

Lupeol: triterpene from Iraqi *Portulaca grandiflora* L (Portulacaceae): Its Extraction, identification (GC/MS), Isolation(CombiFlash), and Structure Elucidation.

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Abstract

The plant known as *Portulaca grandiflora* L belongs to the family of flowering plants known as (Portulacaceae). It is a resilient plant that grows well in harsh environments and serves as a traditional remedy in various nations. Eleven O'clock, Moss-rose, Sunplant, gaddi roja, and Neelakeera are just a few of the popular names that are used to refer to this plant across the globe. The primary concern of the present investigation was establishing the presence of lupeol as a new metabolite in the Iraqi *P. grandiflora* plant, which is triterpenes, have shown significant efficacy as anti-inflammatory, anti-cancer, anti-microbial, cholesterol-lowering, wound healing, and antidiabetic agents. 100 gm of the entire plant was subjected to a Soxhlet Apparatus (Hot method) to extract lupeol through n-hexane. 2gm of the product was the end yield. Lupeol and other phytochemicals have been identified from the normal-hexane part using the GC/MS technique: gas chromatography/mass spectrometry. The process of isolating and purifying the proposed compound included using Combi-Flash NextGen; hexane extract (0.25 g) and silica gel (4 g) were mixed and then placed into cartilage. After that, it was attached to the combi flash gold column with a solvent mixture that consisted of n-hexane: ethyl acetate (90%:10%) for a fraction. The n-hexane fraction is very fruitful due to its abundance of diverse chemicals found in plants belonging to many groups, including fatty acids, volatile oils, phytosterols, triterpenes, aldehyde compounds, terpene alcohols, isomers of vitamin E, diterpenes, alkynes, and alkenes. Identification of isolated compound and Spiking method by HPLC, the melting point, ultraviolet UV spectroscopic, TLC, and Fourier Transform Infrared (FTIR) characteristics of the amorphous powder that was successfully produced showed that it was structurally identical to lupeol. The discovery of Iraqi *P. grandiflora* as a novel natural source of lupeol opened new horizons toward this promising plant for pharmacological activities and pharmaceutical applications.

Keywords: Combi-Flash, Gas chromatography/mass spectrometry, lupeol, *Portulaca grandiflora*.

Introduction

In many countries, Plant therapy has become a cornerstone of scientific research, and the use of herbal medicine is highly growing. ⁽¹⁾ *Portulaca grandiflora* (Portulacaceae) is a small, diffuse, annual, and erect herb extensively cultivated in urban or rural settings, on sandy soil, especially in sunny areas, for its gigantic and brilliantly colored blossoms. ^(2,3) Many nations utilize *P. grandiflora* as a traditional medicine to treat skin rashes, inflammation, ulcers, stomach problems, detoxification, coughing, and urine discharge, in addition to the role in treating hepatitis, Burns, scalds, eczema, and bites from snakes. ^(4,5) The phytochemical constituent of *P. grandiflora* extract disclosed terpenoids, alkaloids, flavonoids, phenolic acids, tannins, glycosides, proteins, carbohydrates, and mucilage. ⁽⁶⁾ Beta cyanins and betaxanthins are produced by *P. grandiflora* and are stored in the stem and flower. Several different phenotypes were seen, with

petal colors varying from white to deep yellow, orange, red, magenta, and pale yellow ⁽⁷⁾ *P. grandiflora* possesses pharmacological activities such as Antimicrobial, Antioxidant, Antidiabetic, anticancer, Anti-atherosclerotic effects, Anti-Parkinson's activity ⁽⁸⁾, and antifungal ⁽⁹⁾. The safest dosage of *P. grandiflora* extract, according to scientific research, is 500 mg daily. ⁽¹⁰⁾ Terpenes are a prominent group of secondary metabolites found in plants, which are primarily composed of isoprene units, each containing five carbon atoms. These isoprene units are combined in various ways to produce a variety of terpenes, including monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), triterpenes (C30), and tetraterpenes (carotenoids, C40). ⁽¹¹⁾ The pentacyclic triterpenoid lupeol is found in a diverse range of fruits and vegetables and has a promising activity as an anti-inflammatory property, antiproliferative, antibacterial, antiproliferative, anti-angiogenic,

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anti-invasive, and cholesterol-lowering agent. Research on both animal and human models has variety of diseases and illnesses, including those associated with healing wounds, diabetes, heart disease, kidney disease, arthritis, and risk factors for heart disease.⁽¹²⁾ as illustrated in Figure 1. Little attention has been paid to exploring the phytochemical content of the 11 o'clock plant, so this study was to investigate different types of

shown that they may have biological effects on a terpenes found in the whole plant in Iraq, specifically the triterpene (lupeol). This compound has various pharmacological activities and traditional uses with potential future applications. The study aimed to extract, isolate, and determine the structure of lupeol obtained from Iraqi *P. grandiflora*

