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RESEARCH ARTICLE

From toxic ornamental to therapeutic prospect: Phytochemical profile and cytotoxic evaluation of n-hexane extract from Iraqi *Senecio rowleyanus*

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Abstract

Senecio rowleyanus H. Jacobsen plant belongs to *Asteraceae* family that contain over 1600 genera and approximately 2500 species that have important pharmacological roles in human life. This study assesses the n-hexane extract of *Senecio rowleyanus*, introduces a new CombiFlash-based method for phytochemical isolation and evaluates its pharmacological effect against breast cancer cells. The whole plant was extracted using maceration in n-hexane and analyzed by Gas Chromatography- Mass Spectrometry (GC-MS). Phytosterols were isolated using CombiFlash column chromatography, yielding several fractions; only fraction B matched lupeol, confirmed by HPLC and FT-IR. Cytotoxicity of the extract was evaluated using the MTT assay on AMJ13 breast cancer cells treated with concentrations (1000, 500, 250, 125, 62.5 and 31.75 µg/mL) for 72 hrs, with doxorubicin as a control. Cell viability was then assessed. GC-MS analysis confirmed the presence of multiple bioactive phytosterols, which revealed a high content of β- and γ-sitosterol (17 %), spathulenol (13 %) and α-amyrin (9 %). with lupeol identified in a specific purified fraction. The n-hexane extract exhibited a cytotoxic effect on AMJ13 cells with an IC_{50} value of 125.3 µg/mL, showing a concentration- and time-dependent reduction in cell viability. The observed cytotoxicity was notable when compared to the standard drug, doxorubicin. *Senecio rowleyanus* cultivated in Iraq demonstrates potent anticancer potential, with lupeol and other phytosterols exhibiting significant cytotoxicity against breast cancer cells. These findings support its promise as a natural candidate for pharmaceutical development, further mechanistic and clinical investigations is requiring.

Keywords: breast cancer cytotoxicity; combi-flash column; GC-MS; lupeol; *Senecio rowleyanus*

Introduction

Medicinal plants have been employed since ancient times for the treatment of a wide range of health conditions. Modern assessments of angiosperms suggest that the number of species ranges from 200000 to 250000, encompassing approximately 300 families and 10500 genera; in spite of the fast-growing literature and research activities in the field of phytochemistry, only small fraction of the total diversity has undergone chemical analysis (1).

Senecio rowleyanus H. Jacobsen, commonly referred to as the String of Pearls (2), is classified under the *Asteraceae* family, which encompasses over 1600 genera and approximately 2500 species (3). This family of plants is distributed across various regions of the globe (4). *Asteraceae* is one of pyrrolizidine alkaloid rich family alongside with *Boraginaceae*, *Fabaceae* and *Orchidaceae* families (5, 6). The genus *Senecio* is characterized by a diverse array of chemical constituents, notably including terpenoids, phenolic compounds, flavonoids, essential oils (EOs) and pyrrolizidine alkaloids (7). Species within the *Senecio* genus exhibit a multitude of biological activities, including but not limited to antibacterial, antimicrobial and cytotoxic properties (8). Essential oils (EOs) represent a significant reservoir of secondary metabolites, which substantiate their extensive pharmacological activity, such as antiviral, insecticidal, antibacterial and allopathic functions. Furthermore, the application of essential oils as natural antioxidants has garnered considerable attention from investigators, given their potential in the prevention and treatment of diseases associated with oxidative damage (9). The essential oil derived from *Senecio rowleyanus* remains largely unexplored, except for a single study conducted in Egypt (10).

Mammary carcinoma represents the most prevalent malignancy and stands as the foremost leading cause of cancer-related mortality among females on a global scale. The condition is typified by the presence of a painless mass or thickening within breast tissue, with the precise etiology of breast cancer remaining elusive; however, multiple risk factors have been identified that may elevate the likelihood of its onset. Mammary carcinoma is categorized into four

principal subtypes predicated on the specific proteins expressed in the malignant cells: Luminal A, Luminal B (HR+/HER2+), HER2+ and Triple-negative (11). The development of resistance to therapeutic agents within cancerous cells, along with the deleterious side effects associated with chemotherapy regimens, constitutes the primary obstacles to successful treatment in oncological care. Considering the limitations inherent in traditional chemotherapy approaches, a subset of cancer patients has begun to explore complementary or alternative therapies that are derived from a diverse array of botanical sources or nutritional components. Therapeutic interventions utilizing medicinal plants have garnered recognition for their efficacy, economic viability and reduced incidence of adverse effects. Numerous phytochemical constituents have demonstrated promising anticancer effects through various mechanisms, triggering programmed cell death in neoplastic cells, the Suppression of angiogenesis (Establish of new blood vessels that supply the tumor) and the disruption of critical signaling pathways that play a pivotal role in cancer advancement and metastasis (12, 13).

Materials and Methods

Chemical and reagent

Hexane 99 % was purchased from JT BAKER /Germany, Ethyl acetate 99.5 % from Chem-Lab NV / Belgium and dimethylsulphoxide (DMSO) from Santacruz Biotechnology/ USA. Doxorubicin from SABA CO./ turkey, lupeol standard from Chem Faces / china. MTT stain was purchased from Bio -World/USA. RPMI 1640, Trypsin/EDTA, Fetal bovine serum, penicillin and streptomycin were purchased from Capricorn/Germany.

