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Essential Oils of *Zygophyllum coccineum* L. Chemical Characterization, Antioxidant, Antibacterial and Cytotoxic Activity on Human Breast Adenocarcinoma (MCF-7)

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ABSTRACT

Background: *Zygophyllum coccineum L.,* a halophytic plant from the *Zygophyllaceae* family, has traditional medicinal uses. Its essential oil (EO) contains diverse bioactive compounds with potential therapeutic value. This study investigates the *chemical composition of Z.* coccineum EO and evaluates its antioxidant, antibacterial and cytotoxic activities.

Methods: EO was extracted from the aerial parts of Z. coccineum via hydro-distillation and analyzed using GC-MS. Antioxidant activity was assessed using DPPH and ABTS assays, while antibacterial effects were tested against Gram-positive and Gram-negative bacteria using the agar diffusion method. Cytotoxicity was evaluated against MCF-7 breast cancer cells using the MTT assay.

Result: GC-MS analysis identified 22 compounds in *Z. coccineum* EO, with palmitic acid (20.36%), 1-(2,3,6-trimethylphenyl)-2-butanone (15.62%) and (E)-3-(2,6,6-trimethylcyclohex-1-en-1-yl) acrylaldehyde (11.47%) as major constituents. The EO exhibited significant antioxidant potency, with IC_{50} values of 19.96 µL/L (DPPH) and 16.70 µL/L (ABTS). It showed strong antibacterial activity, particularly against *Salmonella typhimurium* (20.88 mm inhibition zone). Additionally, the EO also demonstrated cytotoxic towards MCF-7 cells with an IC_{50} of 48.26 µg/mL while exhibiting minimal toxicity toward normal WI-38 cells. Conclusion, *Z. coccineum* EO has a satisfactory bioactive molecule reservoir with potent antioxidant, antibacterial and discriminative anticancer activities. The findings validate its potential pharmaceutical uses.

Key words: Antibacterial, Essential oil, GC-MS, DPPH, MCF-7, *Zygophyllum coccineum*.

INTRODUCTION

Traditional medicine is still well integrated into cultural healthcare systems in the majority of developing countries. All therapeutic medicines were originally produced from plants, whether raw plant components or refined crude extracts, mixes, *etc*. There are more than a thousand known plant species with therapeutic potential and interest in plant-based, eco-friendly medicinal resources increases worldwide (Qadir and Raja, 2021). Essential oils (EOs) with their highly diverse bioactive components represent a significant source of natural therapeutics. European Pharmacopoeia lists several important EOs for treating many disorders (Al-Khaial *et al.*, 2025). EOs have antioxidants, antifungal, antibacterial, antiviral, allelopathic and insecticidal properties (Abd El-Gawed *et al.*, 2020). EOs are also applications in food preservation, aromatherapy, aroma, medication, cosmetic and agricultural. Chemically, EOs consist of complex blend of volatile elements such mono-, sesquit- and diterpenes, phenylpropanoids, hyrdocarbons and others with distinct odours and economic value (Razni *et al.*, 2019).

Halophytes are salt-tolerant plants found in saline environments like salt marshes and the coasts. They possess specialized physiological and biochemical adaptations that enable them to thrive under salt stress (Zahran and El-Amier, 2013). Among them, *Zygophyllum* species play a vital ecological and economic role in arid environment, particularly of the Arabian Peninsula and Saharo-Sindian regions. *Zygophyllum coccineum*, a halophyte within the family Zygophyllaceae known as the "bean caper," is native to regions like North Africa, the Middle East and the Saharo-sindian area. It naturally grows in harsh environments by producing primary and secondary metabolites that function as antioxidants, phytoalexins and stress mediators (Porter, 1974; Zhang *et al.*, 2017). Various parts of *Zygophyllum* plants are abundant in bioactive substances such as phenolic compounds, alkaloids, terpenoids, polysaccharides and fatty acids (Amin *et al.*, 2012; Mohammed *et al.*, 2021). These phytochemicals are associated with therapeutic properties such as antimicrobial, anti-inflammatory and cytotoxic activities (Abd El Raheim

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et al., 2016; Mohammed *et al*., 2021). Moreover, in ancient medicines they were used in the treatment of disorders such as skin diseases, diabetes, high blood pressure, wound healing, anthelmintic, diuretic and gastrointestinal disorders (Gibbons and Orio, 2001). This work aims to describe the biochemical substance of *Z. coccineum* EO and assess its antioxidant, antibacterial and cytotoxic activities.

MATERIALS AND METHODS

Plant material

Aerial parts of *Z. coccineum* were gathered during its flowering period in June 2024 from various populations located in the northern Nile Delta region, Egypt (coordinates: 31°17′11.81″N, 32°10′35.08″E). Healthy, flowering specimens were selectively collected for the study. The identification of the plant was conducted in accordance with the taxonomic references provided by Boulos (1999). A voucher plant sample (code ManU.262603008) was created and kept at the Botany Department Herbarium at the Faculty of Science, Mansoura University, Egypt.