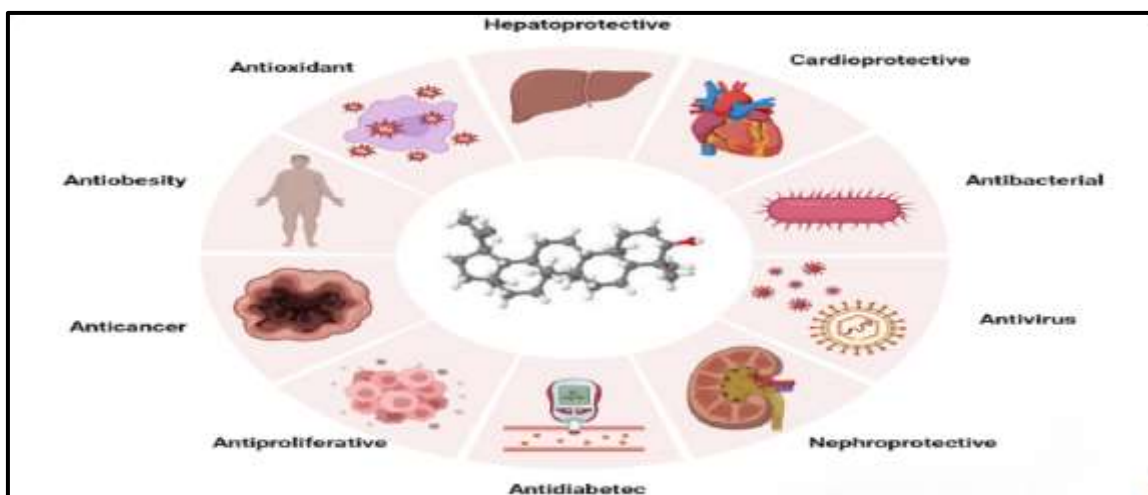


Figure1. Several pharmacological activities of lupeol (created in biorender.com)

Material and Methods

Collection and Authentication of Plant Materials

Between June and July, the whole fresh Iraqi *P. grandiflora* L plant was cultivated from Zayona plant nurseries in Baghdad, Iraq. Dr. Israa Abdul-Razaq, a taxonomist from the College of Biology /Baghdad University, successfully recognized, validated, and verified the authenticity of these newly harvested plants and registered them at BUH No. (17391). The plants underwent a process of cleaning, followed by drying in a shaded area at room temperature. Subsequently, an electrical method was used to crush the plants, and

the weight was measured just before extraction.

Extraction method (Soxhlet Apparatus)

The extraction of various phytoconstituents from *P. grandiflora* was conducted using the Soxhlet Apparatus (Figure 2). A total of 100 gm from coarsely pulverized plant material was densely packed into a thimble and then subjected to extraction using 800 ml of n-hexane at a temperature of 40 °C, and the plant was extracted completely to exhaustion. Then the extract was filtered and concentrated by a rotary evaporator and weighed^(13,14).

