



Chemical composition, antioxidant capacity, and anticancer activity of the essential oil from aerial parts of *Convolvulus lanatus* Vahl

[Composición química, capacidad antioxidante y actividad anticancerígena del aceite esencial de partes aéreas de *Convolvulus lanatus* Vahl]

Mohammed Qahtan Al-Khaial^{1*}, Mustafa M. Fahad², Furqan N. Al-Khateeb¹

¹College of Pharmacy, University of Al Maarif, Ramadi 31001, Iraq.

²Center of Desert Studies, University of Anbar, Ramadi 31001, Iraq.

*E-mail: mohammed.qahtan@uoa.edu.iq

Abstract

Context: Wild plants contribute significantly to ecosystem services, including biodiversity conservation and the provision of natural resources for food, feed, and medicine. *Convolvulus lanatus*, a species native to Egypt, remains underexplored for its phytochemical and therapeutic properties.

Aims: To evaluate the chemical composition, antioxidant capacity, and anticancer potential of the essential oil (EO) extracted from the aerial parts of *C. lanatus* collected in Wadi Araba, Eastern Desert, Egypt.

Methods: The EO was obtained via hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC-MS) to identify its chemical constituents. Antioxidant capacity was assessed through 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging assays. Cytotoxic activity was evaluated against HePG-2 (hepatocellular carcinoma), PC3 (prostate cancer), and HeLa (cervical cancer) cell lines using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay.

Results: GC-MS analysis identified 15 compounds, with coveacin (35.08%) and corymbolone (34.70%) as the predominant constituents. The EO demonstrated notable antioxidant activity, with IC₅₀ values of 49.15 mg/mL (DPPH) and 31.80 mg/mL (ABTS). Cytotoxic effects were observed in a concentration-dependent manner, with IC₅₀ values of 38.93 µg/mL (HePG-2), 57.61 µg/mL (PC3), and 46.72 µg/mL (HeLa).

Conclusions: The EO of *C. lanatus* exhibits significant antioxidant capacity and anticancer activity, potentially attributable to its high content of bioactive terpenes. This study is the first to report on the therapeutic potential of *C. lanatus* EO, suggesting its possible applications in pharmaceutical and natural product development. Further *in vivo* and mechanistic studies are warranted to substantiate these findings.

Keywords: antioxidant capacity; anticancer potential *Convolvulus lanatus*; essential oil; GC-MS.

Resumen

Contexto: Las plantas silvestres contribuyen significativamente a los servicios ecosistémicos, incluyendo la conservación de la biodiversidad y el suministro de recursos naturales para la alimentación humana, animal y animal, y la medicina. *Convolvulus lanatus*, especie nativa de Egipto, permanece poco explorado por sus propiedades fitoquímicas y terapéuticas.

Objetivos: Evaluar la composición química, la capacidad antioxidante y el potencial anticancerígeno del aceite esencial (AE) extraído de las partes aéreas de *C. lanatus* recolectadas en Wadi Araba, Desierto Oriental de Egipto.

Métodos: El AE se obtuvo mediante hidrodestilación y se analizó mediante cromatografía de gases-espectrometría de masas (GC-MS) para identificar sus componentes químicos. La capacidad antioxidante se evaluó mediante ensayos de eliminación de radicales 2,2-difenil-1-picrilhidrazilo (DPPH) y ácido 2,2'-azino-bis(3-etilbenzotiazolina-6-sulfónico) (ABTS). La actividad citotóxica se evaluó frente a las líneas celulares HePG-2 (carcinoma hepatocelular), PC3 (cáncer de próstata) y HeLa (cáncer de cuello uterino) mediante el ensayo de bromuro de 3-(4,5-dimetil-2-tiazolil)-2,5-difenil-2H-tetrazolio (MTT).

Resultados: El análisis GC-MS identificó 15 compuestos, siendo la cropeacina (35,08%) y la corimbolona (34,70%) los constituyentes predominantes. El aceite esencial mostró una notable actividad antioxidante, con valores de CI₅₀ de 49,15 mg/mL (DPPH) y 31,80 mg/mL (ABTS). Se observaron efectos citotóxicos dependientes de la concentración, con valores de CI₅₀ de 38,93 µg/mL (HePG-2), 57,61 µg/mL (PC3) y 46,72 µg/mL (HeLa).