Extraction of EO and GC-MS analysis

The EO was extracted by hydro-distilling 200 g of air-dried *Z. coccineum* aerial parts for 3 hours using a Clevenger apparatus. The EO was separated with n-hexane, dried over anhydrous Na_2SO_4 and stored at ±5°C for further analysis. GC-MS analysis was performed as described by Abd-ElGawad *et al.* (2020) using a TRACE GC Ultra system with a quadrupole mass spectrometer. A 31 m × 0.33 mm column with a 0.26 µm film thickness was used, with helium as the carrier gas at 1.0 mL/min and a split ratio of 1:10. The temperature ramp started at 61°C for 1 min, increasing to 240°C at 4°C/min. Mass spectra were acquired at 71 eV (m/z 41-452). Compound identification was achieved using AMDIS software, NIST and Wiley libraries, retention indices and authentic standards.

Antioxidant activity of the EO

The antioxidant activity of *Z. coccineum* EO was evaluated using the DPPH radical scavenging assay (Miguel, 2010). EO concentrations (5-50 mg/L) were prepared in methanol and mixed with an equal volume of 0.3 mM DPPH solution. After 30 minutes of incubation at 25°C, absorbance was measured at ~518 nm using a spectrophotometer (Analytik Jena, Germany). Vitamin C served as a positive control at concentrations of 1-20 mg/L. The radical scavenging (RS) activity was calculated as:

$$RS\ (\%) = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

Following Re *et al.* (1999), the antioxidant activity of *Z. coccineum* EO was assessed using the ABTS radical scavenging assay. EO concentrations (5-50 mg/mL) were prepared and 2.5 mL of ABTS solution was mixed with 0.3 mL of EO. After 6 minutes of dark incubation, absorbance was measured at ~735 nm. Vitamin C was used as a reference and ABTS inhibition was calculated using a formula similar to that of the DPPH assay.

Antibacterial activity of the EO

Four Gram-negative bacterial strains *Escherichia coli* (PQ836611), *Pseudomonas aeruginosa* (PQ805338), *Salmonella typhimurium* (PQ836615) and *Streptococcus epidermidis* (PQ125228) as well as three Gram-positive strains-*Bacillus cereus* (PQ836613), *Staphylococcus aureus* (PQ836612) and *Staphylococcus haemolyticus* (PQ836616)-were used to measure the antibacterial effectiveness of the EO extracted from *Z. coccineum* aerial parts. Bacterial strains were acquired from the Microbiological Lab., College of Medicine, Mansoura University in Egypt.

The antibacterial activity was evaluated using the agar diffusion method (Lorian, 2005). Filter paper discs (5 mm, Whatman no.1) were impregnated with 10 mg L^{-1} of *Z. coccineum* EO and placed on nutrient agar plates inoculated with 10v CFU/mL of each bacterial strain. Plates were sealed with Para-film® and incubated at ~35°C for 36 hours. Inhibition zones (mm) were measured at three random points. Standard antibiotics (tetracycline (TET), azithromycin (AZM), ampicillin (AMP)) were used for comparison.

MTT assay

Cytotoxic activity of *Z. coccineum* EO was assessed using the MTT assay (Applichem, USA) as described by Bondock *et al.* (2012). MCF-7 human breast cancer cells, obtained from ATCC (Cairo), were used with doxorubicin as a standard chemotherapeutic drug. The assay measures the conversion of MTT to blue formazan by mitochondrial enzymes, indicating cell viability. Cells (2×10^4 cells/mL) were seeded in 96-well plates (200 µL/well) and incubated for ~25 hours before treatment. EO was diluted in 10% DMSO to a final concentration of 4 mg/mL and applied to two cell lines. Treated cells were incubated at 37°C with 5% CO, for 72 hours. After 24 hours of treatment, 20 µL of MTT (5 mg/mL in PBS) was added per well. Formazan crystals were dissolved in 100 µL DMSO and absorbance was measured at 540 nm using a microplate reader (Thermo Scientific Multiscan Spectrum). Cytotoxicity was calculated by comparing the optical densities of treated and untreated cells:

% Cell inhibition=

$$\frac{\text{OD in control well - OD in simple well}}{\text{OD in control well}} \times 100$$

The concentration (in µg/mL) that induces 50% cell death is defined as the IC_{50}.

Data analysis

Each treatment was tested in triplicate for antibacterial and antioxidant activities. Data was analyzed using ANOVA, followed by Duncan's test for mean differences. Results are presented as mean ± standard error. IC_{50} values were calculated using Microsoft Excel 2016.

RESULTS AND DISCUSSION

Chemical composition of *Z. coccineum* EO

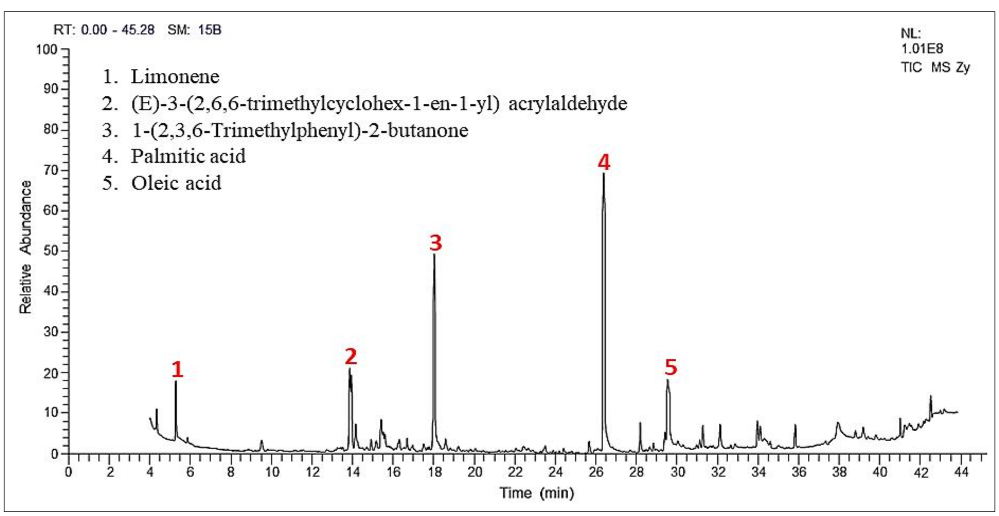
Hydrodistillation of *Z. coccineum* aerial portions yielded a pale yellowish EO with a percentage of 0.049% (v/w), comparable to the 0.044% yield reported for *Z. album* (Kchaou *et al.*, 2016). The extracted EO was investigated utilizing GC-MS to establish its chemical composition (Fig 1). GC-MS analysis identified 22 components, representing 99.97% of the total oil composition (Table 1), a number similar to those identified in the EO of *Z. album* (Kchaou *et al.*, 2016).