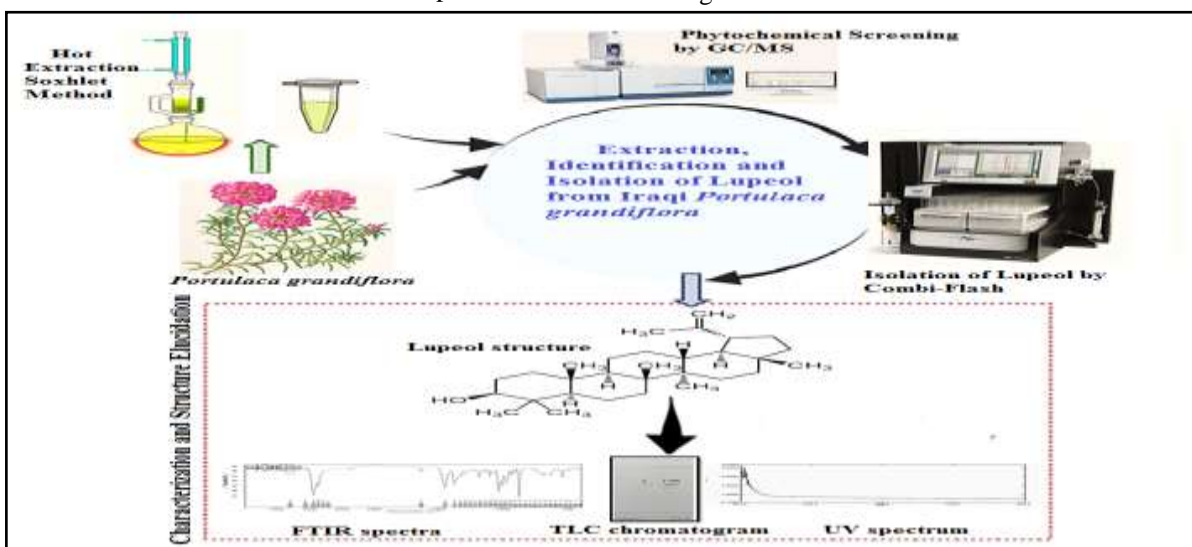


Figure 2. General scheme for extraction, phytochemical identification, isolation, and structural elucidation of proposed lupeol from Iraqi *P. grandiflora*

Preliminary Identifying phytoconstituents

The chemical tests were used to identify phytoconstituent molecules extracted from the *P. grandiflora* n-hexane fraction.

• **Salkowski analysis:** Three milliliters of strong sulfuric acid were combined with 2 milliliters of chloroform was combined with a little quantity of plant hexane extract. Oxidation caused a rusty-brown color to form.

• **Liebermann-Burchard evaluation procedure:** In 5 milliliters of chloroform, dissolve a little quantity of the plant's hexane extract, then dehydrate the chloroform layer using Sodium sulfate (anhydrous). Subsequently, this combination then combined with 10 drops of acetic anhydride and 2 drops of concentrated sulfuric acid. Oxidation of the material causes a change in color to bluish-green, indicating the existence of the steroidal nucleus. ⁽¹⁵⁾

• **Foam test:** plant extract was diluted with 10 ml of distilled water and was agitated in a test tube for

15 seconds and if standing for 15 minutes, indicate the presence of steroidal and triterpene glycoside saponin. ⁽¹⁵⁾

• **Filter paper test:** a small quantity fraction of the plant was individually compressed between two Filter papers and a permanent spot of oil indicated the presence of Fixed oils. ⁽¹⁶⁾

Phytochemical screening of *Portulaca grandiflora* by using GC/MS

Gas chromatography-mass spectrometry was used to examine the n-hexane fraction at the Iraqi Ibn Al-Bitar Centre in Baghdad under the Ministry of Industry and Minerals. By comparing the mass spectra to those in the library, the presence of lupeol in the n-hexane portion of *P. grandiflora* can be determined. Lupeol quantity is determined by dividing the relative peak area by the total peak area. The GC/MS parameters used for the study are shown in Table (1) ⁽¹⁷⁾.

Table 1. GC/MS parameters were used for the study.

Device	Gas chromatography/mass spectrometry instrument from Agilent Technologies, USA (7820A) (5977E)
Column for Analysis	The Agilent HP-5ms Ultra Inert has measures of 30 meters in length, 250 micrometers in inner diameter, and 0.25 micrometers in film thickness.
Volume of injection	1µl
Pressure	11.933 psi
Temperature	GC Inlet Line Temperature: 250 °C Aux heaters Temperature 300 °C
Type of Injection	Splitless
Scan Range	m/z 25-1000
Injector Temperature	250 °C
Carrier Gas	Helium 99.99%
Temperature Programs for Oven	Temperature Ramp 1: 60 °C hold to 3 min. Ramp 2: 60 C to 180 °C 7 min Ramp 3 :180°C to 280°C 8 min Ramp 4: 280°C hold to 5 min.

Isolation of the proposed lupeol from *Portulaca grandiflora* using column chromatography (combi flash NEXTGEN).

The flash chromatography technique of purification is simple and needs development of a little method. Excellent productivity may be achieved via process automation using the Teledyne ISCO Combi-Flash NextGen flash chromatography instrument, generate gradients with customized settings, and achieve peak separation using U.V. light. Gas-pressure solvent

reservoirs may expedite solvent flow, allowing separations to be performed more rapidly compared to traditional gravity-based column chromatography. Based on the spots observed on the thin-layer chromatography (TLC) after using the appropriate solvent system (20% ethyl acetate and 80% n-hexane v/v), a mixture of 0.25 g of hexane extract and 4 g of silica gel is prepared. This silica gel sample is then placed into a vacant cartridge, covered with a frit, and packed onto the Combi-Flash system. The Combi-Flash system