Conclusiones: El aceite esencial de *C. lanatus* exhibe una importante capacidad antioxidante y actividad anticancerígena, posiblemente atribuible a su alto contenido de terpenos bioactivos. Este estudio es el primero en informar sobre el potencial terapéutico del aceite esencial de *C. lanatus*, lo que sugiere sus posibles aplicaciones en el desarrollo de productos farmacéuticos y naturales. Se requieren estudios *in vivo* y mecanísticos adicionales para corroborar estos hallazgos.

Palabras Clave: aceite esencial; capacidad antioxidante; *Convolvulus lanatus*; GC-MS; potencial anticancerígeno.

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AUTHOR INFO

ORCID:

[0000-0001-7072-7924](https://orcid.org/0000-0001-7072-7924) (MQAK)

[0000-0002-7112-1729](https://orcid.org/0000-0002-7112-1729) (MMF)

[0000-0001-7522-6491](https://orcid.org/0000-0001-7522-6491) (FNAK)

Abbreviations: ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH: 2,2-diphenyl-1-picrylhydrazyl; EO: Essential oil; GC-MS: Gas chromatography-mass spectrometry; HePG-2: Human hepatocellular carcinoma; HeLa: Cervical cancer cell line; IC₅₀: Inhibitory concentration; MTT: Methyl thiazolyl tetrazolium; PC3: Prostate cancer cell line.

INTRODUCTION

Wild plants have historically served as vital resources for healing and disease management. Prior to the advent of modern pharmaceuticals, traditional medical systems across ancient civilizations relied extensively on botanical remedies to treat a wide range of ailments (Lila and Dunlap, 2021). Despite substantial advancements in contemporary medicine, the therapeutic potential of wild flora remains an area of significant interest among scientists, pharmacologists, and healthcare professionals (Meesakul et al., 2023; Salama et al., 2022). The biological efficacy of medicinal plants is attributed to the presence of a diverse array of phytoconstituents, including essential oils, phenolics, terpenoids, sterols, alkaloids, tannins, and anthocyanins, which exert various pharmacological effects (Sharifi-Rad et al., 2020). The activity of these compounds is closely associated with their concentration and compositional profile. Recent studies have underscored the broad spectrum of biological activities exhibited by medicinal plants, such as antioxidant, anti-aging, anticancer, antifungal, anti-inflammatory, and antibacterial properties (Abduljabbar et al., 2024; Li et al., 2014).

The development of anticancer agents has a long history, with the use of herbs for cancer treatment documented in traditional medical literature. However, cancer chemotherapy did not emerge as a formal discipline until the 1940s (Cragg et al., 2009). Since then, a variety of anticancer compounds—derived from synthetic chemicals, medicinal plants, and microbial products—have been developed to address the increasing global burden of cancer (Xu et al., 2012). According to GLOBOCAN (2020), one in five individuals is expected to develop cancer during their lifetime, with mortality affecting one in eight men and one in eleven women. These figures are projected to rise due to global aging and evolving lifestyle risk factors.

The family *Convolvulaceae*, commonly known as the bindweed family, comprises approximately 56 genera and 1,600 species worldwide (Boulos, 2000; Sundarapandian et al., 2015). Species within this family are known for their ornamental value, ecological benefits (such as soil stabilization), and pharmacological significance. Many species produce compounds with therapeutic activities, including anti-inflammatory, antidiabetic, and gastrointestinal protective effects (Ambika and Nair, 2019). The genus *Convolvulus* includes nearly 100 species distributed across temperate and subtropical regions. Phytochem-

ical analyses have revealed the presence of alkaloids, flavonoids, coumarins, sterols, saponins, resin glycosides, tannins, and stilbenes in several species (El-Shazly and Wink, 2008; Jenett-Siems et al., 1998; Menemen et al., 2002; Zaman et al., 2022). These compounds have been associated with multiple pharmacological activities, including purgative, antidepressant, antioxidant, hypoglycemic, analgesic, anticancer, antiulcer, and antidiarrheal effects (Atta and Mouneir, 2004; Dhingra and Valecha, 2007; Meng et al., 2002; Sairam et al., 2001; Shabana et al., 1990).