The EO constituents of *Z. coccineum* were analyzed, revealing a diverse range of chemical compounds classified into different groups (Table 1). Monoterpenes were present in moderate amounts, with limonene (7.31%) being the most abundant (Fig 2), followed by β-pinene (2.31%), thymol (2.14%) and safranal (1.13%). The phenolic compounds included 4-vinylphenol (1.06%) and eugenol (2.27%). Caryophyllene oxide, a sesquiterpene, accounted for 4.23% of the total composition. The alkyl benzene group was represented by (E)-2-(buta-1,3-dien-1-yl)-1,3,4-trimethylbenzene (3.04%) and 1-(2,3,6-trimethylphenyl)-2-butanone (15.62%), the latter being the second most abundant compound overall (Figure 2). Aldehydes were dominated by (E)-3-(2,6,6-trimethylcyclohex-1-en-1-yl) acrylaldehyde (11.47%), with nonanal contributing 1.68%.

Fatty acids and their derivatives constituted a significant portion, with palmitic acid (20.36%) being the maximum abundant substance in the oil (Fig 2), followed by oleic acid (7.31%), docosanoic acid (3.28%), 2-mono-linolein (3.42%) and 2-mono-olein (2.8%). Steroids were detected in lower amounts, including β-sitosterol-3-O-β-D-glucopyranoside (0.91%), β-sitosterol (0.76%) and stigmasterol (1.57%). Additionally, the hydrocarbon cyclic alcohol 3,3a,7,7-tetramethyl-3a,5,6,7-tetrahydro-4H-inden-4-ol was present at 3.87% (Table 1).

Palmitic acid was found in a superior concentration (20.36%) in the EO of *Z. eichwaldii* (Mazoochi *et al.*, 2021). Additionally, several identified compounds, including thymol and oleic acid, were also reported as constituents of *Z. eichwaldii* EO. Kchaou *et al.* (2016) reported that nonanal, safranal, thymol and eugenol are commonly dispersed compounds in EOs of *Zygophyllum album*. In this study, oleic acid was found in moderate amounts (7.31%) in the plant extracts, consistent with its presence in the EOs of *Z. oxianum* and *Z. eichwaldii* as reported by previous studies (Sasmakov *et al.*, 2012; Mazoochi *et al.*, 2021). Additionally, Mazoochi *et al.* (2021) documented caryophyllene oxide in *Z. eichwaldii*. Furthermore, limonene, β-pinene, docosanoic acid, 2-mono-linolein and 1-(2,3,6-trimethylphenyl)-2-butanone are commonly found compounds in EOs of various plants, including *Moringa oleifera* (Kuete, 2017), *Pistacia atlantica* (Tahir *et al.*, 2019) and *Cannabis sativa* (Judžentienė *et al.*, 2023).

With the exception of the presence of palmitic and oleic acids, the chemical makeup of the EO in this investigation was completely different from that reported for *Z. eichwaldii* EO (Mazoochi *et al.*, 2021). The genetic distinctions between the two species might be the cause of this variance (Abd-ElGawad *et al.*, 2021). Furthermore, it has been established that climatic and ecological variables have exert a substantial influence on the composition of EO (Abd-El Gawad *et al.*, 2020). The EO of *Z. coccineum* contains various chemical classes, with fatty acids and derivatives making up 40.91% of the composition. Alkyl benzenes account for 18.66%, followed by aldehydes and monoterpenes at 13.15% and 13.14%, respectively. Monoterpenes, known for their fragrance, may aid in plant defense and therapeutic uses. Sesquiterpenes (4.53%), hydrocarbons (3.87%), phenols (3.33%) and steroids (3.24%) are also present, contributing to the oil's stability and pharmacological activities, including antioxidant and anti-inflammatory properties (Salama *et al.*, 2022).



Antioxidant activity of Z. coccineum EO

The EO of *Z. coccineum* exhibited strong antioxidant capacity in both DPPH and ABTS assays (Table 2). A one-way ANOVA indicated significant differences in scavenging activities across concentrations ($p \leq 0.05$) and Duncan's post hoc test revealed that values at 20 and 25 µg/mL were significantly higher than at lower concentrations ($p < 0.05$), confirming a dose-dependent trend. At a concentration of 25 µL/L, *Z. coccineum* EO reduced the DPPH and ABTS colors by 64.05% and 73.59%, correspondingly. Based on IC_{50} values, the EO exhibited IC_{50} values of 19.96 µL/L and 16.70 µL/L for DPPH and ABTS, correspondingly, while vitamin C displayed IC_{50} values of 0.73 µL/L and 0.41 µL/L for the same assays.

