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RESEARCH ARTICLE

Phytochemical analysis, antibacterial and cytotoxic efficacy of *n* -hexane extract from Iraqi cultivated *Jatropha integerrima*: Isolation of stigmasterol and β-carotene using CombiFlash

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Abstract

Jatropha integerrima a member of the Euphorbiaceae family, is well-known for its numerous secondary metabolites with medicinal applications. This study aims to investigate the phytochemical composition of the *n*-hexane extract of the aerial parts of *J. integerrima* grown in Iraq. The work focuses on the identification, separation and characterization of bioactive phytosterols and terpenoids, as well as evaluating the antibacterial activity of the extract against *Staphylococcus aureus* and *Acinetobacter baumannii.* Furthermore, the cytotoxic effect of the extract on the B16 melanoma skin cancer cell line was assessed utilizing the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Two chromatographic techniques, thin layer chromatography (TLC) and gas chromatography-mass spectrometry (GC-MS), are used to detect chemicals and for fractionation and isolation, CombiFlash chromatography and high-performance liquid chromatography (HPLC) were used. Two beneficial compounds, β-carotene and stigmasterol, were successfully isolated. The antibacterial assay revealed that the *n*-hexane extract exhibited significant efficacy, especially against Gram-positive bacteria (*S. aureus*) and demonstrated a notable cytotoxic effect with the highest inhibition rate of 84.00 % on the skin cancer (B16) cell line at a 200 µg/mL concentration. The values of the half maximal inhibitory concentration (IC_{50}) of the extract were 29.86 µg/mL. The isolated components were characterized by HPLC, Fourier transform infrared spectroscopy (FTIR) and ultraviolet-visible (UV-Vis) spectroscopy to verify the existence of stigmasterol and β-carotene. Anti-inflammatory, neuroprotective and antioxidant are the most well-known properties of these compounds. As a result, separating and characterizing these compounds from *J. integerrima* can thus expose significant information on their possible medical use. This study is the first comprehensive analysis of the chemical composition of *J. integerrima* in Iraq, highlighting its promising medicinal value.

Keywords: antibacterial; cancer cell line; CombiFlash column chromatography; HPLC; *J. integerrima*

Introduction

Skin cancer-particularly melanoma-remains one of the leading causes of cancer-related deaths globally (1). Melanoma's worldwide impact has grown significantly during the last several decades. From about 107380 to 289950, the global count of new melanoma cases increased by 170 % between 1990 and 2019 (2). In 2020, an estimated 325000 new melanoma cases result in about 57000 deaths. Forecasts show that by 2040, these figures could rise to 510000 new cases and 96000 deaths annually (3). This increasing trend emphasizes the public health problem that melanoma presents.

Medicinal plants have been used for treating various ailments since ancient times. However, in some regions, the use of medicinal plants is often viewed with skepticism due to a lack of scientific validation and understanding (1). Comprising around 218 genera and more than 6745 species, the Euphorbiaceae family includes the genus *Jatropha*, many of which have commercial and medicinal uses (2). Mostly throughout Africa, Asia, Central and South America, the genus *Jatropha* is found in tropical and subtropical environments (3).

Among these species, one notable species is *J. integerrima*. Indigenous to the Caribbean, particularly Cuba and Hispaniola, the evergreen shrub, also known as spicy jatropha or peregrine, is a popular decorative plant because of its striking red blossoms and adaptability to many climates. Folk medicine has long used various parts of *J. integerrima* to treat bacterial infections, inflammation and skin disorders (4).

Phytochemical research indicates that *J. integerrima* contains a wide spectrum of beneficial secondary metabolites. Studies in several parts of the plant have identified diterpenoids, flavonoids, phenolic acid conjugates, cyclic peptides, phytosterols, sesquiterpenes and coumarins. Many of which have significant biological action (5). Moreover, integerrimide C, isolated from *J. integerrima* latex, has shown remarkable cytotoxic action against tumoral KB cells (6).

Sterols and terpenes are examples of non-polar compounds that are commonly extracted using *n*-hexane. The existence of sterols and terpenes can be initially confirmed by preliminary phytochemical tests and the use of other methods like TLC and GC-MS. Following that, bioactive compounds were separated and purified utilizing advanced chromatographic methods, including CombiFlash chromatography and HPLC, simplifying structure clarification. The isolated chemicals were also characterized and verified using UV-Vis spectroscopy, FTIR and HPLC spike method.

Plant sterol stigmasterol has been related to neuroprotective, antioxidant and anti-inflammatory effects (7). Likewise, a precursor of vitamin A, β-carotene has strong antioxidant and neuroprotective effects (8). Based on previous findings, the antibacterial and cytotoxic potential of the plant's bioactive compounds was evaluated against the B16 melanoma skin cancer cell line. Melanoma is an aggressive kind of skin cancer; with its progression influenced by both environmental and genetic factors (9). With about 324635 new instances of melanoma skin cancer recorded worldwide in 2020, the recent World Health Organization (WHO) estimate shows that the incidence of melanoma is progressively rising (10).

Environmental conditions significantly impact plant phytochemical profiles, especially in different locations. Therefore, the present study aims to extract, identify and isolate bioactive phytosterols and terpenoids from the *n*-hexane extract of *J. integerrima* cultivated in Iraq.

Materials and Methods

Gathering plant parts and authentication

The aerial parts of the plant were collected from the Central Baghdad in April and May 2024. The plant was identified as *J. integerrima* Jacq., family Euphorbiaceae and was recorded under BUH No. 870 in the herbarium of the Department of Biology, College of Science, Baghdad University, Baghdad. The plant materials were cleansed, dried and ground into a fine powder before being used in various applications (Fig. 1).

The plant's extraction process

The powdered aerial parts (250 g) of *J. integerrima* were extracted by maceration (cold technique of extraction) by using 1 L of *n*-hexane (the ratio of 1:4) for 3 days with periodic stirring. Fresh solvent was added daily and the mixture was filtered regularly. A rotary evaporator was then used to dry the filtered extract at a temperature lower than 45 °C and lower pressure. Three grams of crude extract was obtained once the solvent has completely evaporated.

Preliminary phytochemical screening

Two categories of chemical assays were employed for the detection of steroids and terpenoids. Liebermann-Burchard test involved dissolving a tiny bit of *n*-hexane extract in 5 mL of chloroform, then drying the layer over anhydrous sodium sulfate before adding other reagents. Given the next step was to combine the ingredients with two drops of strong sulfuric acid and ten drops of acetic anhydride. Upon further oxidation, the presence of a steroidal nucleus causes the solution to change color from clear to bluish green. Salkowski test: 2 mL of chloroform was used to dilute a minimal quantity of *n*-hexane extract, followed by gradually adding 3 mL of concentrated sulphuric acid (H_2SO_4). Oxidation occurs in stages, resulting in the appearance of a reddish-brown substance (11).

Initial detection of steroids and terpenoids through thin layer chromatography (TLC)

To find bioactive compounds, the *n*-hexane extract of *J. integerrima* underwent TLC, using pre-made silica gel plates GF254 as the stationary phase, diluting the *n*-hexane extract with methanol and spotting against the standards, β-carotene (100 µg/mL), lutein (80 µg/mL), ducasterol (50 µg/mL), lupeol (120 µg/mL) and stigmasterol (90 µg/mL). The following mobile phases (M) were used to develop the plates (12-14): M1: methanol: chloroform (9:1), M2: *n*-hexane: ethyl acetate

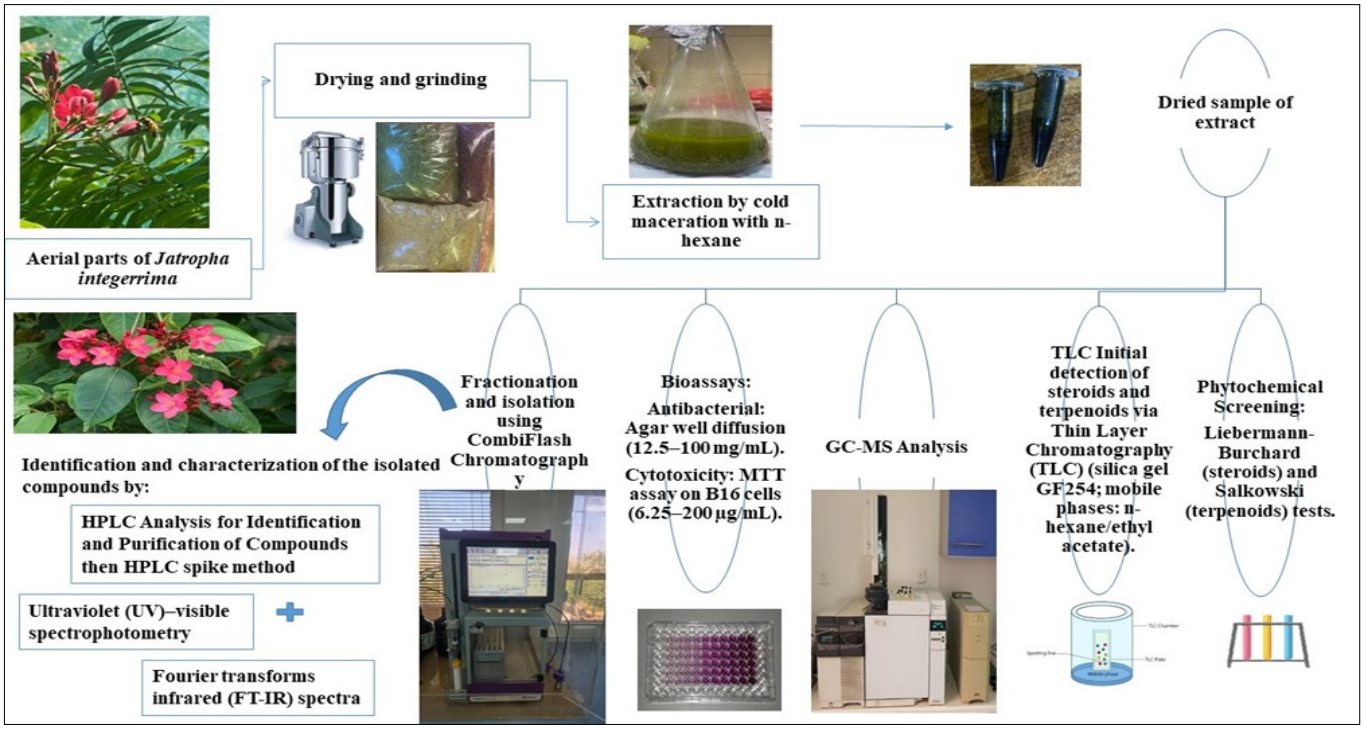


Fig. 1. Scheme of extraction, fractionation, isolation and characterization of terpenoids and sterols from *J. integerrima*.