uses a mixture of solvents consisting of 10% ethyl acetate and 90% n-hexane (volume/volume). The flow rate is 40 mL per minute; 100 test tubes were used (16×160mm), Equilibration Volume: 168.8 ml, Initial Waste: 0.0 ml, Air Purge: 1.0 min, Peak Tube Volume: Max. Non-Peak Tube Volume: Max. Loading Type: Solid Wavelength 1 (red): 254nm, Peak Width: 1 min, Threshold: 0.20 AU Wavelength 2 (purple): 280nm, All Wavelength (orange): 200-300nm, Peak Width: 1 min Threshold: 0.20 AU and using the Lupeol standard, each collected fraction was spotted on a TLC plate.⁽¹⁸⁾

Characterization and structural determination of the isolated proposed lupeol (H1)

The separated white crystal component was subjected to further identification procedures to verify its integrity. The following methods were used for this purpose:

Identification of isolated compound H1 and Spiking method by HPLC

The isolated (H1) from n-hexane fraction of Iraqi *P. grandiflora* identified by HPLC with model SYKAMN (Germany) Fraction collector model = FOXY R1 and Autosampler model = S 5200, C18-ODS (25 cm * 4.6 mm) column and acetonitrile: DW: acetic acid (60: 25: 5) as mobile phase. The isocratic elution was carried out for 10 min at a flow rate of 1ml/min, injection volume was 0.1ml detection was recorded with a UV detector at 210 nm.⁽¹⁹⁾

Thin layer chromatography

Utilizing a thin layer chromatogram and silica gel as GF₂₅₄nm a stationary phase, the separated component was analyzed. Hexane and ethyl acetate were mixed in an 8:2 ratio to form the mobile phase. After spraying the specimens with a 5% H₂SO₄ reagent, they were heated to 110°C for 5-10 minutes to enable visualization.⁽²⁰⁾

Melting point (M.P.)

A material's melting point is the precise temperature at which it changes phase from solid to liquid; at the melting point, the solid and liquid phases are in equilibrium. Used to identify unknown compounds by comparing the melting points of isolated constituents to the melting point of its standard.⁽²¹⁾ The Electrical Melting point apparatus (Stuart, England) was used for the measurement.

Fourier transforms infrared (FT-IR) spectra.

Fourier transform infrared spectroscopy is a method used to analyze how a molecule interacts with light in the infrared portion of the electromagnetic spectrum, specifically in the range of 4000 to 400 cm⁻¹.⁽²²⁾ The instrument used for the measurement is found at the Environmental and Water Research Department/Ministry of Sciences and Technology, and the structural assignments

were correlated for distinctive bands by attenuated total reflection (ATR) technique.

Ultraviolet (UV)-visible spectrophotometry:

Ultraviolet spectrophotometry is measured by a computerized ultraviolet spectrophotometer using methanol as a reference/standard, and the model is SHIMADUZU, with a range of 1900-4000 nm. Used to identify the isolated constituent (H1).⁽²³⁾

Results and Discussion

Despite the fact that *P. grandiflora* is an ornamental and therapeutic plant cultivated in Iraq, it contains many important secondary metabolites, beta-xanthine, phenolic acids, and flavonoids that were taken in previous studies.^(6,7) Thus, this is the first study in Iraq on selected terpene compounds, particularly the triterpenoid (lupeol), for its various pharmacological effects. The whole plant was extracted using the Soxhlet process, which relies on heat to facilitate the penetration of solvent through the plant powder. The selection of this strategy was based on the fact that *P. grandiflora* has a great tolerance to elevated temperatures.^(2,3) The specific method of extraction is determined by several criteria, such as the consistency and moisture level of extracting plant material, as well as the desired chemical to be separated. So, the determination of an optimal technique of extraction is essential and, in some instances, relies on extract-specific use. To maximize the extraction yield which gives 2 gm from 100 gm of the plant, the use of n-hexane, a nonpolar solvent, is very effective for extracting nonpolar components, particularly lupeol.⁽¹⁴⁾

Preliminary phytochemical investigation

Chemical analyses were used to determine the presence of steroids, terpenes, saponin, and fixed oil compounds in *P. grandiflora*, the results are shown in Table 2

Table 2. Findings from the hexane fraction chemical tests

Name of tests on n-hexane fraction	results
Liebermann Burchard reaction	+
Salkowski reaction	+
Foam test	+
Filter paper test	+

Gas chromatography/mass spectrometry (GC / MS) analysis

The GC-MS chromatogram of the n-hexane extract of *P. grandiflora* exhibits varying numbers of peaks, each representing distinct chemical components. The molecular formula, molecular weight, and peak area are the three basic tools used to identify phytochemical substances; nevertheless,

because of a lack of authentic samples and undetected. ⁽²⁴⁾ Lupeol is one of the recognized chemicals, determined by comparing its mass

pertinent library data, some GC-MS peaks went spectra with library spectra. Figure 3 and Table 3 provide a summary of these detected constituents.