The pronounced antioxidant potency of *Z. coccineum* EO in this work may be ascribed to its major constituents, including palmitic acid, oleic acid, 1-(2,3,6-trimethylphenyl)-2-butanone, caryophyllene oxide, limonene and docosanoic acid, which may act either individually or synergistically (Abd-ElGawad *et al.*, 2020; Salama *et al.*, 2022; El Hachlafi

Table 1: The essential oil constituents of the coastal *Z. coccineum* were identified through GC-MS analysis.

Constituents	Rt	Area %	Mol. Wt.	Mol. F.
		Monoterpene		
β-Pinene	4.35	2.31	136	$C_{10}H_{16}$
Limonene	5.3	7.31	136	$C_{10}H_{16}$
Safranal	5.81	1.13	150	$C_{10}H_{14}O$
Thymol	9.53	2.14	150	$C_{10}H_{14}O$
		Phenol		
4-Vinylphenol	10.33	1.06	120	C_8H_8O
Eugenol	28.25	2.27	164	$C_{10}H_{12}O_2$
		Sesquiterpene		
Caryophyllene oxide	15.42	4.23	220	$C_{15}H_{24}O$
		Alkyl benzene		
(*E*)-2-(buta-1,3-dien-1-yl)-1,3,4-trimethylbenzene	14.16	3.04	172	$C_{13}H^{16}$
1-(2,3,6-Trimethylphenyl)-2-butanone	18.04	15.62	190	$C_{13}H_{18}O$
		Aldehyde		
(*E*)-3-(2,6,6-trimethylcyclohex-1-en-1-yl) acrylaldehyde	13.86	11.47	178	$C_{12}H_{18}O$
Nonanal	35.83	1.38	142	$C_9H_{18}O$
		Fatty acids and its derivatives		
Palmitic acid methyl ester	25.66	2.16	270	$C_{17}H_{34}O_2$
Palmitic acid	26.37	20.36	256	$C_{16}H_{32}O_2$
Nonadecanoic acid	29.4	1.57	298	$C_{19}H_{38}O_2$
Oleic acid	29.54	7.31	282	$C_{18}H_{34}O_2$
Docosanoic acid	32.12	3.28	340	$C_{22}H_{44}O_2$
2-Mono-linolein	33.96	3.42	354	$C_{21}H_{38}O_4$
2-Mono-olein	34.1	2.80	356	$C_{21}H_{40}O_4$
		Steroids		
β-Sitosterol-3-O-β-D-glucopyranoside	42.38	0.91	577	$C_{35}H_{60}O_6$
β –sitosterol	43.62	0.76	415	$C_{29}H_{50}O$
Stigmasterol	44.19	1.57	412	$C_{29}H_{48}O$
Hydrocarbon "cyclic alcohol"				
3,3a,7,7-tetramethyl-3a,5,6,7-tetrahydro-4*H*-inden-4-ol	13.89	3.87	192	$C_{13}H_{20}O$
Total		**Σ= 99.97**		
Fatty acids and its derivatives		40.91		
Alkyl benzene		18.66		
Aldehyde		13.15		
Monoterpene		13.14		
Sesquiterpene		4.53		
Hydrocarbon		3.87		
Phenol		3.33		
Steroids		3.24		

Rt: Retention time, Mol. Wt: Molecular weight, Mol. F: Molecular formula.

et al., 2024). Notably, palmitic acid, the predominant compound, has been reported at a high concentration (14.85%) in *Reichardia tingitana*, which exhibited strong antioxidant activity (Salama *et al*., 2022).

Antibacterial activity of *Z. coccineum* EO

The EO demonstrated differential antibacterial effects against the tested strains (Table 3). With an inhibitory zone of 20.88 mm, *S. typhimurium* showed the greatest sensitivity to the EO among the tested bacterial strains, demonstrating its strong antibacterial action. Similarly, *E. coli* and *B. cereus* exhibited considerable sensitivity, with clear zones of 16.84 mm and 17.91 mm, correspondingly. These findings suggest that the EO possesses potent antibacterial properties, particularly against certain enteric and foodborne pathogens (Table 3).

On the other hand, *P. aeruginosa* exhibited the lowest sensitivity to the EO, with an inhibition zone of only 8.47 mm, suggesting a higher level of resistance. This is consistent with previous reports indicating that *P. aeruginosa* is often more resistant to plant-derived antimicrobials due to its robust efflux pump system and biofilm formation capabilities. Among the Gram-positive bacteria tested, *S. aureus* demonstrated moderate sensitivity, with an inhibition zone of 10.97 mm, whereas *B. cereus* showed a stronger inhibitory response (Table 3).