(70:20), M3: *n*-hexane: ethyl acetate (8:2), M4: toluene: ethyl acetate (5:5) and detection was performed using 5 % H_2SO_4 and anisaldehyde-sulfuric acid spray reagents, followed by heating to detect the spots. Then the spots' retention factor (Rf value) is calculated according to the equation:

$$Rf = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent front}} \quad \text{(Eqn. 1)}$$

Where, Rf denotes the retention factor. Distance travelled by the compound is measured from the baseline to the centre of the compound spot. Distance travelled by the solvent front is measured from the baseline to the highest point reached by the solvent. The Rf value of the sample spots was compared with the standard spot (15).

Gas chromatography - mass spectrometry (GC-MS) analysis

An Agilent Technologies GC-MS was used to analyze the *n*-hexane extract. The instrument, equipped with a non-polar column was used to identify phytochemical compounds based on their mass spectra and retention times. The mobile phase was run on ultra-pure helium gas (99.99 %) with a flow rate of 1 mL/min, with split ratio of 80:1 and the temperature were set to 260 °C. The overall run time was 48 min (16, 17).

Fractionation and isolation using CombiFlash chromatography

Flash chromatography was performed using a CombiFlash NEXTGEN system. This automated system enables efficient compound purification with programmable gradient settings, UV-based peak detection and automated fraction collection. The use of pressurized solvent flow accelerates separation compared to traditional gravity-based column chromatography. First, 0.25 mg of the *n*-hexane extract was mixed with 2 g of silica gel, then silica sample was placed into an empty cartridge, capped and packed onto the system with a 24 g silica column at a flow rate of 20 mL/min. The run was initiated based on the spots observed on TLC, using a suitable solvent system (20 % ethyl acetate and 80 % *n*-hexane, v/v) to elute the column, with a gradient rate until reaching 100 % ethyl acetate for 45 min. This process resulted in 57 fractions of 15 mL each. The obtained fractions were then analyzed using TLC plate (18).

Identification and characterization of the isolated compounds by HPLC analysis

HPLC was used to further purify the CombiFlash fractions, if necessary. The purified compounds were then subjected to HPLC to confirm the characteristics of β-carotene and stigmasterol. The UV/Vis absorption analysis was performed to compare the active components with the standard materials. The equipment was used to determine the retention times of both the standards and the samples. The HPLC system was a SYKAMN model from Germany. The fraction collector model was FOXY R1 and the autosampler model was S5200. Nucleosil C18 columns (Supelco, 5 µm 250 mm × 4.6 mm internal diameters) were used for separation. The mobile phase consisted of acetonitrile and water (2:8, v/v) under isocratic conditions, with a flow rate of 1 mL/min. The injection volume was 100 µL. For UV scanning, the detection wavelength was set at 260 nm (19).

HPLC spike method

The standard addition method, commonly known as the spike method, involves co-injecting an isolated compound with a reference standard to confirm its identity, ensuring identical chromatographic behaviour and retention time (20).

Thin layer chromatography (TLC)

The isolated component was analyzed using a TLC, silica gel GF 254 nm as the stationary phase. The mobile phase consisted of a mixture of hexane and ethyl acetate in an 8:2 ratio. To visualize stigmasterol, the TLC plate was sprayed with 5 % H_2SO_4 reagent and then heated at 110 °C for five to ten min. β-carotene was directly visualized under visible light.

Fourier transform infrared (FTIR) spectra

Fourier transform infrared spectroscopy is used to examine a molecule's interactions with light in the infrared spectrum, specifically between 4000 and 400 cm^{-1} (21). Using the CombiFlash sample from *J. integerrima*, the attenuated total reflection (ATR) technique was applied to support the structural assignments of β-carotene and stigmasterol by analysing their characteristic absorption bands.

Ultraviolet (UV)–visible spectrophotometry

UV–Vis spectrophotometry was performed using methanol as blank, a computerized UV spectrophotometer with a wavelength range of 220-600 nm is utilized to ascertain the identity of the separated component (22). After the separated β-carotene and stigmasterol were initially measured, the standards were also investigated under like conditions and referenced accordingly.

Antibacterial activity assay

The antibacterial efficacy of the *n*-hexane extract of *J. integerrima* was examined utilizing the agar well diffusion technique against two bacterial strains: *S. aureus*, a Gram-positive bacterium; *A. baumannii*, a Gram-negative bacterium.

Preparation of bacterial suspension: Strains of bacteria were cultured in nutrient broth at 37 °C for 24 hr and standardized to 0.5 McFarland turbidity (approximately 1.5×10^8 CFU/mL). The preparation of Muller Hinton (MH) involved dissolving 38 g of powder in 1 L of distilled water and heating the mixture over a burner while stirring. The mixture was autoclaved at 121 °C for 15 min to achieve sterility. It was then cooled to 50 °C, poured into a Petri dish and allowed to solidify for about 15 min. After solidification, the plates were stored inverted at 4 °C. The next step was to put around 20 mL of MH agar into sterile Petri plates. For this study, we used sterile cotton swabs to extract the bacteria from their respective stock cultures (23). To the agar, different concentrations of *n*-hexane extract (12.5, 25, 50 and 100 mg/mL) were introduced after the organisms had been cultured in a lab. The test organisms and extract were grown on plates and left overnight at 37 °C to assess the antibacterial action. The average zones of inhibition (mm) were then measured and recorded (24, 25).

Cytotoxicity evaluation

The cytotoxic effects of *n*-hexane extract were assessed against the B16 melanoma skin cancer cell line cultured in RPMI-1640 supplemented with fetal bovine serum, penicillin and streptomycin. Cells were passaged using trypsin-EDTA and

reseeded at 80 % confluence under 37 °C in a humidified 5 % CO_2 incubator (26, 27). Cells were seeded at a density of 1×10^4 cells per well. Twenty-four hr later, using *n*-hexane extract concentrations (200, 100, 50, 25, 12.5 and 6.25 µg/mL), cell viability after 72 hr of treatment was measured. The percentage of cytotoxicity was calculated using the inhibition rate of cell growth and the IC_{50} value. The inhibition rate of cell growth was calculated as follows (28, 29):

$$\text{Inhibition rate} = A - B/A*100 \qquad \text{(Eqn. 2)}$$

Where, A is the optical density of the control and B is the optical density of the samples. The morphological changes of the dead cells were seen and documented under an inverted microscope with 40x magnification (Optika, Italy).

Statistical analysis

GraphPad Prism 6 was used in the statistical analysis of the acquired data employing an unpaired t-test. The values were expressed as triple mean ± SD.

Results and Discussions

Although *J. integerrima* is cultivated in Iraq as an ornamental, it is also a rich source of numerous significant secondary metabolites, including flavonoids, terpenoids, alkaloids and phenolic compounds, that have therapeutic potential against variety of diseases (4). Additionally, the growing resistance to synthetic drugs has sparked a fresh interest in researching the therapeutic potential of plants. This makes it the first study in Iraq to examine the antibacterial activity and cytotoxic activities, along with a phytochemical analysis, specific sterol and terpene components for their varied pharmacological effects, especially triterpenoid (*β*-carotene) and steroids (stigmasterol).

The plant's extraction process

Extractions were made from the plant's aerial parts by using a maceration technique with *n*-hexane, a nonpolar solvent effective for isolating nonpolar compounds. The process yielded 9 g of extract from 250 g of dried plant material, corresponding to a 3.6 % yield. The extraction technique depends on the target chemical to be separated, as well as the consistency, moisture degree of the obtained plant material and the ability of the plant to withstand the heat. Thus, selecting the optimum extraction technique is crucial and occasionally relies on the intended use of the extract.

Preliminary phytochemical screening

The Salkowski and Libermann Burchard reactions confirmed that terpenoids and steroids were present in the aerial component of *J. integerrima* (Table 1).

Table 1. Results of chemical testing for *J. integerrima n*-hexane extract.

	Liebermann-Burchard reaction	Salkowski reaction
The aerial parts of the plant	+	+

Thin layer chromatography (TLC)

β-carotene, stigmasterol, lupeol, lutein and ducasterol, were detected in the *n*-hexane extract of *J. integerrima,* aerial parts using TLC (Fig. 2). Rf values of these compounds were compared with the standards values (Table 2).

Table 2. Rf value of the compounds in *n*-hexane extract of *J. integerrima* cultivated in Iraq compared to standards.

Standard	Rf (retardation factor) value of standard	Rf (retardation factor) value of *n*-Hexane extract
Stigmasterol	0.45	0.45
β-carotene	0.95	0.95
Lutein	0.13	0.13
Lupeol	0.52	0.52
Ducasterol	0.23	0.22

Gas chromatography - mass spectrometry analysis (GC-MS)

The chromatogram displayed 48 peaks, indicating the presence of 48 distinct chemical constituents in the extract (Table 3 and Fig. 3). The most abundant bioactive ingredient in our sample extract, including sterols, tocopherols and terpenoids. The sterol group was represented by stigmasterol, 22,23-dihydro stigmasterol and campesterol. Tocopherols present in the form of vitamin E, *β*-tocopherol and *γ*-tocopherol. Terpenoids were phytol, squalene and *α*-amyrine. The biologically active fatty acids and esters were also detected in the extract such as methyl palmitate, 9,15-octadecadienoic acid, methyl ester and n-eicosane.