In Egypt, approximately 20 species of *Convolvulus* are reported, including *Convolvulus lanatus*, a spiny plant endemic to arid desert regions (Boulos, 2000). Despite its traditional medicinal use, the phytochemical composition and biological activity of *C. lanatus*, particularly its essential oil (EO), remain largely unexplored. Preliminary studies have suggested the presence of alkaloids, phenolics, coumarins, and phytosterols in this species, yet comprehensive pharmacological evaluations are lacking.

Given the increasing interest in natural compounds with antioxidant and anticancer activities, the present study aimed to investigate the chemical profile and biological activities of *C. lanatus* EO. The essential oil was extracted from the aerial parts of the plant collected from the Eastern Desert of Egypt and analyzed using gas chromatography-mass spectrometry (GC-MS). The antioxidant potential was evaluated using DPPH and ABTS radical scavenging assays, while cytotoxic activity was assessed *in vitro* against human hepatocellular carcinoma (HePG-2), prostate carcinoma (PC3), and cervical carcinoma (HeLa) cell lines. The findings of this study are expected to provide new insights into the pharmacological value of *C. lanatus* EO and its potential as a source of bioactive compounds for therapeutic use.

MATERIAL AND METHODS

Chemicals

The following analytical-grade reagents and chemicals were used: 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate (K₂S₂O₈), ethanol (C₂H₅OH) (Sigma-Aldrich, USA), dimethyl sulfoxide (DMSO) (Merck, Germany), methanol (CH₃OH) (Fisher Scientific, UK), phosphate-buffered saline (PBS), Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin solution, trypsin-EDTA solution (Gibco,

Thermo Fisher Scientific, USA), and MTT cell proliferation assay kit (Abcam, UK).

Plant material

Specimens of *Convolvulus lanatus* Vahl were collected in March 2023 from Wadi Araba in the northern region of Egypt's Eastern Desert (28°59'7.28"N, 32°10'31.28"E) (Fig. 1). Identification was performed in accordance with Boulos (2000), and a voucher specimen (Mans. 0030312004) was deposited at the herbarium of the Faculty of Science, Mansoura University, Egypt. The collected aerial parts were cleaned to remove debris and dried in the shade at ambient temperatures (23–30°C). Dried material was subsequently ground into fine powder using a microfine grinder (IKFA® MF 13 Basic Microfine Grinder Drive, Breisgau, Germany).

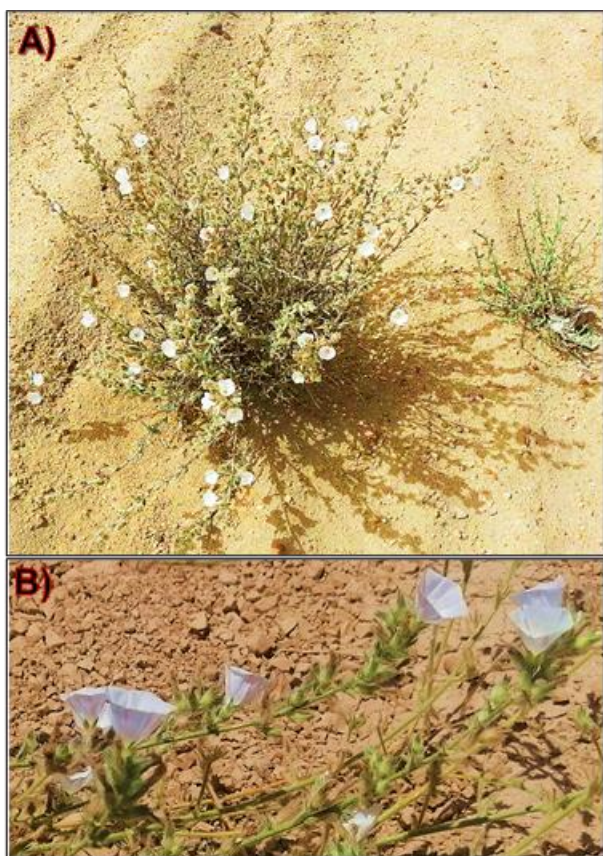


Figure 1. *Convolvulus lanatus* Vahl.