Plant material

The whole plant of *S. rowleyanus* (including flower & root) were collected in July 2024 from Baghdad city taxonomic identification of the plant is done by Prof. Sakina Abbas Aliwi, Department of Biology - College of Science - University of Baghdad the root is meticulously cleansed to eliminate any foreign material and then the whole plant left to dry for approximately 14 days.

Extraction method

Two hundred grams of shade-dried, pulverized whole plant material would be defatted by maceration with 500 mL of n-hexane for 24 hrs and then replace the old solvent with new one and repeat the process for three time, then collect the fractions (1500 mL) and allowed to dry using a rotary evaporator (IKARV 10 D S99 R / USA).

Gas Chromatography-Mass Spectrometer (GC-MS) analysis

GCMS analysis was done for the n-hexane extract using Agelint Technologies(7820A) Gas chromatography system coupled with a mass spectrometer (5977E) USA. Analytical column, Agelint HP-5ms Ultra lneit (30 m length x 250 µm inner diameter x 0.25 µm film thickness) was used. Carrier gas, Helium; injection temperature, 250 ˚C; Oven program temperature; initial Ramp 1, 70 ˚C; Ramp 2, 70 ˚C to 180 ˚C for 7 ˚C/min; Ramp 3, 180˚C to 280˚C for 8 ˚C/min; Ramp 4, 180˚C to 300˚C for 7 ˚C/min; Ramp 5, 300 ˚C hold to 7 min. Injection type, splitless; injection volume 1µL; Aux heateres temperature, 310 ˚C; GC inlet line temperature, 250 ˚C; pressure 11.933 psi; scan range, m/z 25-1000. GC-MS analysis was carried out at the Ministry of Industry and Mineral/ Research and Development Authority/ Abn Al-Bitar Research Center. This methodology capitalizes on the distinct characteristics of compounds by segregating them according to their varying degrees of polarity and boiling points. Such disparities facilitate the temporal detection of different compounds, leading to variations in retention times. The retention times, in conjunction with spectral data, are employed in conjunction with the National Institute of Standards and Technology (NIST) data library to infer the identity of the molecules or compounds present within the analyzed mixture (14).

Combi flash column

Flash chromatography represents a rapid and efficient methodology for the separation of organic compounds, enabling the isolation of several grams of material within an exceedingly brief time frame (15). In this technique mixture of 0.1g n-hexane and 2 g silica gel (60-120 mesh) dissolved in sufficient amount of dichloromethane then allow solvent to evaporate at room temperature. A silica gel sample is introduced into an unoccupied cartridge, consequently, the material was wrapped in a frit and consequently aligned onto the combi-flash system. The combi-flash apparatus employs a solvent mixture composed of 10 % ethyl acetate and 90 % n-hexane (volume/volume). The flow rate is maintained at 70 mL per min; a total of 152 test tubes were utilized (15*150), with a column silica mass of 120 g, a runtime of 35 min, initial waste measured at 0.0 column volumes (CV), an air purge duration of 1 min, with peak tube volume maximized, non-peak tube volume also maximized and the loading type categorized as solid. The wavelength designated as 1 (red) was set to 254 nm, with a peak width of 1 minute and a threshold established at 0.20 AU, while the wavelength designated as 2 (purple) was calibrated to 280 nm and all wavelengths (orange) were recorded from 200-300 nm, maintaining a peak width of 1 minute and a threshold of 0.20 AU. Each fraction that was collected was subsequently spotted onto a TLC plate and run using the same solvent system n-hexane: ethyl acetate (90:10). Within the context of a flash chromatography system, various processes can be automated to achieve enhanced productivity, gradients can be established with programmable parameters, peaks can be separated through the utilization of ultraviolet light and columns alongside collecting tube racks can be automatically identified, contingent upon the system in use. Its compact design positions it as an exemplary personal system, thereby rendering it particularly appropriate for implementation in chemical fume hoods and other enclosed indoor environments (16).

High-Performance Liquid Chromatography (HPLC) analysis of selected fraction from combi-flash column

HPLC technique constitutes a highly sensitive, specific and effective qualitative analytical approach, thereby facilitating the definitive identification of the constituents of the probable compound in fraction samples, based on retention

time when compared with a lupeol standard that was subjected to identical HPLC conditions (17). The column used for HPLC separation was a Nucleosil C18 column (Supelco, 5µm, 250mm× 4.6 mm i.d.). Detection of the mobile phase was acetonitrile - water (20:80, v/v) was employed as the mobile phase in isocratic mode at a flow rate of 1 mL/min, with an injection volume of 100 µL. Detection was wavelength of 260 nm.

Fourier transform infrared spectroscopy analysis

Fourier transform infrared spectroscopy stands out for its simplicity, economical nature and the lack of rigorous sample preparation procedures or the need for supplementary chemicals. Conversely, this technique often necessitates the employment of a spectral library or the expertise of a knowledgeable user for the interpretation of spectra, as each compound exhibits distinct and unique patterns of spectroscopic bands (18). The fundamental principle underlying this technique entails that the absorption of infrared (IR) radiation by a specific type of sample (whether chemical, microbiological, etc.) results in modifications of the vibrational modes of the chemical bonds present within the sample (19).