Fractionation and isolation using CombiFlash chromatography

CombiFlash chromatogram of *n*-hexane extract fractions from the instrument is presented in Fig. 4. Fifty seven fractions recorded from CombiFlash test tube spotting on TLC plates revealed that tube number 9 gave a spot similar to *β*-carotene standard and tubes numbered 16-20 gave a spot similar to

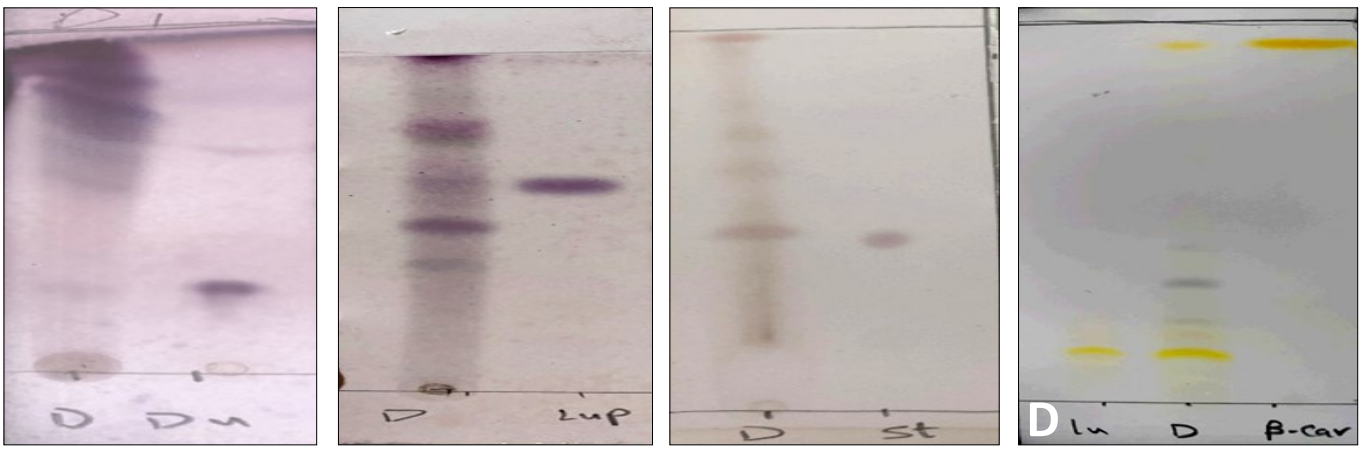


Fig. 2. Thin-layer chromatogram of *n*-hexane extract of *J. integerrima*..

Table 3. The GC-MS analysis of the *n*-hexane extract of *J. integerrima* cultivated in Iraq.

Peak	Name of compound	Similarity index %	Retention time (min)	Area %
1	Phthalic acid, butyl undecyl ester	83	24.53	1.23
2	Neophytadiene	91	25.371	0.28
3	Methyl palmitate	96	25.926	0.26
4	9,15-octadecadienoic acid, methyl ester, (Z,Z)-	99	28.468	0.34
5	Phytol	91	28.873	1.87
6	*β*-selinene	86	30.876	0.48
7	*α*-selinene	95	32.567	0.18
8	*n*-eicosane	84	36.838	0.20
9	Squalene	96	38.124	2.84
10	*δ*-tocopherol	97	39.328	3.09
11	*β*-tocopherol	98	40.309	3.81
12	*γ*-tocopherol	94	40.459	1.79
13	Vitamin E	99	41.378	9.13
14	Campesterol	99	42.638	8.10
15	Stigmasterol	95	43.09	13.19
16	Stigmasterol, 22,23-dihydro-	99	43.998	23.56
17	α-amyrine	91	44.434	4.8

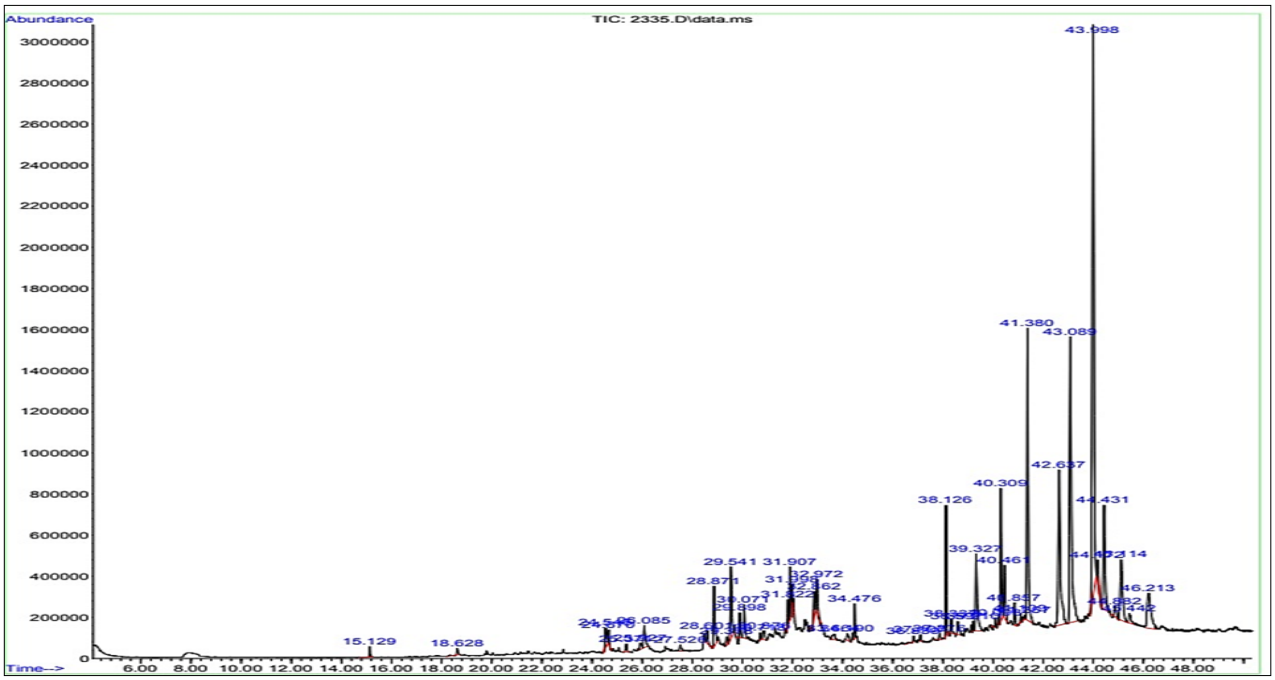


Fig. 3. The GC-MS analysis of the *n*-hexane aerial parts of *J. integerrima.*

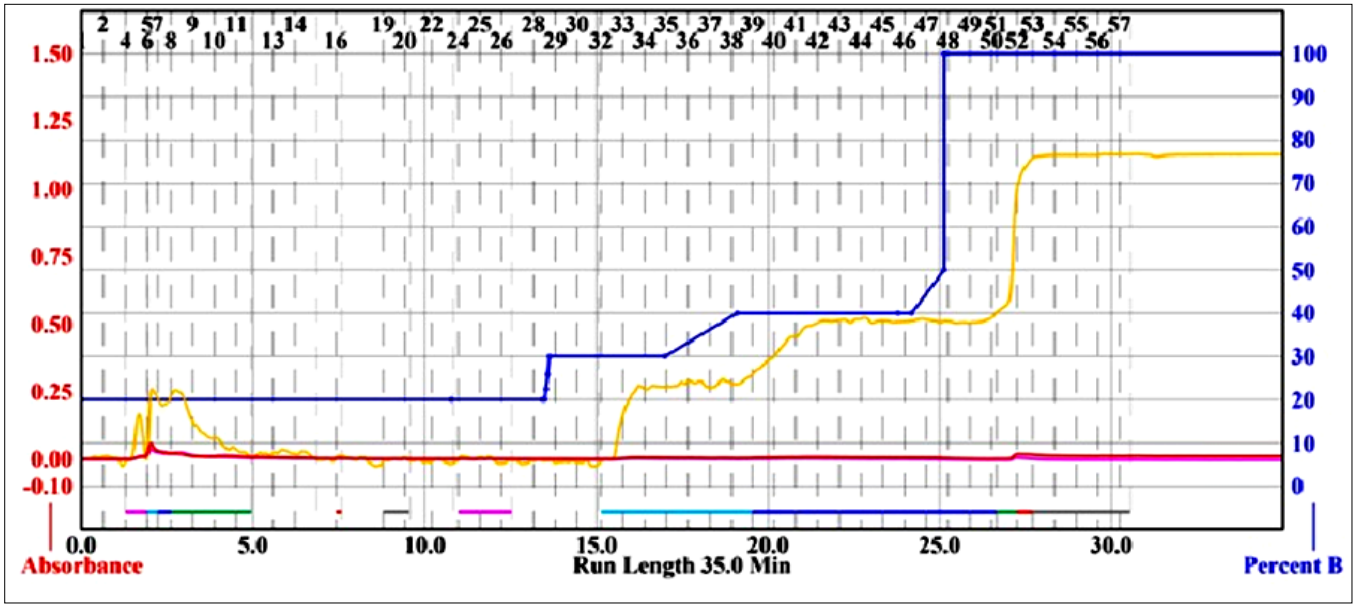


Fig. 4. CombiFlash chromatogram of *n*-hexane extract of *J. integerrima* cultivated in Iraq.

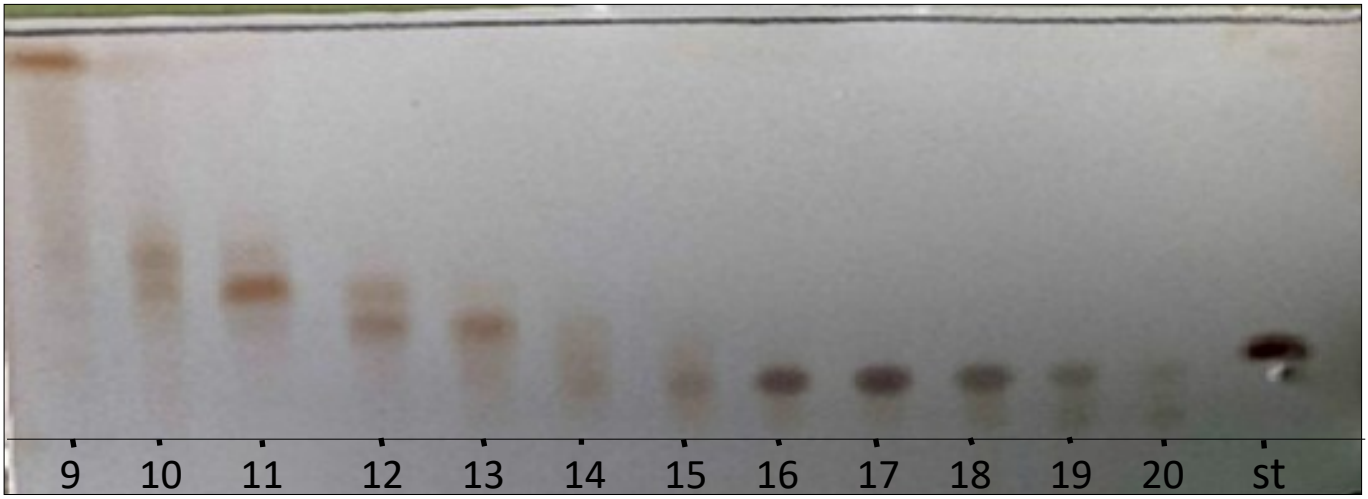


Fig. 5. A thin layer chromatogram showing the fractions that were obtained after the *n*-hexane extract was cleaned up using CombiFlash column chromatography phase created in (hexane: ethyl acetate 80:20) using silica gel GF254 nm as the stationary phase. Visualized using 5 % H_2SO_4 spray reagent, then heated for 5 to 10 min at 110 °C.

stigmasterol standard presented in Fig. 5.