(A) General view of the wild shrub, (B) Close-up of a blossoming branch.

Essential oil extraction (EO)

Hydrodistillation was performed on 100 g of dried aerial parts using a 5-L Clevenger apparatus for 4 hours. The resulting EO was separated, treated with three drops of *n*-hexane, and dehydrated using 0.5 g of anhydrous sodium sulfate (Na₂SO₄). This proce-

dures was repeated for three different plant samples (200 g each). The EO was stored in dark amber vials at 4°C until chemical profiling and biological evaluations were conducted.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Chemical profiling of EO samples was conducted using a TRACE Ultra gas chromatograph coupled with an ISQ™ EC single quadrupole mass spectrometer (Thermo Scientific™, USA), as previously described by Abd-ElGawad et al. (2022). A TR-6 MS column (30 m × 0.25 mm ID) was used. Helium served as the carrier gas at a flow rate of 1.0 mL/min with a 1:10 split ratio. The oven temperature was initially set to 60°C for 1 min, then ramped to 240°C at 4°C/min. The injector and detector temperatures were both maintained at 210°C. A 1 µL aliquot of EO (diluted in hexane, 1:10 v/v) was injected. Electron ionization (EI) was performed at 70 eV with a mass range of 40–450 m/z. Identification of constituents was based on retention indices (relative to C8–C22 n-alkanes) and spectral matching with the NIST and Wiley libraries using the AMDIS software.

Antioxidant capacity assays

DPPH radical scavenging assay

The DPPH radical scavenging activity of the EO was determined as described by Miguel (2010). EO solutions at concentrations of 5–50 mg/L were prepared in methanol. An equal volume of freshly prepared 0.3 mM DPPH solution was mixed with each EO dilution and incubated for 30 min at 25°C. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Analytik Jena, Germany). Ascorbic acid served as the positive control at concentrations of 1–20 mg/mL. Radical scavenging activity was calculated using the equation [1].

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

Where A_{control} and A_{sample} are the control and EO sample absorbances, respectively.

ABTS radical scavenging assay

ABTS scavenging activity was measured following Re et al. (1999). EO concentrations of 5–50 mg/mL were prepared. A 0.2 mL EO solution was mixed with 2 mL of freshly prepared ABTS radical solution and incubated in the dark for 6 min. Absorbance was read at 735 nm using a Spectronic 22D spectrophotometer (Milton Roy, USA). Ascorbic acid was used as the reference antioxidant. Scavenging percentages were calculated using the formula [1].

MTT cytotoxicity assay

The cytotoxic potential of *C. lanatus* EO was evaluated against human hepatocellular carcinoma (HePG-2, HTB-22), prostate carcinoma (PC3, CRL-1435), and cervical carcinoma (HeLa, CCL-2) cell lines obtained from ATCC. The assay was performed using an MTT assay kit (Applichem, USA) as described by Bondock et al. (2012) and Terblanche et al. (2017). Doxorubicin served as a positive control.

Cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin. Suspensions (2×10^4 cells/mL) were seeded in 96-well plates (200 μ L/well) and incubated at 37°C in a humidified 5% CO₂ atmosphere for 24 h. EO was dissolved in 10% DMSO, diluted to 4 mg/mL, and applied to the cells at varying concentrations. After 72 h of incubation, 20 μ L of 5 mg/mL MTT solution in PBS was added to each well. Following 4 h of incubation, the medium was discarded, and the formazan crystals were solubilized in 100 μ L DMSO. Absorbance was recorded at 540 nm using a microplate reader (Thermo Scientific™ Multiskan Spectrum).

Cytotoxicity and selectivity index (SI) were calculated using the following equations [2] and [3].

$$\% \text{ Inhibition} = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100 \quad [2]$$

$$\text{SI} = \frac{\text{IC}_{50} \text{ (normal cell line)}}{\text{IC}_{50} \text{ (cancer cell line)}} \quad [3]$$

Where IC₅₀ is the concentration (in μ g/mL) that results in 50% cell death.