Cell culture

The mammary carcinoma cell line (AMJ13) has been derived from a female Iraqi patient diagnosed with breast neoplasm. This specific cell line was derived from the primary tumor of a 70-year-old Iraqi female patient, whose histological assessment confirmed the presence of infiltrating ductal carcinoma (20). The cell line was maintained in Minimum Essential Medium (MEM) (US Biological, USA), augmented with 10 % (v/v) Fetal Bovine Serum (FBS) (Capricorn-Scientific, Germany), alongside 100 IU of penicillin and 100 µg of streptomycin (Capricorn-Scientific, Germany) and was incubated in a humidified environment at 37 °C. Exponentially proliferating cells were utilized for experimental procedures (21, 22).

- **Cytotoxicity assay of n-hexane fraction**

Cell lines were inoculated at a cellular density of 10000 cells per well in a 96-well microplate (NEST Biotech, China) and subsequently incubated at 37 °C for a duration of 72 hrs until the achievement of monolayer confluence. The assessment of cytotoxicity was conducted using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Elabscience, China). The cells were subjected to a spectrum of varying concentrations (1000, 500, 250, 125, 62.5, 31.75 µg). Following 72 hrs of incubation, 28 µL of MTT dye solution (2 mg/mL) was introduced into each well. The duration of incubation was prolonged for an additional three hours. A total of 100 µL of dimethyl sulfoxide (DMSO) was introduced to each well and was allowed to incubate for 15 min. The optical density was measured at 492 nm utilizing a microplate reader. The percentage of cytotoxicity was computed employing the following formula:

$$\text{Cytotoxicity \%} = (\text{OD Control} - \text{OD Sample})/\text{OD Control} \times 100$$

where OD Control represents the mean optical density of the untreated wells and OD Sample denotes the optical density of the treated wells (23).

Statistical analysis

The obtained data were statically analyzed using Tukey's ANOVA multiple comparisons test with GraphPad Prism 8. The results were expressed as the mean ± SD of triplicate measurements (23, 24).

Results and Discussion

Gas Chromatography-Mass Spectrometer (GC-MS)

Phytochemical yield of 200 g of Senecio rowleyanus using n-hexane solvent was 8 g of pale-yellow color extract, with pleasant aromatic odor. This extract is rich in different bioactive compound according to GC-MS result, the most abundant compound found in n-hexane extract is beta and gamma. -sitosterol (17 %), followed by spathulenol (13 %) and alpha-amyrin (9 %); and a lot of other bioactive compound that is found in small percent as show in Table 1 and Fig. 1.

Combiflash separation of lupeol

This method was selected based on previous studies and involved the use of flash chromatography-a fast and efficient purification technique that utilizes pressurized gas (50–200 psi) and fine silica gel particles. It was applied to isolate bioactive compounds from the n-hexane fraction of *Senecio rowleyanus*. Compared to traditional gravity-fed column chromatography, flash chromatography offers several advantages, including quicker separation, lower solvent consumption and higher chemical yields. An isocratic solvent system of n-hexane: ethyl acetate (90:10) was used for the elution, as shown in Fig. 2. The collected fractions were analyzed using Thin-Layer Chromatography (TLC). One of the fractions, labeled compound B, produced a distinct spot with an Rf value of 0.397, which closely matched that of the lupeol standard - suggesting that the isolated compound is likely lupeol. Additional identification methods were employed to confirm the identity of compound B. The successful isolation and confirmation of lupeol from *S. rowleyanus* using

Table 1. GS-MS result of *Senecio rowleyanus*

Compound name	Similarity index	Retention time in minute	Peak area %	Molecular weight g/mol	Chemical formula
Alfa-Copaene	99	10.790	1.92	204.35	$C_{15}H_{24}$
Alloaromadendrene	97	12.339	0.39	204.35	$C_{15}H_{24}$
Alpha-Muurolene	98	13.006	1.01	204.35	$C_{15}H_{24}$
(-)-Spathulenol	91	14.555	13.30	220.35	$C_{15}H_{24}O$
Squalene	90	29.252	1.41	410.7	$C_{30}H_{50}$
Stigmasterol	99	35.942	0.48	412.7	$C_{29}H_{48}O$
Gamma-Sitosterol	96	36.531	17.52	414.7	$C_{29}H_{50}O$
Beta-Sitosterol	98	36.531	17.52	414.7	$C_{29}H_{50}O$
Alpha-Amyrin	90	37.388	9.11	426.7	$C_{30}H_{50}O$
Lupeol	99	39.863	0.53	426.7	$C_{30}H_{50}O$