HPLC analysis for identification and purification of compounds

CombiFlash fractions were HPLC eluted and were matched with the approved reference substances. The HPLC study revealed that the *β*-carotene flash fraction number 9 included only *β*-carotene (Fig. 6). Alternatively, combi flash fractions 16–20 have a mix of chemicals and stigmasterol, depending on the standard for stigmasterol and the amount of time that the substance stays in solution (Fig. 7).

Identification and characterization of the isolated compound

HPLC analysis and spiking method

The retention time (Rt) obtained under identical chromatographic settings of the extracted *β*-carotene and stigmasterol against the true standards of *β*-carotene and stigmasterol, therefore enabling one to distinguish them qualitatively (Table 4). The isolated β-carotene and stigmasterol were then spiked using standards and a strong peak was visible in the HPLC chromatogram (Fig. 8, 9).

Thin layer chromatography (TLC)

The TLC profile showed co-migration of the separated compound with the standard, confirming identity of desired compounds (Fig. 10).

FTIR spectra

FTIR spectroscopy was performed to compare synthetic reference standards and natural compounds isolated *via* combi-flash from *J. integerrima.* FTIR spectra of the isolated compounds closely matched those of *β*-carotene and stigmasterol standards (Fig. 11A, B and 12A, B).

Ultraviolet spectroscopy (UV)

The UV spectrum was obtained for the isolated and the standard, the maximum absorbance spectrum of *β*-carotene and stigmasterol was revealed to be the same at the same wavelength (Fig. 13, 14). The previously studies data from several analytical techniques showed that isolated molecule possessed all the qualities exactly matching for *β*-carotene and stigmasterol standards, confirming *β*-carotene and stigmasterol.

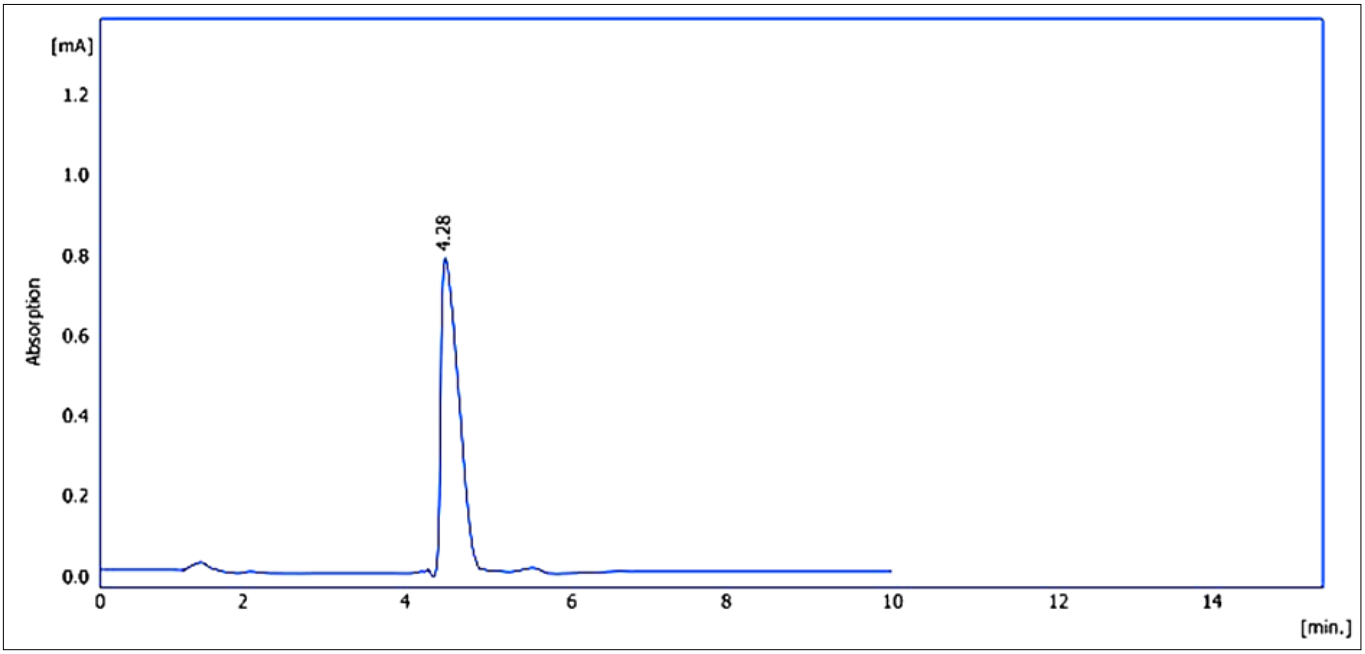


Fig. 6. HPLC chromatogram of fraction 9 (*β*-carotene) from CombiFlash extract.

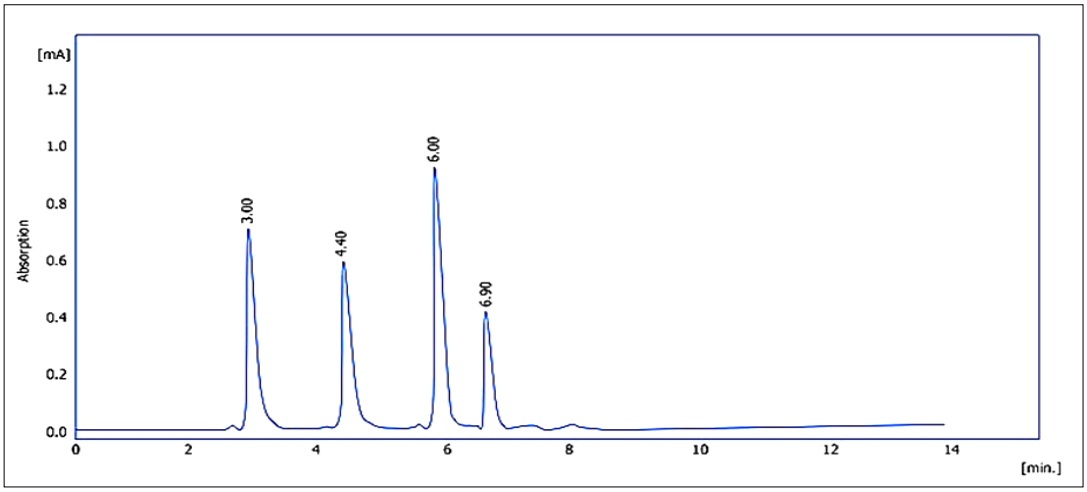


Fig. 7. HPLC chromatograms of fractions 16-20 (stigmasterol) from CombiFlash extract.

Table 4. Retention times (in min) for *β*-carotene and stigmasterol.

Standard	isolates Rt (min)	Standards Rt (min)
β-carotene	4.28	4.25
Stigmasterol	6.10	6.09

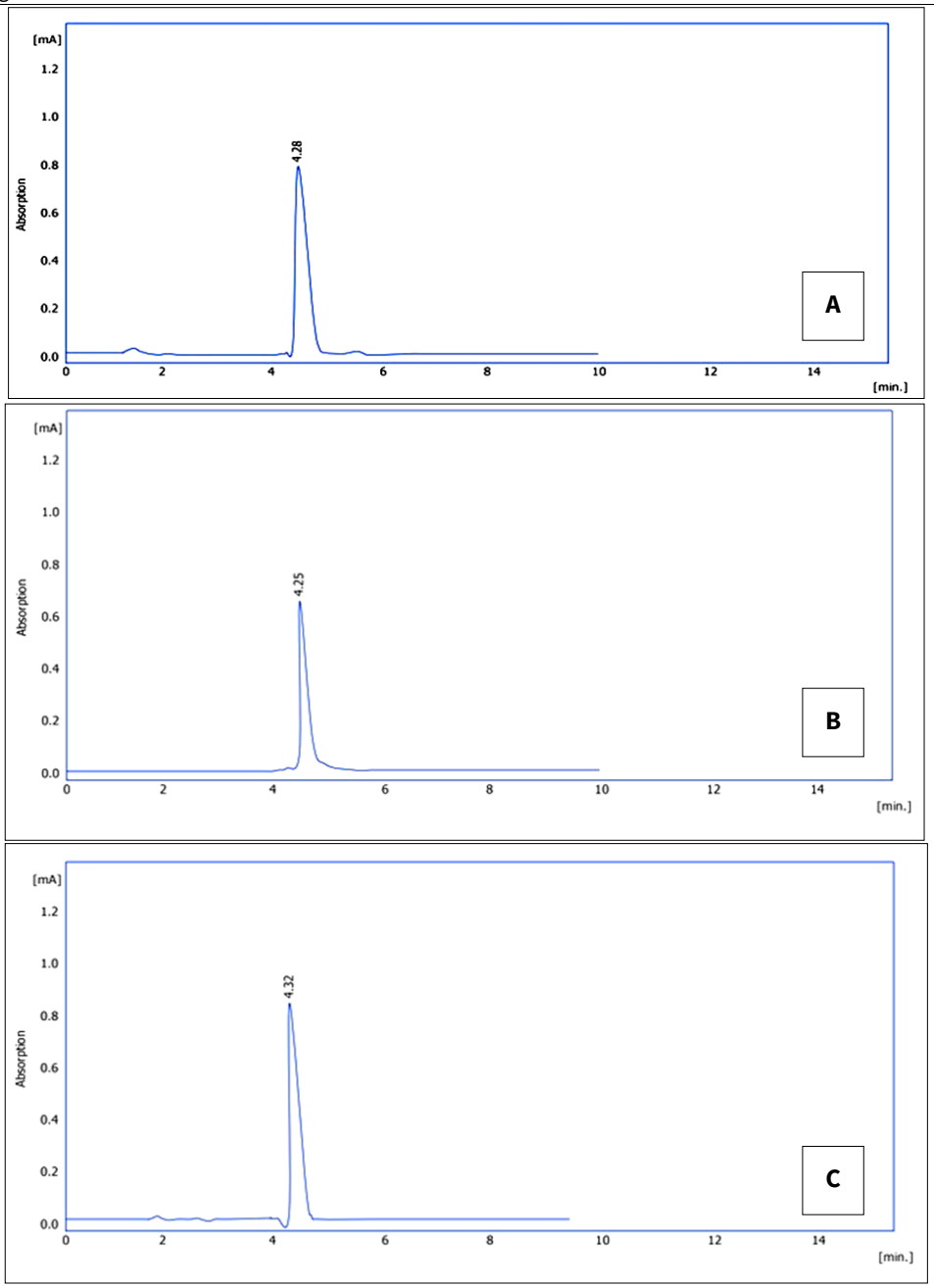


Fig. 8. HPLC chromatogram (**A**) isolated *β*-carotene, (**B**) *β*-carotene standard, (**C**) isolated *β*-carotene with *β*-carotene standard HPLC spike.