An SI value >3 was considered indicative of high selectivity towards cancer cells (Chothiphat et al., 2019).

Statistical analysis

All experiments were performed in triplicate. Data were expressed as mean $\pm$ standard deviation (SD). Statistical analysis was conducted using one-way analysis of variance (ANOVA), and means were compared using the least significant difference (LSD) test at a significance level of $p < 0.05$. All analyses were conducted using CoStat version 6.711 (CoHort Software, Monterey, CA, USA).

RESULTS AND DISCUSSION

GC-MS analysis of EO

The chemical constituents of the EO extracted from *Convolvulus lanatus* aerial parts were identified using GC-MS analysis. The chromatographic profile (Fig. 2) revealed the presence of 15 compounds representing 100% of the total composition (Table 1). The dominant constituents were croweacin (35.08%) and corymbo-

lone (34.70%), eluting at retention times of 17.59 and 22.75 minutes, respectively. Additional major components included lup-20(29)-en-3-ol acetate (8.78%), 1-bisabolone (5.54%), methyl commate B (2.82%), and hexahydrofarnesyl acetone (2.72%), collectively accounting for 19.86% of the total identified compounds.

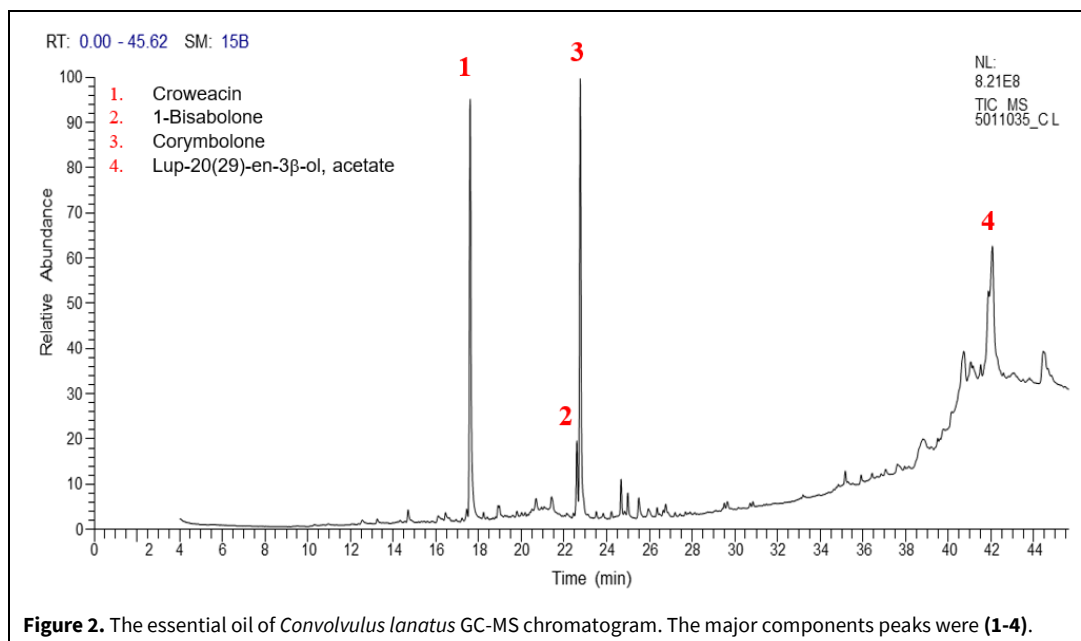
The EO compounds were categorized into four chemical groups. Terpenes were the most abundant (61.30%), followed by shikimate derivatives (36.11%). Fatty acid derivatives and steroids were present in minor proportions. This compositional profile highlights the predominance of terpene-based secondary metabolites in the EO of *C. lanatus*.