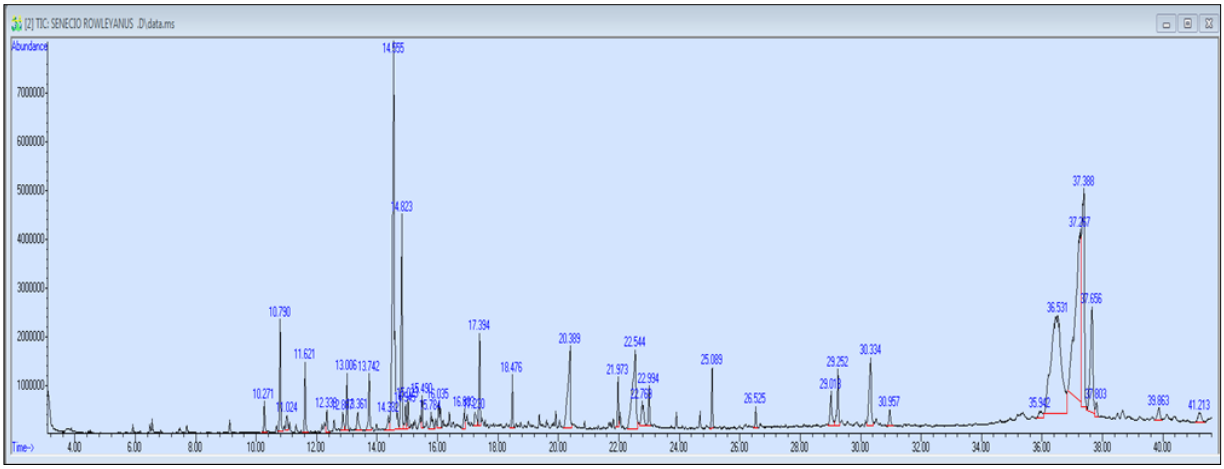


Fig. 1. GC-MS of n-hexane Extract of *Senecio rowleyanus.*

automated flash chromatography highlights the reliability and efficiency of this method, supporting findings from earlier research (25).

Identification of combi-flash column isolated lupeol by:

1-spiking method by HPLC

The isolated fraction B, obtained from the n-hexane extract, underwent analytical assessment after the introduction of a lupeol standard, which, upon evaluation via HPLC chromatography, manifested a distinct and pronounced peak that aligned with the retention time specified in Fig. 3 and Table 2.

2- Fourier transform infrared spectroscopy for the isolated compound

The infrared spectral analysis and the distinctive infrared absorption bands of the isolated compound B produced findings that were congruent with those of the standard lupeol, as illustrated in the corresponding Fig. 4 and Table 3.

Cytotoxic effect of n-hexane fraction

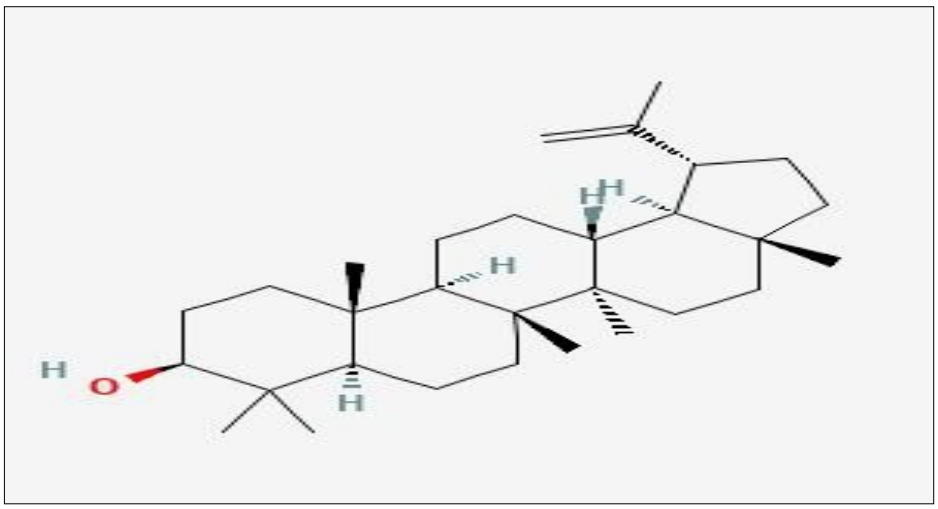
n-Hexane is a non-polar solvent commonly used to extract lipid-soluble compounds due to the principle of "like dissolves like." This property makes it particularly effective in isolating hydrophobic bioactive compounds such as lupeol, β -sitosterol, γ-sitosterol, α-amyrin and various essential oils (26, 27). These compounds are widely recognized for their cytotoxic properties against various cancer types, including breast cancer (28, 29). In addition, a study on *Senecio rowleyanus* cultivated in Egypt investigated the plant's essential oils extracted using n-hexane. The extract was tested against brain and liver cancer cell lines and the findings revealed a strong anticancer effect, further highlighting its potential as a natural source for cancer treatment (10). Among the bioactive constituents, lupeol has been the subject of extensive research for its antitumor activities. It has demonstrated the ability to induce apoptosis, inhibit cell proliferation and reduce migration and invasion of

Table 2. Retention time result of isolated lupeol and lupeol standard and spiking the fraction with lupeol standard

Standard used	Rt of standard peak (min)	Rt of isolated lupeol (min)	Rt of isolated B spiked with stander
Lupeol	4.00	4.05	4.08

Table 3. Characteristic FT-IR absorption bands (cm^{-1}) of the isolated lupeol

Functional group	Frequency wave number (cm^{-1})	Main attributed
Alcoholic O-H	3342.0	O-H Stretching vibration
C-H	3075.8	Alkene C-H stretching
C-H	2942.9	Alkane C-H stretching
C-H	2352.5	Alkane C-H stretching
C=C	1633.6	Alkene C=C stretching
C-H	1452.8, 1371.9	Alkane C-H bending
C-O	1292.5, 1245.9, 1092.5	alcoholic C-O stretching