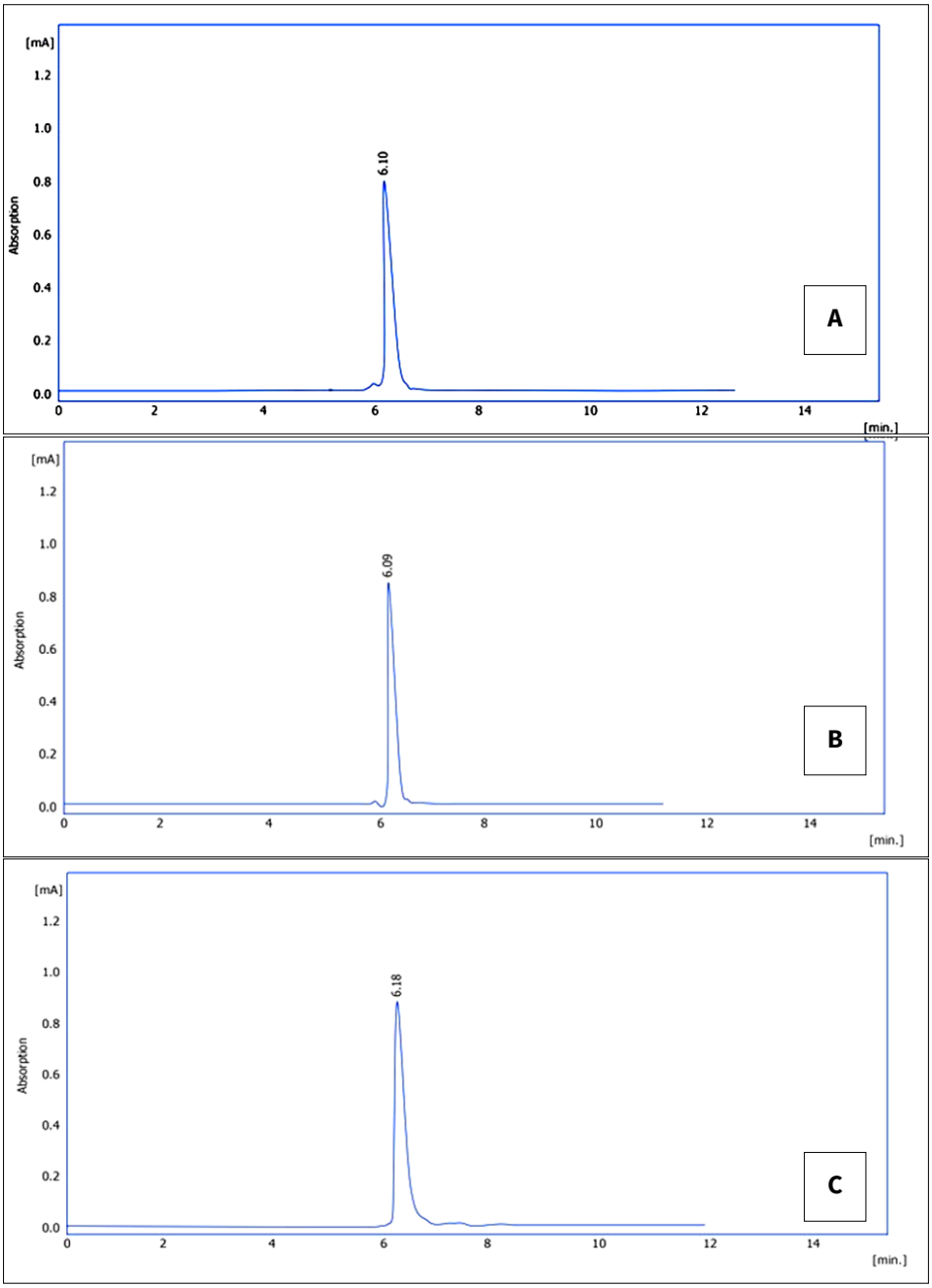


Fig. 9. HPLC chromatogram (**A**) isolated stigmasterol, (**B**) stigmasterol standard, (**C**) isolated stigmasterol with stigmasterol standard HPLC spike.

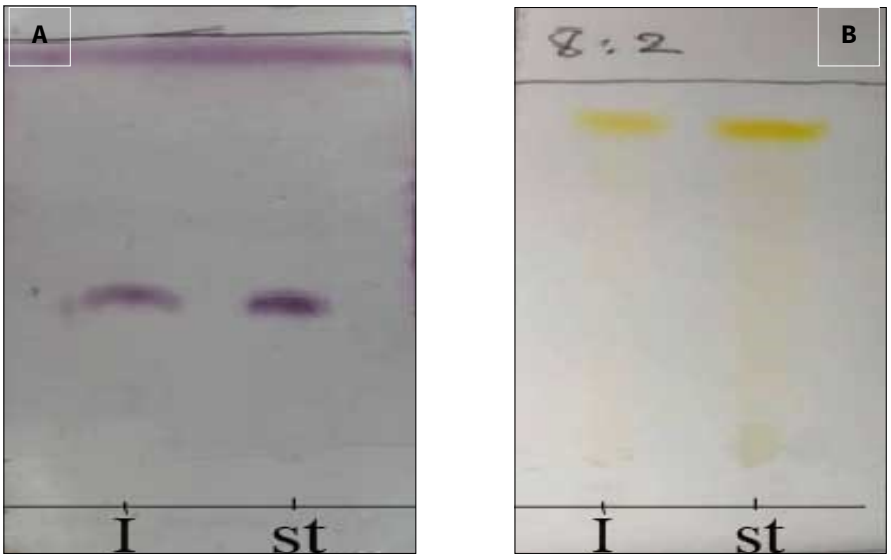


Fig. 10. TLC of the isolated compounds with the standard. (A) stigmasterol and the standard are visualized using 5 % H_2SO_4 spray reagent, then heated for 5 to 10 min at 110 °C. (B) β-carotene and the standard visualized by the eye. Both were developed in the mobile phase: *n*-hexane: ethyl acetate (80:20) using silica gel GF254 nm as the stationary phase.

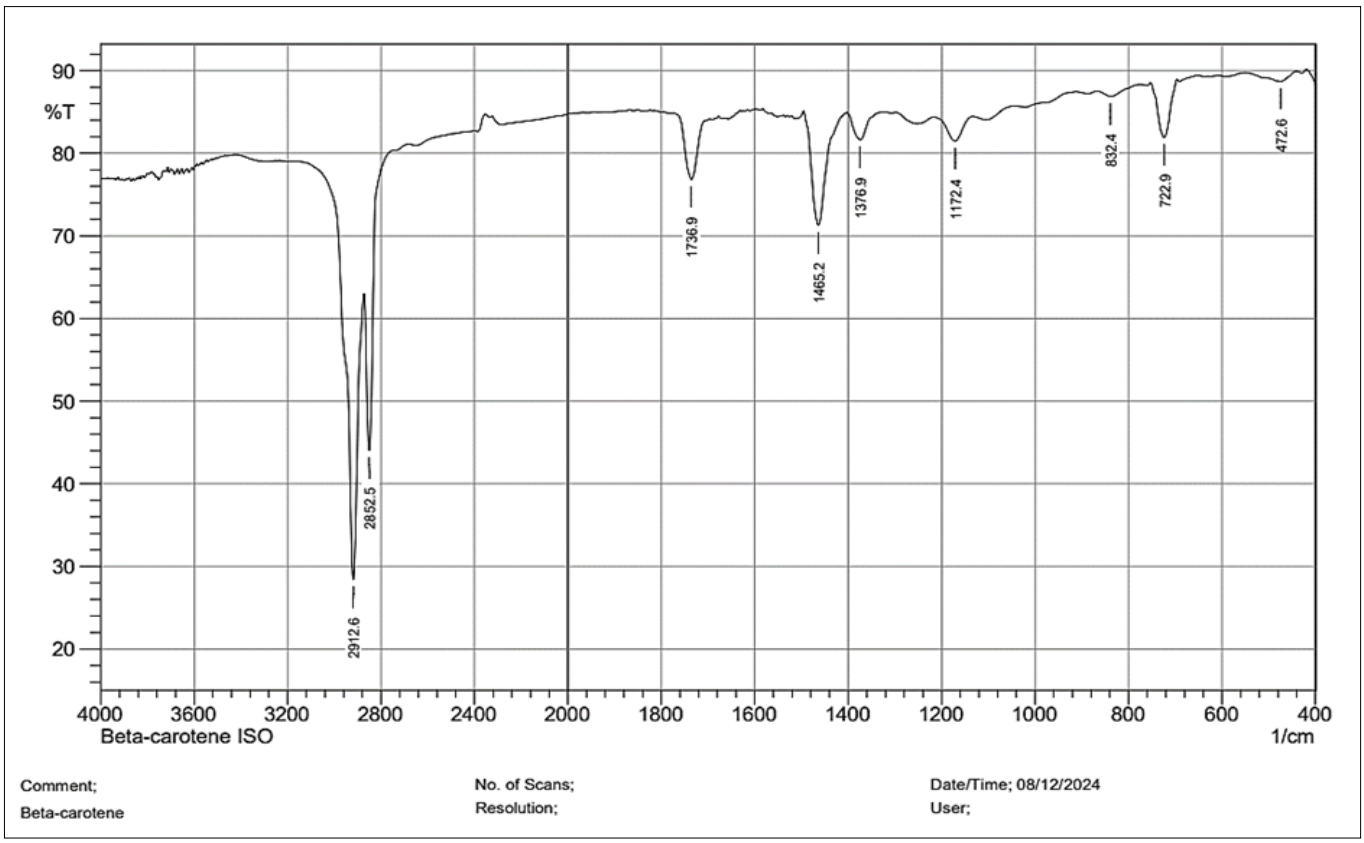


Fig. 11(A). FTIR spectra of isolated *β*-carotene.

