumor and normal cells (Table 3). The results revealed significant inhibition percentages at higher concentrations. As a result, the *Z. coccineum* extract shown good cytotoxic effectiveness against HePG-2 and PC3 tumor cells at a concentration of 100 µg/mL, with% inhibitions at 54.82 and 61.71%, respectively. The results are supported by decreased inhibition against the WI-38 normal cell line with 13.93 % inhibition, which expresses the concentration that indicated 50% of the cell's inhibition of growth. The results were determined from the curves formed by plotting the percentages of cell growth vs. amount of drug (µg). Therefore, the effect of cytotoxic activity increases as the extract concentration and IC<sub>50</sub> values decrease. The IC<sub>50</sub> values of the *Z. coccineum* MeOH extract were 38.68, 49.65, and >100 g/ml-1 for HepG-2, PC3, and WI-38 normal cells, respectively. To compare the outcomes of the studied samples against the diverse types of cancers, doxorubicin was used as a benchmark medicine, with IC<sub>50</sub> values of 5.24, 8.80, and >100 g/ml-1 for HepG-2, PC3, and WI-38, respectively (Table 3).

Based on the IC<sub>50</sub> values, The extract obtained from this plant has a noncytotoxic effect for healthy cells (WI-38) and moderate cytotoxic activity on two human tumor cells (HepG-2 and PC3). According to Elbadry *et al.* [41] and Mohammed *et al.* [8], *Z. coccineum* extract showed cytotoxic effects against the HeLa and MCF-7 cell lines, and against MCF-7, HCT-116 and HepG2 cell-lines, respectively. This result agrees with result obtained by Salama et al.

[42,43] who studied, the antiproliferative activities of organic extracts prepared from *Reichardia tingitana* and *Rumex vesicarius* were tested in vitro against HeLa, MCF7 and PC3 cells by using the MTT assay.

**Table 3.** The percentages of inhibition at different concentrations against the studied tumor and normal cell lines.

Samples	Conc. (µg/mL)	In Vitro Cytotoxicity		
		HePG-2	PC3	WI-38
Doxorubicin	100	93.34	91.62	10.99
	50	88.55	81.82	9.77
	25	85.28	75.52	7.02
	12.5	68.72	58.42	5.11
	6.25	50.80	41.92	2.92
	3.125	39.20	25.59	1.51
	1.56	25.82	23.14	0.00
	<sup>(a)</sup> IC <sub>50</sub>	5.24	8.80	>100
<i>Zygophyllum coccineum</i>	100	54.82	61.71	13.93
	50	45.67	38.47	10.71
	25	41.22	29.25	8.94
	12.5	33.03	24.06	5.94
	6.25	26.20	15.48	2.60
	3.125	14.88	7.75	2.05
	1.56	2.65	0	0
	IC <sub>50</sub>	38.68	49.65	>100

#### 4. Conclusions.

Despite extensive research on plant extracts over the last two decades, the bioactivities and mechanisms of action of plant extracts are still incompletely known. In this study, six compounds were found in the methanolic extracts of *Z. coccineum*; six components were discovered by GC-MS analysis. It was found that the molecule 2-Ethylhexanoic acid had a high composition percentage (82.98%) in the fatty acid and derivatives category, 91b,5,5,6a-Tetramethyl-octahydro-1-ox a-cyclopropa[a]inden-6-one was found by composition percentage (8.13%) in the terpene category, and 6H-[1]Benzopyrano[4,3-C]isoquinoline-6,11(5H)-dione was found by composition percentage (2.90%) in the category of alkaloid. *Z. coccineum* extract demonstrated beneficial biological characteristics such as cytotoxic and antioxidant properties. The highest antioxidant activity was seen in *Z. coccineum* extract, indicating that it has a high ability to capture free radicals in the DPPH solution. The two tumor

cells, HepG-2 and PC3 cells, showed increased potential anticancer activity with *Z. coccineum* extract. As a result, the biological activity of *Z. coccineum* supports its use as a traditional therapy for a variety of diseases, including cancer treatment.

#### 5. Conflicts of interest

“There are no conflicts to declare.”

#### 6. Acknowledgments

“None”

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