The presence of croweacin and corymbolone in this EO is consistent with previous reports on *Ammi visnaga*, *Myristica fragrans*, *Astragalus chrysostachys*, and *Cyperus articulatus* (Assis et al., 2020; Carolina and Maman, 2016; Ghasemian-Yadegari et al., 2019; Khalfallah et al., 2011; Li and Sheng, 2013). Other identified compounds, such as 1-bisabolone, lup-20(29)-en-3-ol acetate, and hexahydrofarnesyl acetone, have also been reported in several medicinal plant species (Elshikh et al., 2023; He et al., 2020; Khan et al., 2021; Yeo et al., 2016), suggesting a potential pharmacological basis for the EO's biological activities.

Antioxidant capacity

The antioxidant potential of *C. lanatus* EO was evaluated using DPPH and ABTS radical scavenging assays. Both assays revealed a concentration-dependent scavenging activity (Fig. 3). At 50 mg/mL, the EO achieved 61.07% DPPH and 75.36% ABTS radical inhibition. The corresponding IC₅₀ values were 49.15 mg/mL (DPPH) and 31.80 mg/mL (ABTS), indicating a stronger effect in the ABTS assay. These values suggest a moderate antioxidant potential when compared to ascorbic acid.

The antioxidant capacity may be attributed to the EO's chemical profile, particularly its high content of croweacin, corymbolone, 1-bisabolone, lup-20(29)-en-3-ol acetate, methyl commate B, and hexahydrofarnesyl acetone. These compounds have been reported to possess radical scavenging activity in prior studies involving other plant species (Bednarczyk-Cwynar et al., 2016; Carolina and Maman, 2016; Đorđević et al., 2013; Ferraz et al., 2006; Ghasemian-Yadegari, et al., 2019; Jiangseubchatveera et al., 2015; Khalfallah et al., 2011; Kumar et al., 2022; Pérez-González et al., 2019; Sarhadi et al., 2020; Swain, 2024; Zhanzhaxina et al., 2020). It is likely that synergistic interactions among these components further enhanced the antioxidant potential observed in this study (Essa et al., 2021).

**Table 1.** Essential oil composition of *Convolvulus lanatus* identified by GC-MS.

No.	Compound	Category	Rt (min)	Molecular weight	Molecular formula	Area (%)
Shikimate						
1	Methyleugenol	Shikimate	14.68	178	C ₁₁ H ₁₄ O ₂	1.03 ± 0.04
2	Croweacin	Shikimate	17.59	192	C ₁₁ H ₁₂ O ₃	35.08 ± 0.25
Terpenes						
3	5,5-Dimethyl-4-[3-methyl-1,3-butadienyl]-1-oxaspiro[2.5]octane	Monoterpene	17.43	206	C ₁₄ H ₂₂ O	0.81 ± 0.01
4	(-)-Spathulenol	Sesquiterpene	18.90	220	C ₁₅ H ₂₄ O	0.82 ± 0.02
5	Caryophyllene oxide	Sesquiterpene	18.96	220	C ₁₅ H ₂₄ O	0.69 ± 0.01
6	Epiglobulol	Sesquiterpene	20.67	222	C ₁₅ H ₂₆ O	1.23 ± 0.06
7	Shyobunol	Sesquiterpene	21.40	222	C ₁₅ H ₂₆ O	1.38 ± 0.07
8	1-Bisabolone	Sesquiterpene	22.59	220	C ₁₅ H ₂₄ O	5.54 ± 0.12
9	Corymbolone	Sesquiterpene	22.75	236	C ₁₅ H ₂₄ O ₂	34.70 ± 0.23
10	Hexahydrofarnesyl acetone	Sesquiterpene	24.66	268	C ₁₈ H ₃₆ O	2.72 ± 0.09
11	3-Methyl-4-(1,3,3-trimethyl-7-oxa-bicyclo[4.1.0]hept-2-yl)-but-3-en-2-on	Sesquiterpene	25.49	222	C ₁₅ H ₂₆ O	1.81 ± 0.05
12	Methyl commate B	Triterpene	41.85	470	C ₃₁ H ₅₀ O ₃	2.82 ± 0.08
13	Lup-20(29)-en-3β-ol, acetate	Triterpene	42.06	468	C ₃₂ H ₅₂ O ₂	8.78 ± 0.14
Fatty acid derivatives						
14	Methyl palmitate	Fatty acid derivatives	26.35	270	C ₁₇ H ₃₄ O ₂	0.78 ± 0.01
Steroid						
15	Ursodeoxycholic acid	Steroid	41.15	392	C ₂₄ H ₄₀ O ₄	1.81 ± 0.04