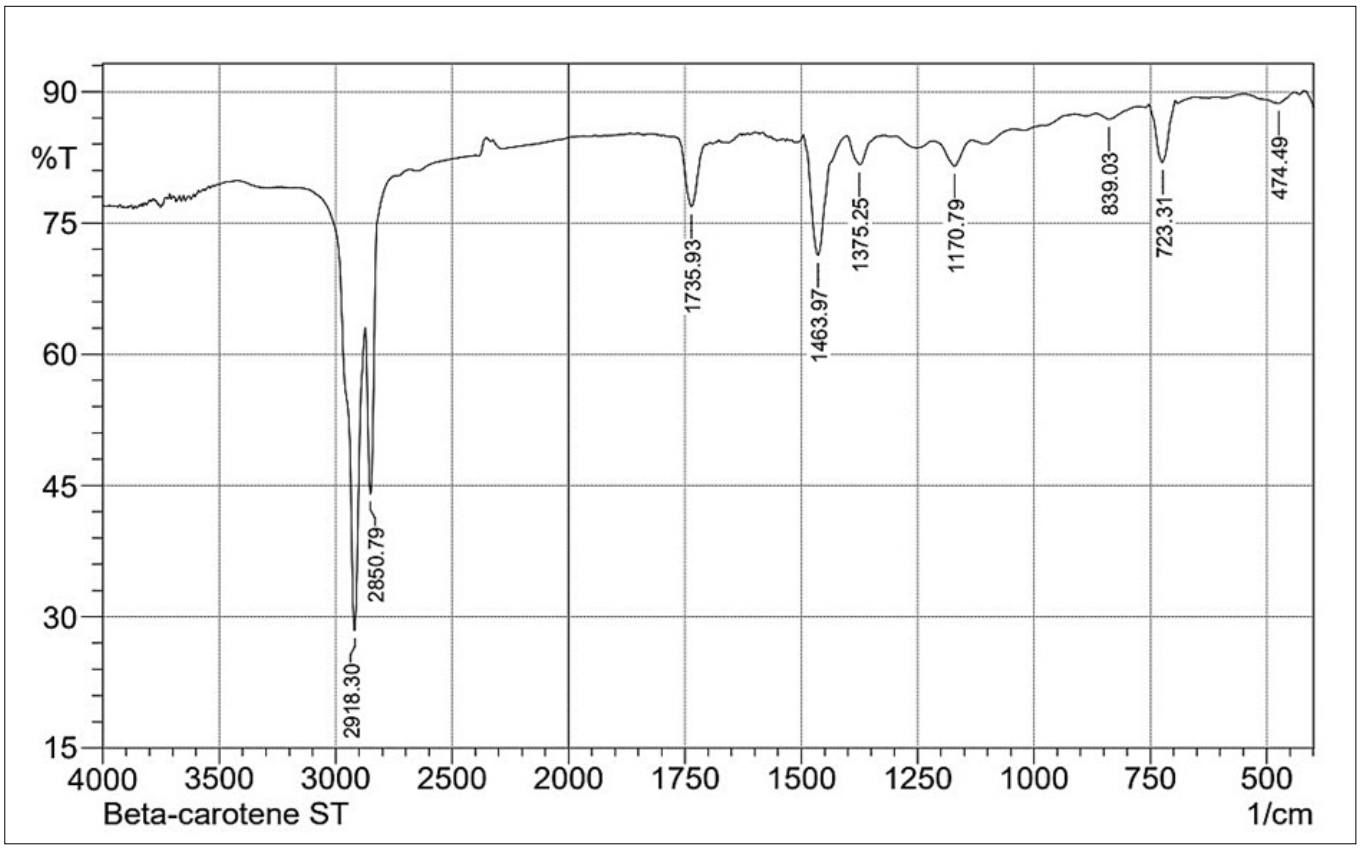


Fig. 11(B). FTIR spectra of β-carotene standard.

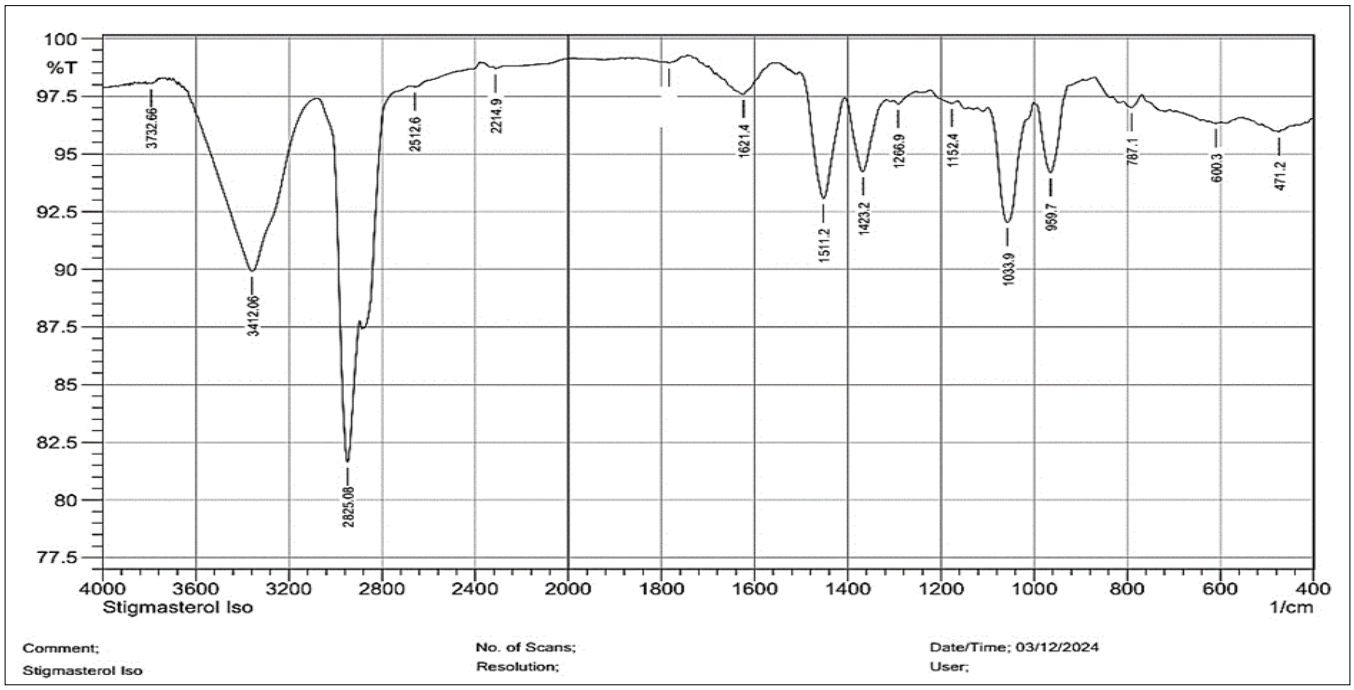


Fig. 12(A). FTIR spectra of isolated stigmasterol.

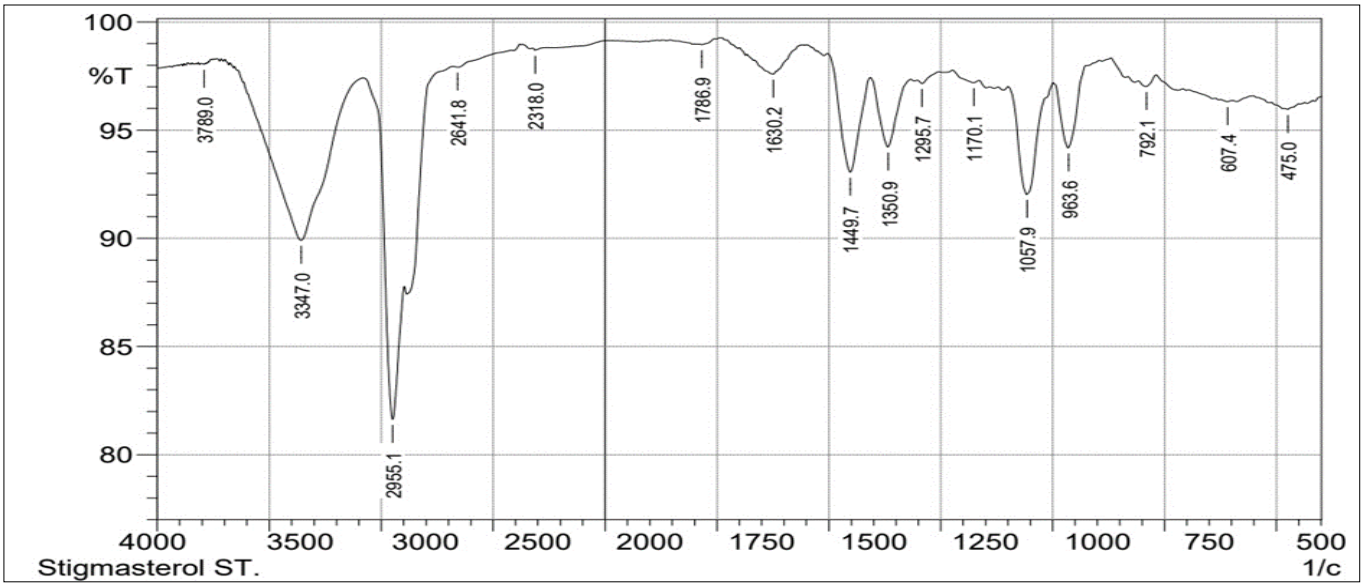


Fig. 12(B). FTIR spectra of stigmasterol standard.