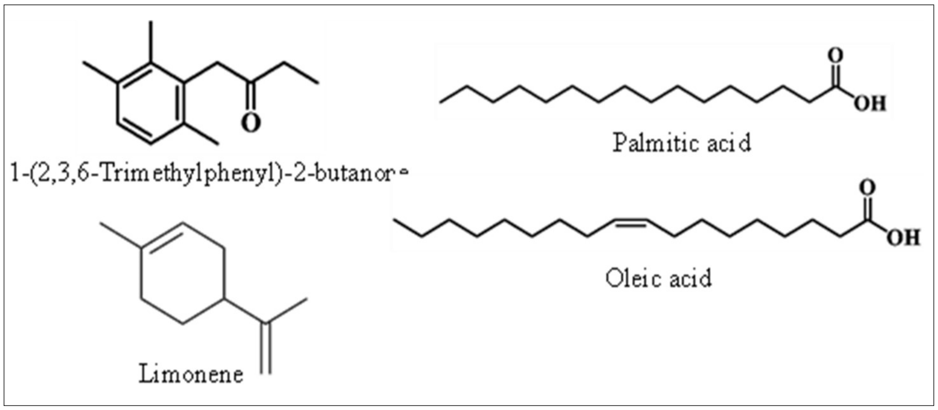


Fig 2: The biochemical structures of the primary detected substances in the EO of *Z. coccineum* were determined through GC-MS analysis.

Table 2: DPPH and ABTS scavenging activity along with IC_{50} values by *Z. coccineum* essential oil. Duncan's multiple range test at $p \leq 0.05$.

Treatment	Concentration (µL/L)	DPPH		ABTS	
		Scavenging (%)*	IC50 (µL/L)	Scavenging (%)	IC50 (µL/L)
Z. coccineum (Essential oil)	5	2.67±0.12^{E}	19.96	4.43±0.16^{E}	16.70
	10	25.14±1.48^{D}		29.94±1.23^{D}	
	15	33.61±1.76^{C}		48.13±1.69^{C}	
	20	50.86±2.01^{B}		64.52±1.95^{B}	
	25	64.05±2.15^{A}		73.59±2.07^{A}	
Vitamin C		0.73		0.41	

Table 3: The antibacterial activity of the EO obtained from the aerial shoot of *Z. coccineum* was assessed in comparison to selected reference antibiotics.

Microbes	*Z. coccineum*	MIC(10 mg/ml)	TET	AZM	AMP
Gram-negative bacteria					
Escherichia coli	16.84±0.45^{B}	0.035	14.91±0.35^{A}	12.88±0.27^{B}	15.77±0.25^{B}
Pseudomonas aeruginosa	8.47±0.06^{D}	0.051	0.00C	7.37±0.07^{C}	11.35±0.08^{C}
Salmonella typhimurium	20.88±0.82^{A}	0.042	9.27±0.27^{B}	0.00D	0.00E
Gram-positive bacteria					
Bacillus cereus	17.91±0.66^{B}	0.039	8.48±0.18^{B}	14.95±0.28^{A}	8.25±0.15^{D}
Staphylococcus aureus	10.97±0.12^{C}	0.058	13.31±0.23^{A}	15.28±0.31^{A}	21.94±0.12^{A}

The data are the mean of three replications ± standard error and they show the diameter of the inhibitory zone (mm). Duncan's multiple range test at the $p<0.05$ level.

ANOVA confirmed significant differences in bacterial inhibition zones ($p \leq 0.05$). Duncan's test identified *S. typhimurium* as significantly more susceptible than *P. aeruginosa* and *S. aureus* ($p < 0.05$).

The selected antibiotics demonstrated varying degrees of activity versus the tested bacterial strains, with Gram -ve bacteria generally exhibiting higher resistance than Gram-positive strains. This trend aligns with findings from previous research (Breijyeh *et al.*, 2020; Salama *et al.*, 2022; Abduljabbara *et al.*, 2024), which attribute the observed resistance to the structural differences in bacterial cell walls (Breijyeh *et al.*, 2020). Gram -ve bacteria's outer membrane serves as an extra barrier, preventing antibiotics from penetrating and enhancing their resistance. The reference antibiotic tetracycline exhibited the strongest inhibition across all tested bacteria, with MIC values extending from 0.035 to 0.058 mg/ml. Azithromycin and ampicillin, however, showed inconsistent effects, with *S. typhimurium* and *P. aeruginosa* being resistant to at least one of these antibiotics. Interestingly, the EO demonstrated higher efficacy against *S. typhimurium* compared to both azithromycin and ampicillin, indicating its potential as an alternative antimicrobial agent.

The antibacterial activity of the EO may be attributed to its major bioactive components, including limonene, 1-(2,3,6-trimethylphenyl)-2-butanone. (E)-3-(2,6,6-trimethylcyclohex-1-en-1-yl) acrylaldehyde, palmitic acid and oleic acid. These compounds may exert their antimicrobial effects either individually or through synergistic interactions. A similar study by Han *et al.* (2021) demonstrated potent inhibitory activity against *S. aureus*. Additionally, according to Kuete (2017), 1-[2,3,6-trimethylphenyl]-2-butanone is a significant component of *Moringa oleifera* EO and has strong microbicidal, anti-inflammatory and antidiabetic effects. Moreover, palmitic and oleic acids have also shown antibacterial activity against various Gram-positive and Gram-negative oral pathogens (Huang *et al.*, 2010). Furthermore, Sharma *et al.* (2021) identified palmitic acid as a major compound (15.57%) in *Capsicum annuum* oleoresin, where it exhibited antimicrobial potential towards *B. subtilis, E. coli, P. aeruginosa and S. aureus*.

EOs with high concentrations of caryophyllene oxide have also exhibited significant antimicrobial properties (Bhaisare *et al.*, 2016; Abd-ElGawad *et al.*, 2022). Moreover, EOs intense in fatty acids and monoterpenes have been reported to possess substantial antimicrobial activity (Marchese *et al.*, 2017).