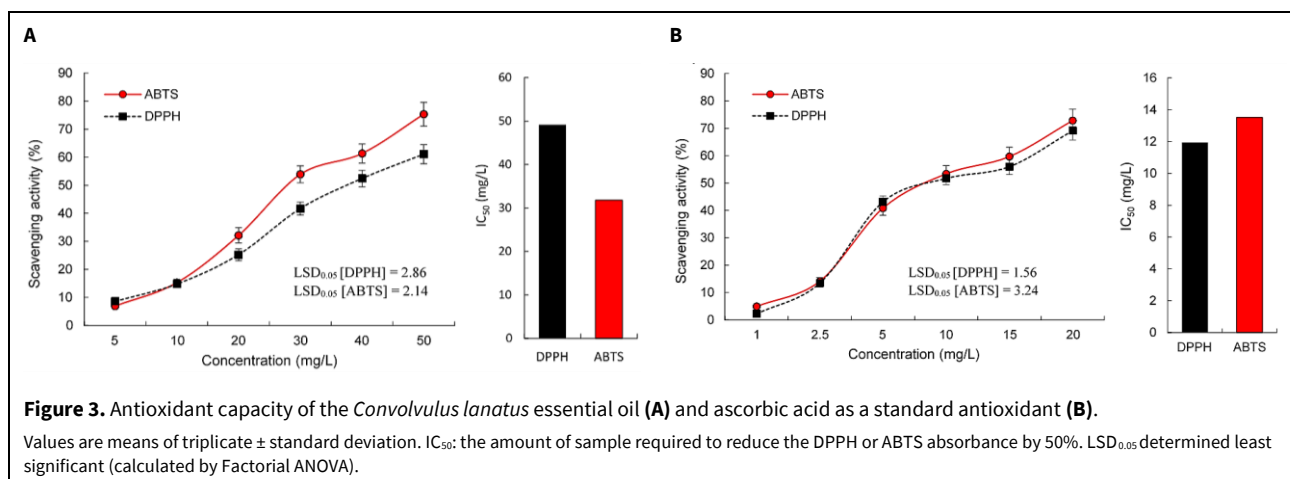


Figure 3. Antioxidant capacity of the *Convolvulus lanatus* essential oil (A) and ascorbic acid as a standard antioxidant (B).

Values are means of triplicate $\pm$ standard deviation. IC₅₀: the amount of sample required to reduce the DPPH or ABTS absorbance by 50%. LSD_{0.05} determined least significant (calculated by Factorial ANOVA).