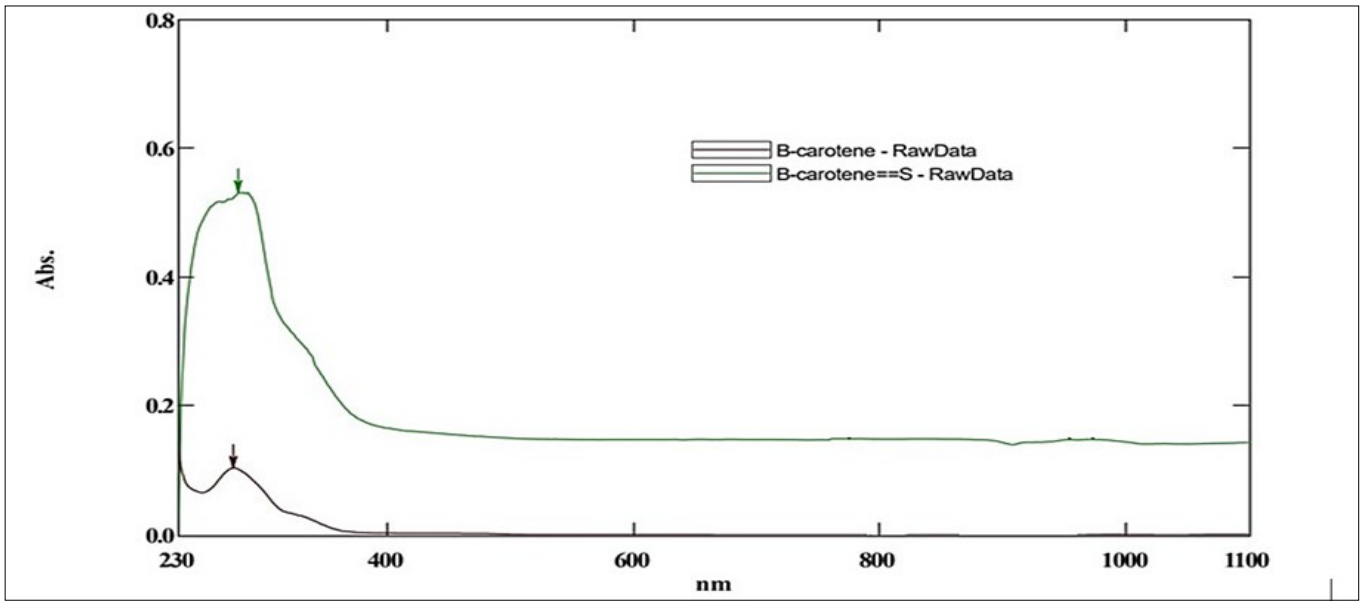


Fig. 13. UV spectrum of the *β*-carotene standard and the isolated *β*-carotene from the *n*-hexane extract.

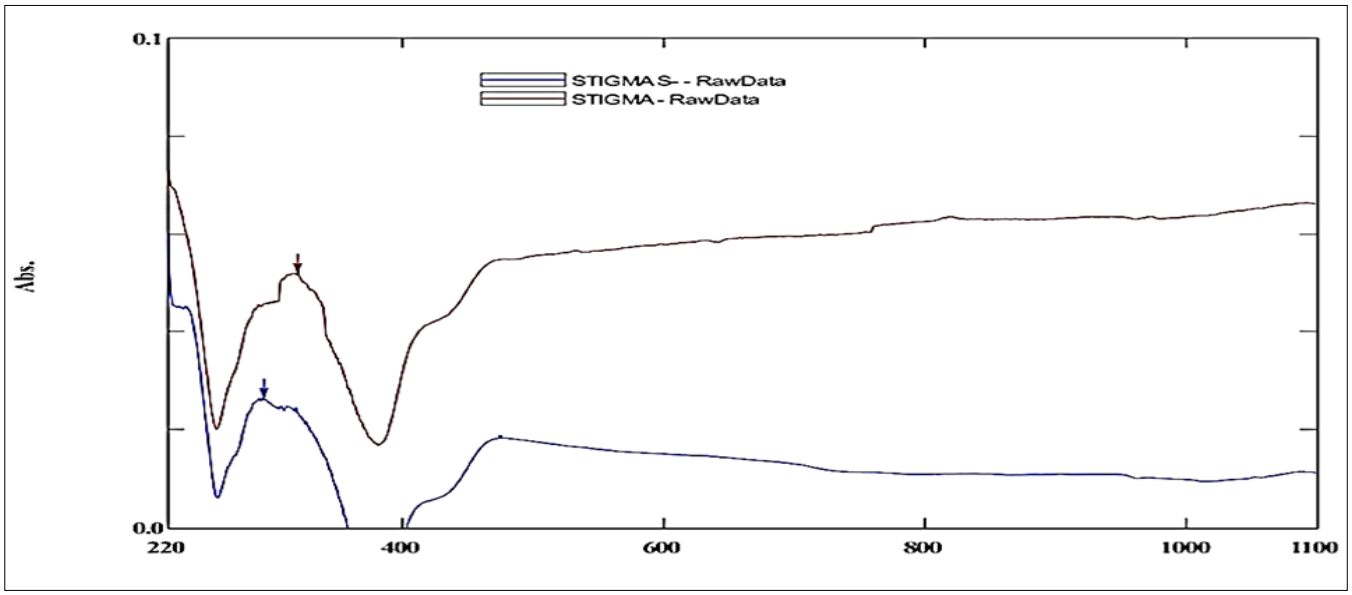


Fig. 14. UV spectrum of the stigmasterol standard and the isolated stigmasterol from the *n*-hexane extract.

Antibacterial activity

The antibacterial assay considerable activity of the *n*-hexane extract of *J. integerrima* cultivated in Iraq, especially against Gram-positive bacteria such as *S. aureus* (Table 5). The zone of inhibition ranged from 6 mm (negative control) to 19 mm at the highest concentration (100 mg/mL), indicating a dose-dependent response. *A. baumannii,* showed the inhibition zones ranged from 6 mm (negative control) to 8 mm. Moreover, the dose-dependent antibacterial action, highest concentration (100 mg/mL) produced the greatest inhibition zones (Fig. 15, 16). The results suggest significant potential of the extract as an antibacterial agent, especially at higher concentrations.

Table 5. Antibacterial analysis (zone of inhibition (mm) of *J. integerrima n*-hexane extract against *S. aureus* and *A. baumannii.*

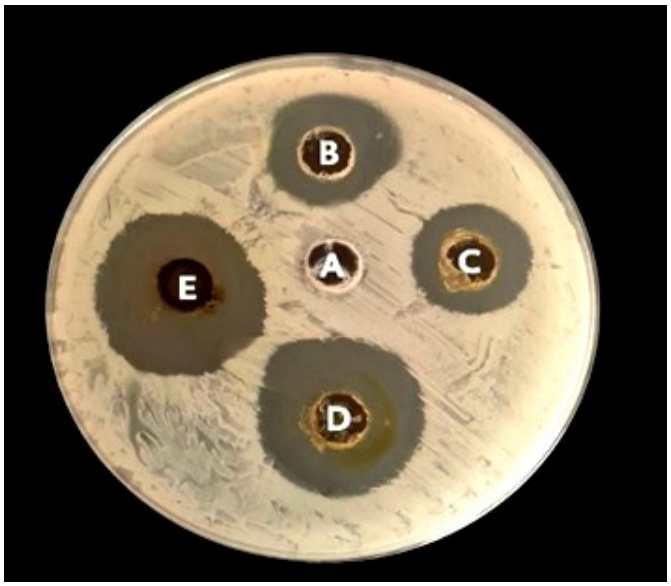
Concertation / Bacterial	0 (A)	12.5 (B)	25 (C)	50 (D)	100 (E)
S. aureus	6 mm	16 mm	17 mm	18 mm	19 mm
A. baumannii	6 mm	6 mm	6.1 mm	6.2 mm	8 mm

Cytotoxic assessment against melanoma skin cancer cells B16

In the MTT assay, the extract exhibited significant cytotoxic effects on the B16 cell line and the significance level (± SD) of three independent measurements was used to reflect the findings. The highest inhibition rate of 84.13± 2.395 % on the skin cancer (B16) cell line at concentration of 200 µg/mL, indicating potent anticancer activity, while the IC_{50} = 29.86 µg/mL (Fig. 17). There is a detectable morphological change such as detachment or shrinkage in the cell was seen (Fig. 18).

This study successfully isolated stigmasterol and β-carotene from the *n*-hexane extract by CombiFlash for the first time in Iraq. Contemporary chromatographic and spectroscopic methods enable precise identification of bioactive components.

Additionally, the GC-MS analysis revealed a diverse profile of bioactive compounds are known for their significant pharmacological properties. β-carotene serves as a potent antioxidant and a precursor to vitamin A. Scavenging free radicals and enhancing cellular defense mechanisms have demonstrated a reduction in oxidative stress and prevention of neurodegenerative diseases (30). Carotene's anti-



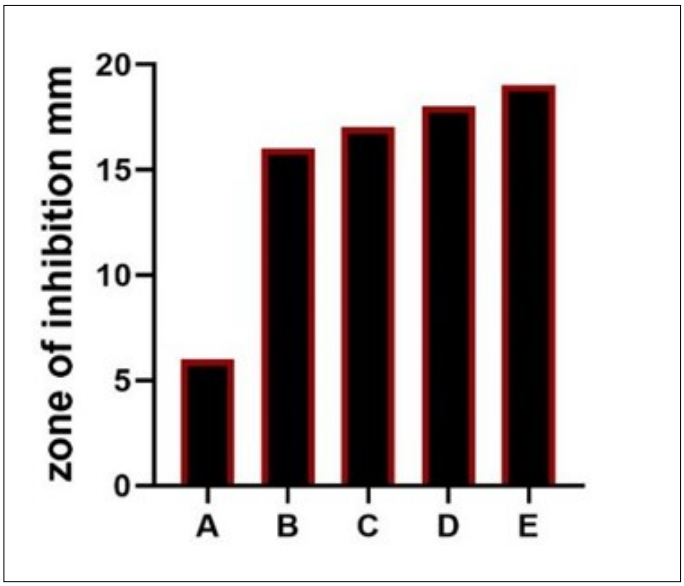


Fig. 15. Antibacterial activity of *n*-hexane extract of *J. integerrima* against *S. aureus.* (**A**) Control (**B**) 12.5 % (**C**) 25 % (**D**) 50 % (**E**) 100 %.