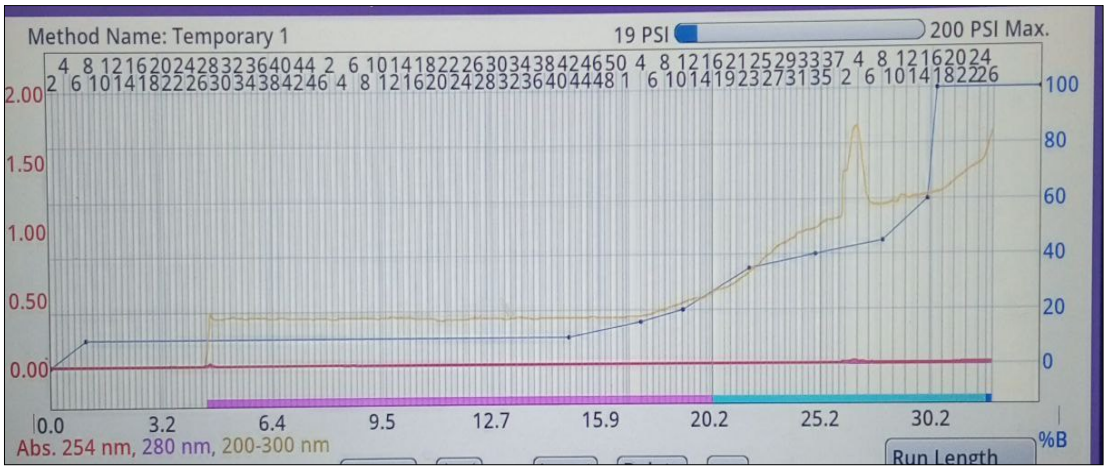


Fig. 2. Combi flash chromatogram of n-hexane fraction.

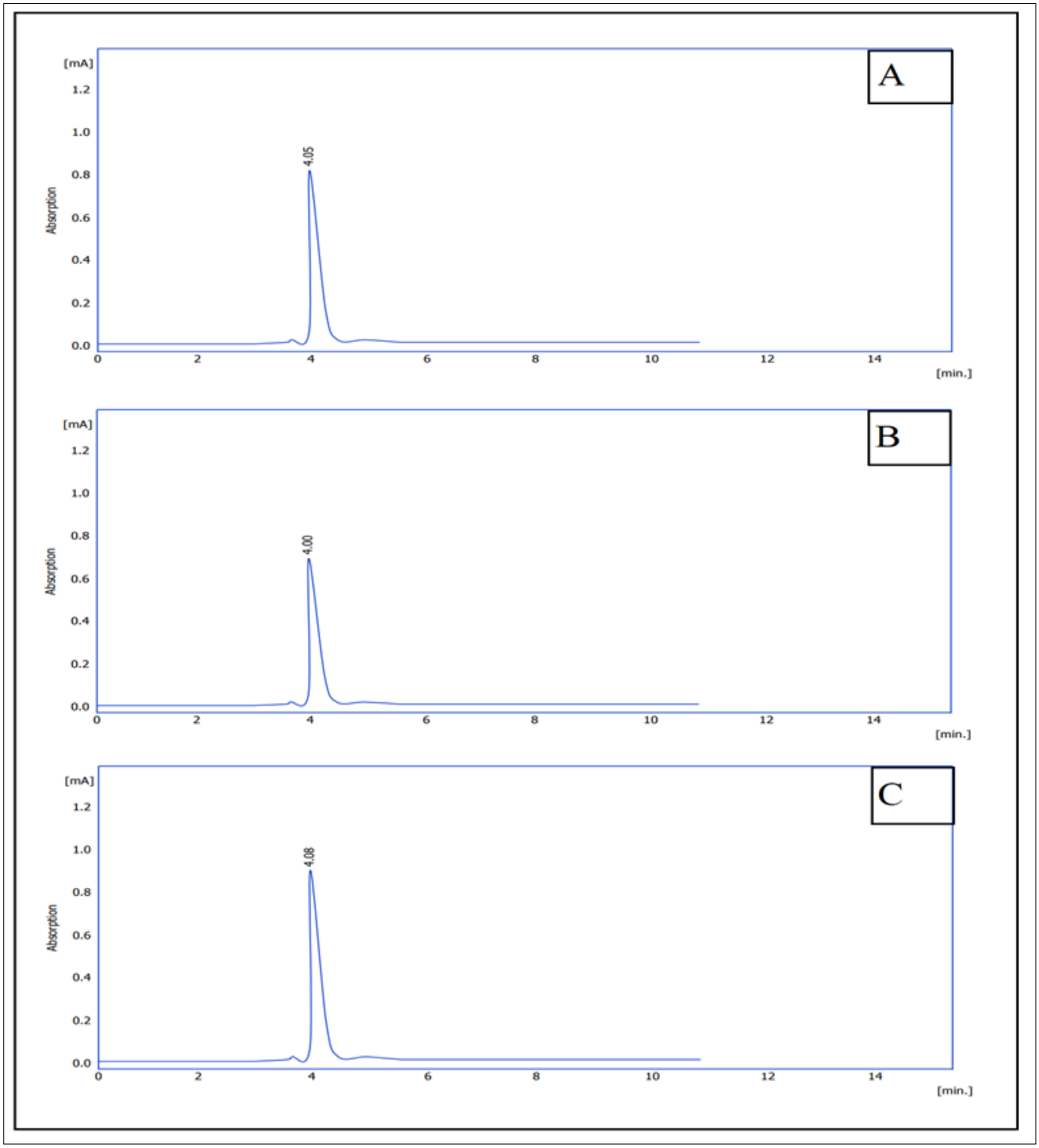


Fig. 3. HPLC chromatography of (A) the isolated lupeol compound, (B) the lupeol standard, (C) the isolated lupeol spiked with the lupeol standard.