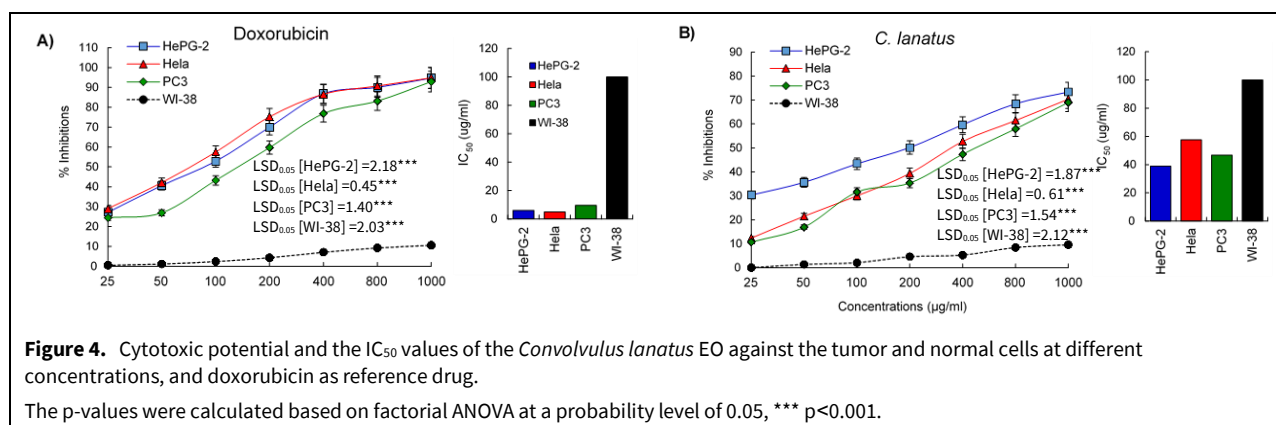


Figure 4. Cytotoxic potential and the IC₅₀ values of the *Convolvulus lanatus* EO against the tumor and normal cells at different concentrations, and doxorubicin as reference drug.

The p-values were calculated based on factorial ANOVA at a probability level of 0.05, *** p<0.001.

Cytotoxic activity

The cytotoxic effects of *C. lanatus* EO were assessed against HePG-2 (hepatocellular carcinoma), PC3 (prostate carcinoma), and HeLa (cervical carcinoma) cell lines using the MTT assay (Fig. 4). The EO exhibited dose-dependent cytotoxicity, with IC₅₀ values of 38.93 μg/mL (HePG-2), 57.61 μg/mL (PC3), and 46.72 μg/mL (HeLa). No significant toxicity was observed in the WI-38 normal human fibroblast cell line (IC₅₀ > 100 μg/mL), indicating selective cytotoxicity toward cancer cells.

These findings suggest that *C. lanatus* EO has stronger anticancer effects on HePG-2 and HeLa cell lines than on PC3 cells. The EO's effectiveness was comparable to that of doxorubicin, which served as the reference drug (IC₅₀: 6.03, 5.12, and 9.60 μg/mL for HePG-2, PC3, and HeLa, respectively). The selectivity index (SI) values of the EO were 2.57 (HePG-2), 1.74 (PC3), and 2.14 (HeLa), indicating preferential cytotoxicity towards cancer cells, particularly HePG-2 and HeLa. According to standard interpretation (Maungchanburi et al., 2022; Tronina et al., 2023), SI values >2 denote promising selective anticancer activity, and values >3 are considered highly selective.

The cytotoxic activity of *C. lanatus* EO may be attributed to its terpene-rich profile, as terpenoids have been reported to exert anticancer activity through induction of apoptosis, cell cycle arrest, and modulation of oxidative stress (Bazou et al., 2008; Mohammadi-Cheraghbabadi and Hazrati, 2023). Previous studies also support the presence of cytotoxic phytochemicals in members of the *Convolvulaceae* family, including *C. arvensis* and other related species (Atta and Mouneir, 2004; Fan et al., 2022).

CONCLUSION

Fifteen chemical constituents were identified in the essential oil of *Convolvulus lanatus* aerial parts via GC-MS analysis. The most abundant compounds were coveacin (35.08%) and corymbolone (34.70%), followed by lup-20(29)-en-3β-ol acetate (8.78%), 1-bisabolone (5.54%), methyl commate B (2.82%), and hexahydrofarnesyl acetone (2.72%). These constituents primarily belonged to the terpene and shikimate-derived categories, suggesting a bioactive profile with potential therapeutic relevance.

The EO demonstrated moderate antioxidant capacity with IC₅₀ values of 49.15 mg/mL (DPPH) and 31.80 mg/mL (ABTS). Additionally, it exhibited promising cytotoxic effects against three human cancer cell lines, with IC₅₀ values below 60 µg/mL, and showed selective toxicity favoring cancer cells over normal fibroblasts.

These findings support the therapeutic potential of *C. lanatus* EO, particularly due to its high content of biologically active terpenes. Further investigations, including mechanistic studies and *in vivo* validations, are warranted to explore its applicability as a source of natural antioxidant and anticancer agents.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Al-Khaial MQ	Fahad MM	Al-Khateeb FN
Concepts or ideas	x	x	x
Design	x	x	x
Definition of intellectual content	x	x	x
Literature search	x	x	x
Experimental studies	x	x	x
Data acquisition	x	x	
Data analysis	x		x
Statistical analysis	x	x	x
Manuscript preparation		x	x
Manuscript editing	x	x	
Manuscript review	x	x	x

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