Cytotoxic activity of *Z. coccineum* EO

The EO of *Z. coccineum* exhibited dose-dependent cytotoxicity against MCF-7 breast cancer and normal WI-38 cells at different concentrations, with doxorubicin as a standard (Fig 3). The highest inhibition rate was recorded at 70.96% at 800 µg/mL, while the IC_{50} value for MCF-7 cells was calculated to be 48.26 µg/mL. In contrast, its effect on normal WI-38 cells was minimal, with greatest inhibition of 9.32% at 1000 µg/mL and an IC_{50} value exceeding 100 µg/mL, indicating selective toxicity toward cancer cells. Doxorubicin exhibited stronger cytotoxicity, achieving complete inhibition (100%) at 800 µg/mL, with an IC_{50} of 5.24 µg/mL for MCF-7 cells. However, it also affected normal cells, showing 11.61% cytotoxicity at 1000 µg/mL.

These findings indicate that EOs exhibits promising anticancer potential with selective cytotoxicity, suggesting its potential as a natural medicinal agent to treat breast cancer. According to published guidelines, cytotoxicity is classified based on IC_{50} values as follows: IC_{50} less than 10 µg/mL is considered very toxic, IC_{50} between 10 and 100 µg/mL is classified as potentially toxic, IC_{50} ranging from 100 to 1000 µg/mL is deemed hypothetically harmful and IC_{50} greater than 1000 µg/mL is regarded as potentially non-toxic (Gad-Shayne, 2009). This classification provides a valuable reference for evaluating the cytotoxic potential of phytoconstituents of extracts and essential oils in biomedical applications.

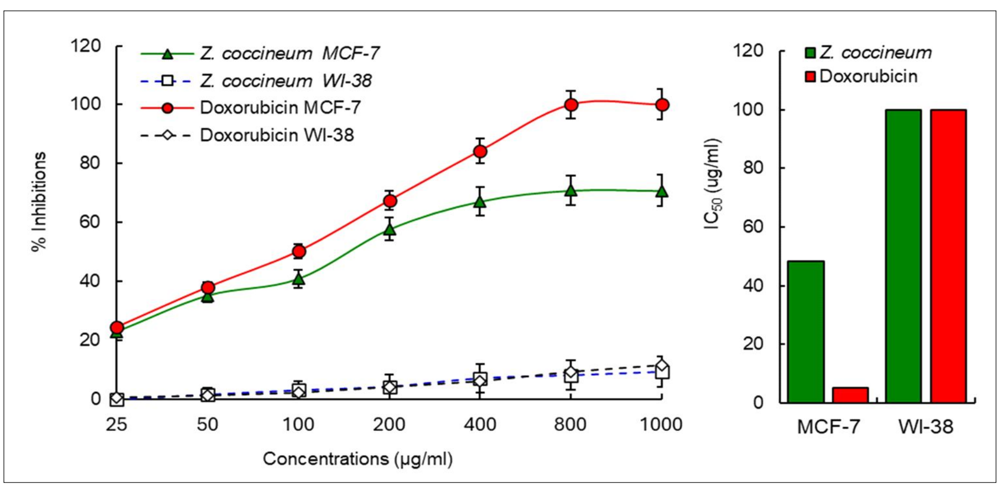


Fig 3: The cytotoxic action and IC_{50} values of *Z. coccineum* EO were assessed against MCF-7 tumor cells and natural cells at varying concentrations, with doxorubicin used as a control.

The principal chemical substance in the EOs analyzed are responsible for the cytotoxicity of the oils employed in this investigation. Several factors influence the cytotoxic mechanism, such as the sample intensity, nature of the isolated components, phytochemical content, nanoparticle model, size and collection and the nature of the tumor and non-cancerous cell line. Cytotoxicity may also be related to protein loss (El-Amier *et al.*, 2023).

The main component of EOs, palmitic acid, has cytotoxic properties, especially against cancer cells, via a number of pathways, including oxidative stress and apoptosis induction (Biswas *et al.*, 2022). According to Kuete (2017), 1-[2,3,6-trimethylphenyl]-2-butanone, derived from *Moringa oleifera* essential oil, exhibited anticancer activity. In addition, limonene induced cytotoxic effects on multiple cancer cell lines, including those of the colon, prostate, lung and breast (Chaudhary *et al.*, 2012). Oleic acid and limonene have also been said to enhance the action of conventional chemotherapy drugs such as doxorubicin and paclitaxel (Menendez *et al.*, 2005).

CONCLUSION

This investigation identifies the chemical profile and bioactivity of *Z. coccineum* essential oil (EO) and its potential pharmaceutical and therapeutic applications. The EO contained high levels of bioactive compounds according to GC-MS analysis, the chief ingredients of which were palmitic acid, 1-(2,3,6-trimethylphenyl)-2-butanone and (E)-3-(2,6,6-trimethylcyclohex-1-en-1-yl) acrylaldehyde. The EO exhibited high antioxidant activity, which suggests its potential as a natural antioxidant agent. Besides, its high antibacterial activity, especially against *S. typhimurium*, makes it a candidate for use as an alternative antimicrobial agent. In addition, the EO was selectively cytotoxic to MCF-7 human breast cancer cells but exhibited low toxicity toward natural WI-38 cells, showing its promise as a natural anticancer agent. These results indicate that *Z. coccineum* EO is a satisfactory source of bioactive substances for food preservation, cosmetic and pharmaceutical industries.