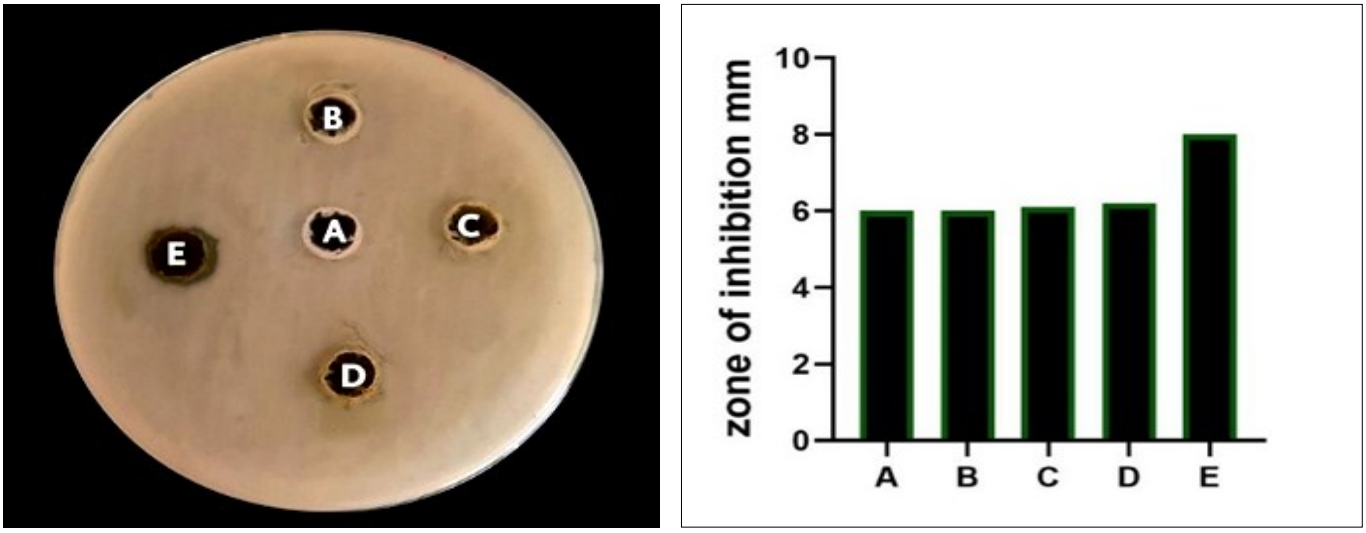


Fig. 16. Antibacterial activity of *n*-hexane extract of *J. integerrima* against *A. baumannii* (**A**) Control (**B**) 12.5 % (**C**) 25 % (**D**) 50 % (**E**) 100 %.

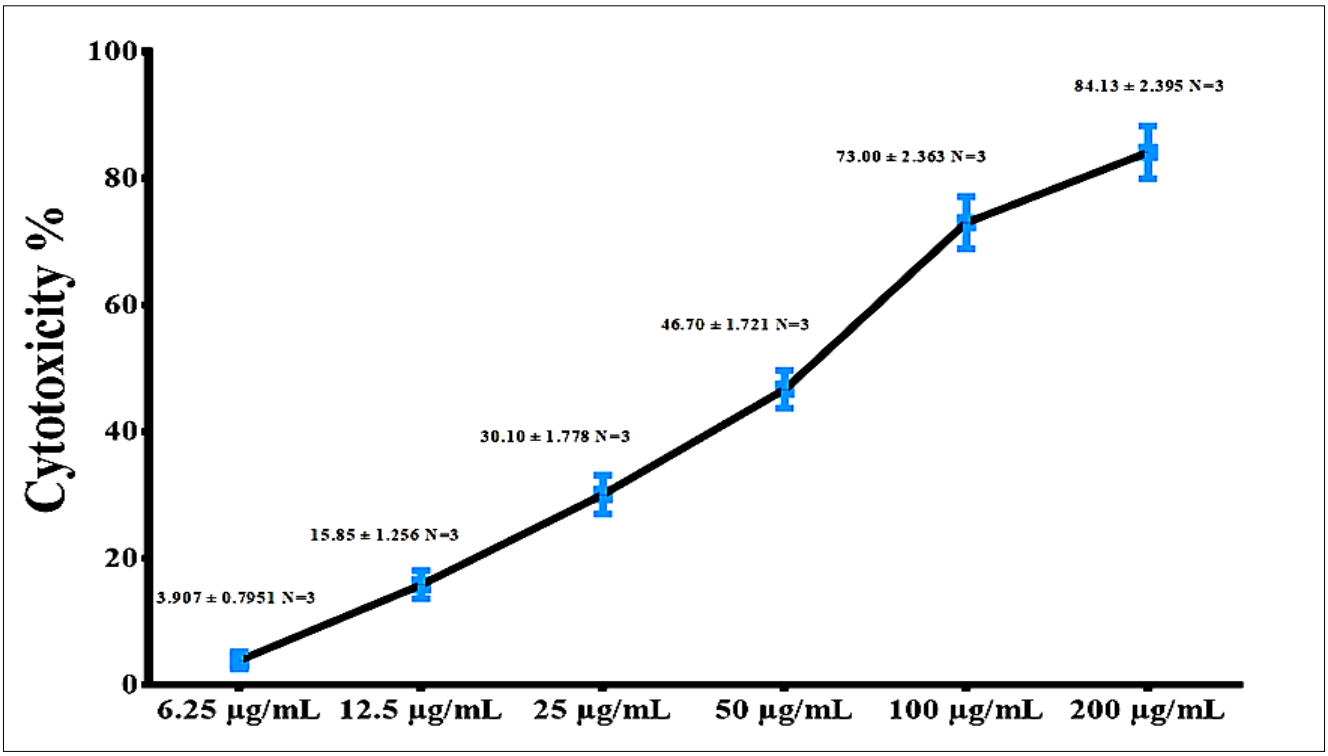


Fig. 17. Cytotoxicity effects at various doses (200, 100, 50, 25, 12.5, 6.25 µg/mL) from the *n*-hexane extract of *J. integerrima* against the B16 cell line. Mean ± SD of three-fold observations was used to express the data.

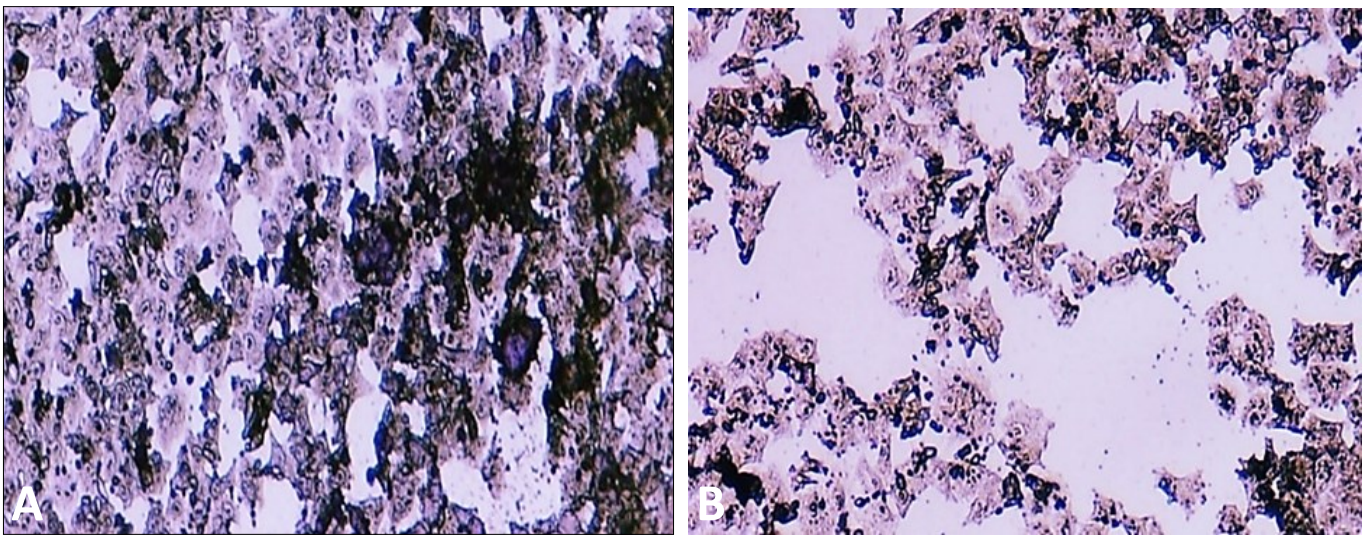


Fig. 18. Effect of *n*-hexane extract of *J. integerrima* on the morphology of skin cancer cells (B16 cell) (**A**) prior treatment, (**B**) following treatment with the extract.

inflammatory and anti-cancer qualities also make it a useful therapeutic agent (31). Stigmasterol, a phytosterol structurally similar to cholesterol, has demonstrated significant anti-inflammatory, anti-diabetic and neuroprotective effects (32). Studies have shown that it lowers long-term illness risk, including disorders such as Parkinson's and Alzheimer's, alters cholesterol processing and enhances cardiovascular health by means of modification of cholesterol levels (33). Furthermore, motivating potential uses in pharmaceutical formulations yields antibacterial and anticancer effects (34). Also essential is vitamin E, whose free radical scavenging action helps to prevent skin cancer (35). The results show that *J. integerrima* extract has broad-spectrum antibacterial action, higher inhibition against Gram-positive bacteria (*S. aureus*) is seen relative to Gram-negative bacteria (*A. baumannii*). This greater susceptibility of Gram-positive bacteria is likely due to their cell wall structure, which lacks the outer membrane found in Gram -negative bacteria-a barrier that can limit the penetration of compounds present in the *n*-hexane extract. Terpenoids and sterols, including stigmasterol and *β*-carotene, are well-known for their antibacterial qualities, so the activity might be ascribed to either oxidative stress or possibly disturbance of bacterial membranes or inhibition of enzymes (36, 37). All of these findings validate that the extract of *J. integerrima* has biologically active chemicals that may have a function in the anticancer especially against melanoma B16 skin cancer cell line and antibacterial activities displayed in our study.

Conclusion

This study demonstrated that *J. integerrima* is a promising source of bioactive compounds with potential therapeutic applications. Moreover, it may serve as a valuable source of natural antibacterial agents, supporting its traditional use in treating infections. Natural sources like *J. integerrima* offer a promising starting point for the development of new, potent anticancer drugs with minimal side effects, bringing fresh hope to cancer treatment strategies. However, further *in vitro* and *in vivo* studies, as well as additional research, are required to fully evaluate its biological potential and medicinal effects.

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Authors' contributions

The present study was designed and manuscript reviewed by NMI. The literature search, clinical and experimental studies, data and statistical analysis, manuscript preparation and editing were done by MJM**.** Data analysis was conducted in College of Pharmacy, University of Baghdad and Department of Pharmacy. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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