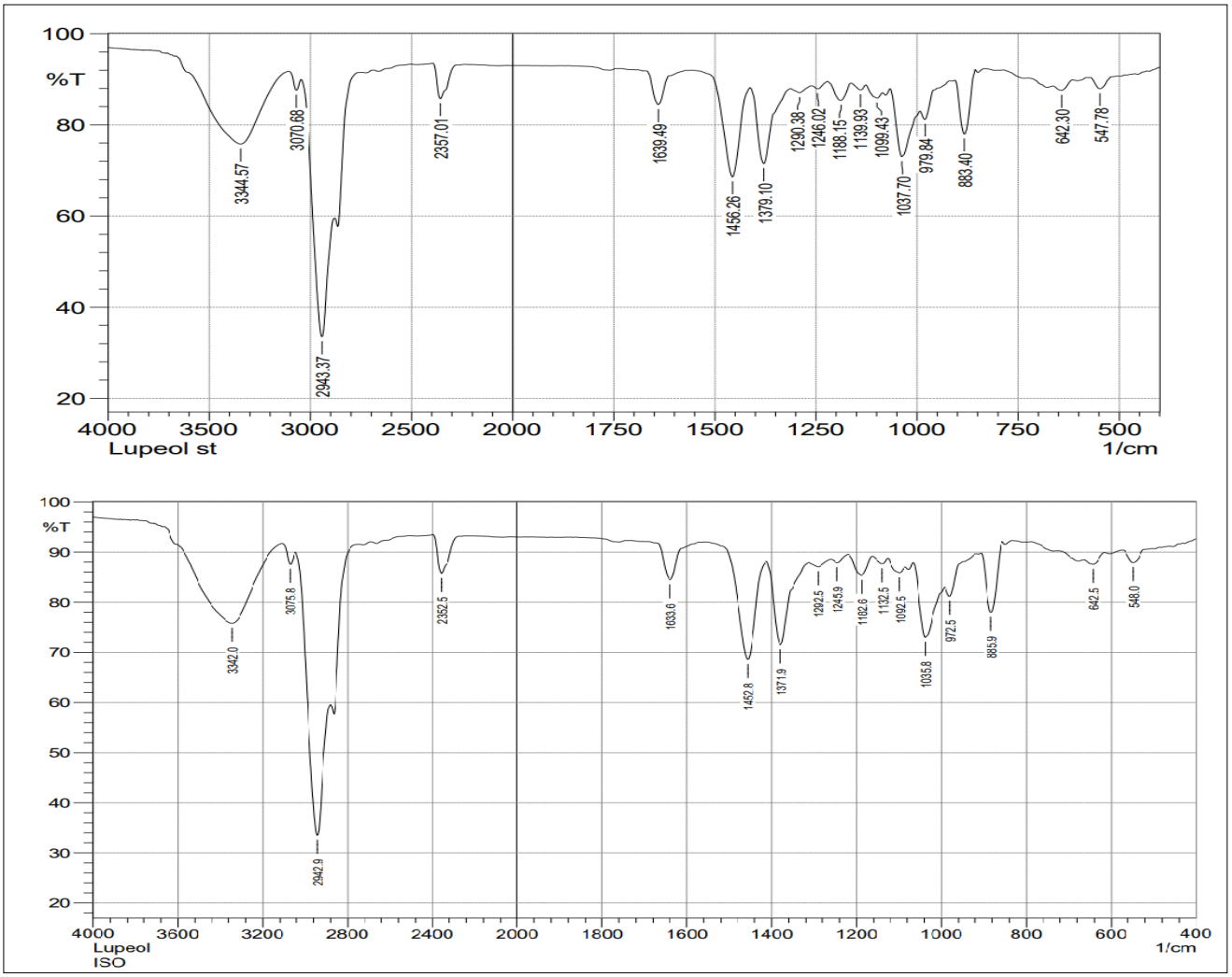


Fig. 4. FT-IR spectra of isolated lupeol and lupeol standard.

cancer cells. In breast cancer specifically, lupeol was found to induce apoptotic effects and inhibit proliferation in MCF-7 and MCF-10A cell lines (30). This study evaluates the cytotoxic effect of the n-hexane fraction of *Senecio rowleyanus* using the AMJ13 breast cancer cell line. The results, shown in Fig. 5, reveal significant cytotoxic activity in a concentration-dependent manner. The n-hexane fraction effectively limited the proliferation of breast cancer cells, with an IC_{50} value of 125.3 µg/mL. The IC_{50} (half maximal inhibitory concentration) refers to the concentration of a substance required to reduce a biological or biochemical function by 50 %. In cytotoxicity assays, it indicates the concentration at which cell viability is reduced by half compared to untreated cells, serving as a key indicator of a compound's potency. To assess the therapeutic potential of the *S. rowleyanus* n-hexane fraction, its IC_{50} value was compared with that of doxorubicin, a widely used chemotherapeutic agent. As shown in Fig. 6, the IC_{50} of doxorubicin was 111.5 µg/mL, indicating stronger cytotoxic activity than the plant extract. Nevertheless, the n-hexane fraction remains a promising candidate for anticancer therapy due to its natural origin and bioactive constituents. Fig. 7 presents visual observations of AMJ13 breast cancer cells after treatment with both the n-hexane fraction and doxorubicin, highlighting morphological changes associated with cytotoxic effects.