Conflict of interest

All authors declared that there is no conflict of interest.

REFERENCES

Abd, El. Raheim, M., Alam, A., Nour, Y.S. and Radwan, A.M. (2016). Evaluation of Antimicrobial and Antioxidant activities of *Matricaria recutita*, *Ricinus communis* and *Zygophyllum coccineum* Extracts. Bulletin of Environment, Pharmacology and Life Sciences. 5: 30-33.

Abd-ElGawad, A.M., El Gendy, A.E.-N.G., Assaeed, A.M., Al-Rowaily, S.L., Alharthi, A.S., Mohamed, T.A., Nassar, M.I., Dewir, Y.H. and Elshamy, A.I. (2021). Phytotoxic effects of plant essential oils: A systematic review and structure-activity relationship based on chemometric analyses. Plants. 10: 36.

Abd-ElGawad, A.M., El-Amier, Y.A., Bonanomi, G., Gendy, A.E. N.G.E., Elgorban, A.M., Alamery, S.F. and Elshamy, A.I. (2022). Chemical composition of Kickxia aegyptiaca essential oil and its potential antioxidant and antimicrobial activities. Plants. 11(5): 594.

Abd-ElGawad, A.M., Elshamy, A.I., El-Amier, Y.A., El Gendy, A.E.-N.G., Al-Barati, S.A., Dar, B.A., Al-Rowaily, S.L. and Assaeed, A.M. (2020). Chemical composition variations, allelopathic and antioxidant activities of *Symphyotrichum squamatum* (Spreng.) Nesom essential oils growing in heterogeneous habitats. Arabian Journal of Chemistry. 13: 4237-4245.

Abduljabbara, B.T., El-Zayat, M.M., El-Halawany, E.S.F. and El-Amier, Y.A. (2024). Selenium nanoparticles from *Euphorbia retusa* extract and its biological applications: Antioxidant and antimicrobial activities. Egyptian Journal of Chemistry. 67(2): 463-472.

Al-Khaial, M.Q., Fahad, M.M. and Al-Khateeb, F.N. (2025). Chemical composition, antioxidant capacity and anticancer activity of the essential oil from aerial parts of convolvulus lanatus vahl. Journal of Pharmacy and Pharmacognosy Research. 13(5): 1400-1408.

Amin, E., Wang, Y.H., Avula, B., El-Hawary, S.S., Fathy, M.M., Mohammed, R. and Khan, I.A. (2012). Simultaneous determination of saponins and a flavonoid from aerial parts of *Zygophyllum coccineum* L. Journal of AOAC International 95(3): 757-762.

Bhaisare, D.B., Thyagarajan, D., Churchil, R.R. and Punniamurthy, N. (2016). *In-vitro* antimicrobial efficacy of certain herbal seeds essential oils against important poultry microbes. Indian Journal of Animal Research. 50(4): 561-564. doi: 10.18805/ijar.7089.

Biswas, P., Datta, C., Rathi, P. and Bhattacharjee, A. (2022). Fatty acids and their lipid mediators in the induction of cellular apoptosis in cancer cells. Prostaglandins and Other Lipid Mediators. 160: 106637.

Bondock, S., Adel, S., Etman, H.A. and Badria, F.A. (2012). Synthesis and antitumor evaluation of some new 1,3,4-oxadiazole-based heterocycles. European Journal of Medicinal Chemistry. 48: 192-199.

Boulos, L. (1999). Flora of Egypt. Cairo: Al Hadara Publishing.

Breijyeh, Z., Jubeh, B. and Karaman, R. (2020). Resistance of Gram-negative bacteria to current antibacterial agents and approaches to resolve it. Molecules. 25: 1340.

Chaudhary, S.C., Siddiqui, M.S., Athar, M. and Alam, M.S. (2012). D-Limonene modulates inflammation, oxidative stress and Ras-ERK pathway to inhibit murine skin tumorigenesis. Human and Experimental Toxicology. 31(8): 798-811.

El Hachlafi, N., Elbouzidi, A., Batbat, A., Taibi, M., Jeddi, M., Addi, M., Naceiri Mrabti, H. and Fikri-Benbrahim, K. (2024). Chemical composition and assessment of the anti-inflammatory, antioxidant, cytotoxic and skin enzyme inhibitory activities of *Citrus sinensis* (L.) Osbeck essential oil and its major compound limonene. Pharmaceuticals. 17(12): 1652.

El-Amier, Y.A., Abduljabbar, B.T., El-Zayat, M.M., Sarker, T.C. and Abd-ElGawad, A.M. (2023). Synthesis of metal nanoparticles *via Pulicaria undulata* and an evaluation of their antimicrobial, antioxidant and cytotoxic activities. Chemistry. 5(4): 2075-2093.

Gad-Shayne, C. (2009). Alternatives to *in vivo* studies in toxicology and General and Applied Toxicology. 6. John Wiley and Sons Inc.

Gibbons, S., Oriowo, M.A. (2001). Antihypertensive effect of an aqueous extract of *Zygophyllum coccineum* L. in rats. Phytotherapy Research. 15(5): 452-455.

Han, Y., Chen, W., Sun, Z. (2021). Antimicrobial activity and mechanism of limonene against *Staphylococcus aureus*. Journal of Food Safety. 41(5): e12918.