Conclusion

Senecio rowleyanus cultivated in Iraq contains several bioactive phytochemicals, including lupeol, β-/γ-sitosterol and α-amyrin. The use of CombiFlash column chromatography proved effective for partial purification, with lupeol being successfully isolated. Moreover, the n-hexane extract demonstrated promising cytotoxic effects against breast cancer cells, supporting its potential as a natural source of anticancer agents. Further studies are necessary to elucidate the mechanisms of action and assess the therapeutic potential. Future research should explore its effectiveness as an adjuvant therapy alongside conventional chemotherapy. For example, combining its bioactive compounds with drugs like doxorubicin may enhance cytotoxic effects against cancer cells while potentially reducing the adverse side effects commonly associated with chemotherapy.

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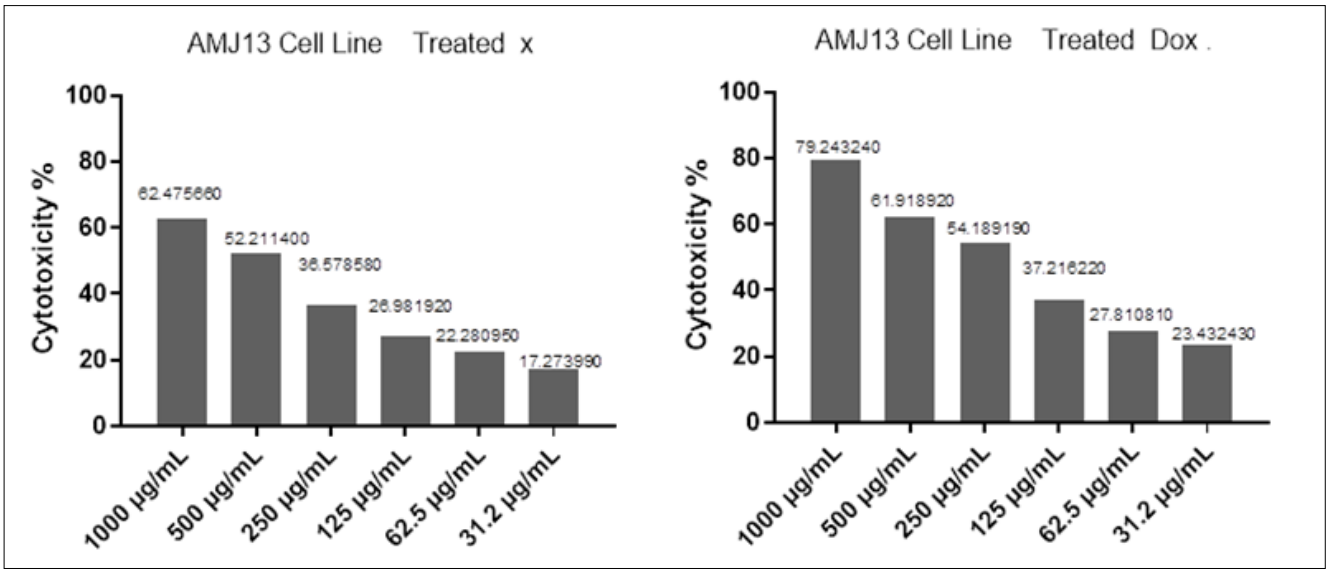

Fig. 5. Inhibitory curve showing the effect of (*S. rowleyanus* n-hexane extract in comparison with doxorubicin) on cell viability. The x-axis represents the concentration of the test compound (in µg) and the y-axis represents the percentage of cell death (cytotoxicity).