Huang, C.B., George, B., Ebersole, J.L. (2010). Antimicrobial activity of n-6, n-7 and n-9 fatty acids and their esters for oral microorganisms. Archives of Oral Biology. 55(8): 555-560.

Judžentienë, A., Garjonytë, R., Bûdienë, J. (2023). Phytochemical composition and antioxidant activity of various extracts of fibre hemp (*Cannabis sativa* L.) cultivated in Lithuania. Molecules. 28(13): 4928.

Kchaou, M., Ben Salah, H., Mnafgui, K., Abdennabi, R., Gharsallah, N., Elfeki, A., Damak, M., Allouche, N. (2016). Chemical composition and biological activities of *Zygophyllum album* (L.) essential oil from Tunisia. Journal of Agricultural Science and Technology. 18(6): 1499-1510.

Kuete, V. (2017). *Moringa oleifera* and medicinal spices and vegetables from Africa, 485-496. Academic Press.

Lorian, V. (2005). Antibiotics in Laboratory Medicine. Lippincott Williams and Wilkins, Baltimore, Philadelphia, USA.

Marchese, A., Arciola, C.R., Barbieri, R., Silva, A.S., Nabavi, S.F., Tsetegho Sokeng, A.J., Izadi, M., Jafari, N.J., Suntar, I., Daglia, M., Nabavi, S.M. (2017). Update on monoterpenes as antimicrobial agents: A particular focus on p-cymene. Materials. 10(8): 947.

Mazoochi, A., Pourmousavi, S.A., Bamoniri, A. (2021). Essential oil analysis and biological activities of the aerial parts of *Zygophyllum eichwaldii* CA Mey., a native plant from Iran. Journal of Medicinal Plants. 20(79): 85-98.

Menendez, J.A., Vellon, L., Colomer, R., Lupu, R. (2005). Oleic acid, the main monounsaturated fatty acid of olive oil, suppresses Her-2/neu (erbB-2) expression and synergistically enhances the growth inhibitory effects of trastuzumab (Herceptin™) in breast cancer cells with Her-2/neu oncogene amplification. Annals of Oncology. 16(3): 359-371.

Miguel, M.G. (2010). Antioxidant and anti-inflammatory activities of essential oils: A short review. Molecules. 15(12): 9252-9287.

Mohammed, H.A., Khan, R.A., Abdel-Hafez, A.A., Abdel-Aziz, M., Ahmed, E., Enany, S., Mahgoub, S., Al-Rugaie, O., Alsharidah, M., Aly, M.S., Mehany, A.B. (2021). Phytochemica profiling, *in vitro* and in silico anti-microbial and anti-cancer activity evaluations and Staph GyraseB and h-TOP-IIβ receptor-docking studies of major constituents of *Zygophyllum coccineum* L. aqueous-ethanolic extract and its subsequent fractions: An approach to validate traditional phytomedicinal knowledge. Molecules. 26(3): 577.

Porter, D.M. (1974). Disjunct distributions in the new world Zygophyllaceae. Taxon. 23(2-3): 339-346.

Qadir, S.U., Raja, V. (2021). Herbal medicine: Old practice and modern perspectives and Phytomedicine. Academic Press. 149-180. doi:10.1016/B978-0-12-824109-7.00001-7

Razni, D., Rouisset, L. and Benyagoub, E. (2019). Chemical indices and antibacterial properties of some essential oils of apiaceae and lauraceae spices in southwest of Algeria. Asian Journal of Dairy and Food Research. 38(2): 105-113. doi: 10.18805/ajdfr.DR-130

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine. 26(9-10): 1231-1237.

Salama, S.A., Al-Faifi, Z.E., El-Amier, Y.A. (2022). Chemical composition of *Reichardia tingitana* methanolic extract and its potential antioxidant, antimicrobial, cytotoxic and larvicidal activity. Plants. 11(15): 2028.

Sasmakov, S.A., Gazizov, F.Y., Putieva, Z.M., Wende, K., Alresly, Z., Lindequist, U. (2012). Neutral lipids, phospholipids and biological activity of extracts from *Zygophyllum oxianum*. Chemistry of Natural Compounds. 48: 11-15.

Sharma, P.K., Fuloria, S., Alam, S., Sri, M.V., Singh, A., Sharma, V.K., Kumar, N., Subramaniyan, V., Fuloria, N.K. (2021). Chemical composition and antimicrobial activity of oleoresin of *Capsicum annuum* fruits. Mindanao Journal of Science and Technology. 19(1).

Tahir, N.A., Azeez, H.A., Hama Amin, H.H., Rashid, J.S., Omer, D.A. (2019). Antibacterial activity and allelopathic effects of extracts from leaf, stem and bark of Mt. Atlas mastic tree (*Pistacia atlantica* subsp. *kurdica*) on crops and weeds. Allelopathy Journal. 46(1): 121-132.

Zahran, M.A., El-Amier, Y.A. (2013). Non-traditional fodders from the halophytic vegetation of the deltaic Mediterranean coastal desert, Egypt. Journal of Biological Sciences. 13(4): 226-233.

Zhang, H., Li, X., Nan, X., Sun, G., Sun, M., Cai, D. and Gu, S. (2017). Alkalinity and salinity tolerance during seed germination and early seedling stages of three alfalfa (*Medicago sativa* L.) cultivars. Legume Research-An International Journal. 40(5): 853-858. doi: 10.18805/lr.v0i0.8401.