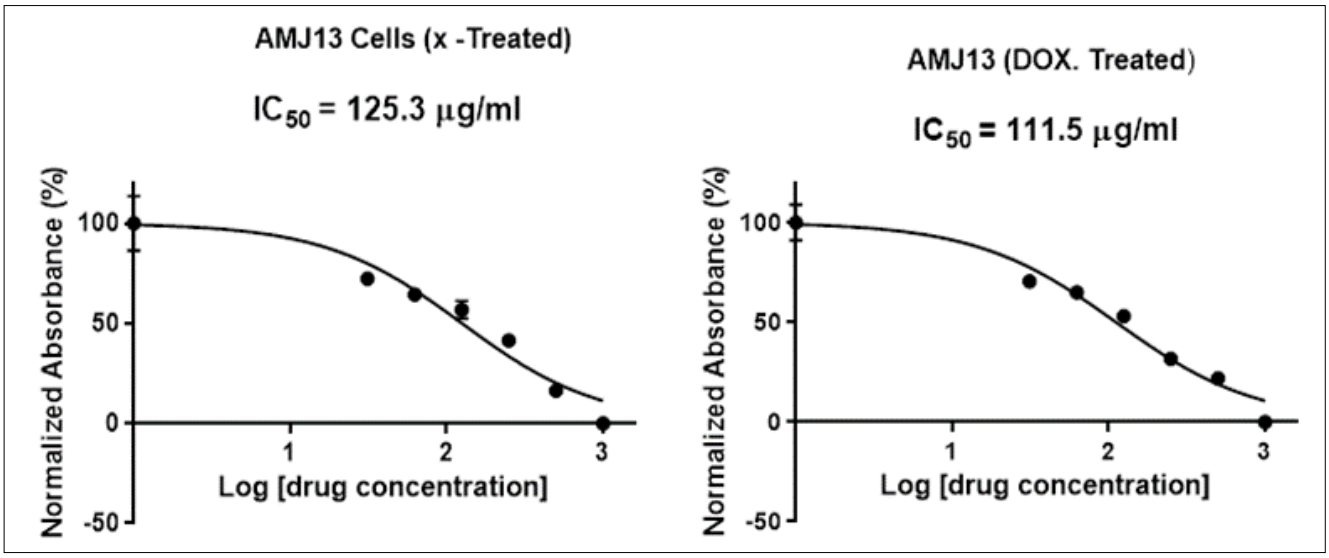

Fig. 6. Dose-response curve used to determine the IC50 value of [*S.rowleyanus* n-hexane fraction and doxorubicin] on cells. The x-axis represents the concentration of the test compound (in µg) and the y-axis represents the percentage of cell inhibition. The IC50 value, represented by the Log of drug concentration of the test n-hexane fraction that inhibits 50 % of cell viability.

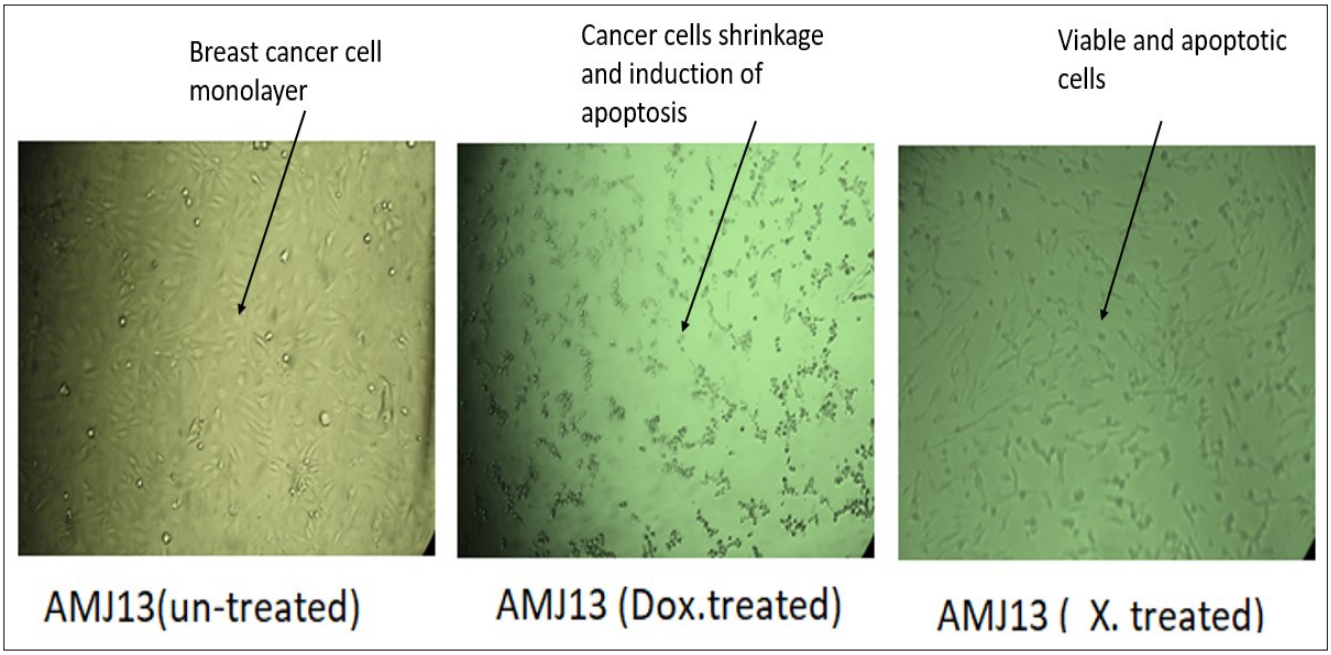

Fig. 7. Observations the effect of *Senecio rowleyanus* n-hexane fraction and doxorubicin against breast cancer cells in comparison with control (un-treated).

Authors' contributions

NAC designed the experiment, conducted laboratory work, and performed data analysis. EJK provided guidance on methodology, reviewed the results, and contributed to the final interpretation of the findings. Both authors collaborated on manuscript preparation and approved the final version.

Compliance with ethical standards

Conflict of interest: No conflicts of interest.

Ethical issues: None, since there were no human or animal studies in the publication, ethical approval is not required for this study.

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