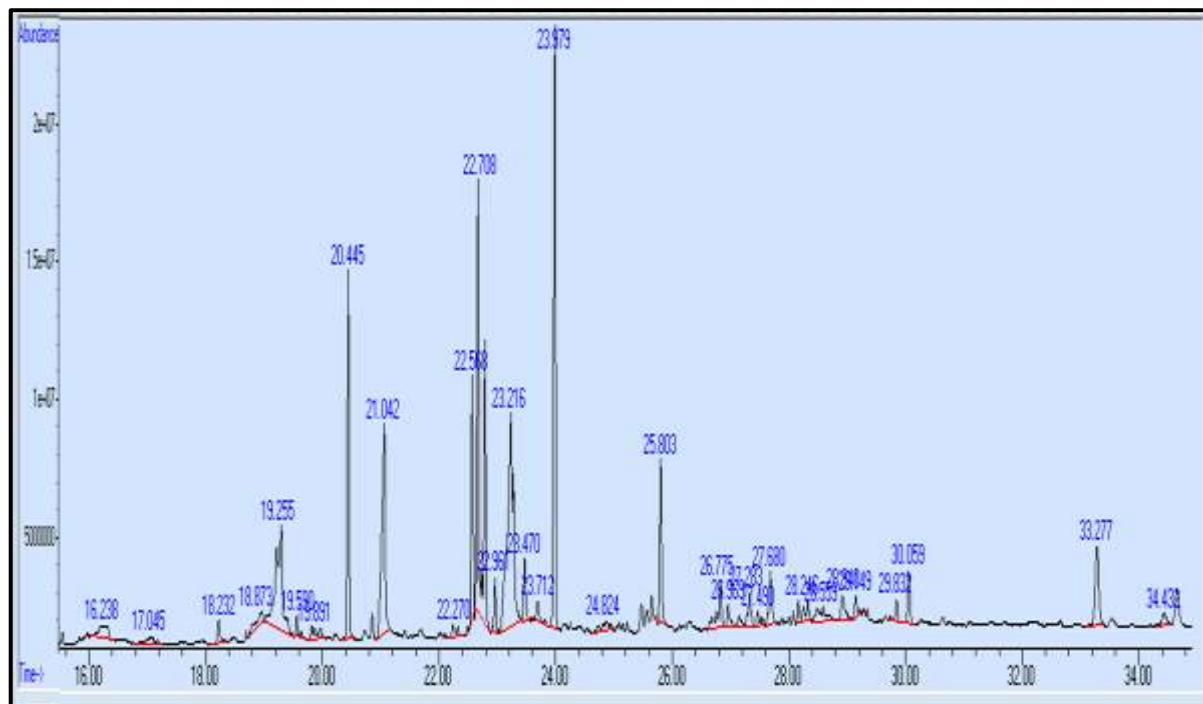


Figure 3. Examining the normal-hexane extract with GC-MS of Iraqi *Portulaca Grandiflora*

Table 3. Phytochemical substances have been found in the n-hexane extract of Iraqi *P. grandiflora*.

No. of peak	Name of compound	Chemical classes	R.T	Similarity index	Pharmacological activity
1	1-Hexadecanol	Terpene Alcohol	16.241	90	antioxidant activity. ⁽²⁵⁾
3	Tetradecanoic acid	Myristic acid, Saturated fatty acids	18.232	95	anti-oxidants, nematicide, hypocholesterolemic, cancer-preventive, and lubricant. ⁽²⁶⁾
4	9,12-Octadecadienoic acid (Z,Z), Linolenic acid.	Saturated fatty acids	18.872	99	protects the liver, reduces inflammation, wards against cancer, kills nematodes, alleviates allergies, and is antieczemic. ⁽²⁷⁾
5	9-Octadecenoic acid, (E)-	monounsaturated omega-9 fatty acid. (Elaidic Acid)	19.255	95	anticancer, antitumor, arachidonic acid-inhibitor. ⁽²⁸⁾
7	N-Hexadecanoic acid	palmitic acid, Saturated fatty acids	19.893	91	potent inhibitory activity against both Gram-positive and Gram-negative bacteria. ⁽²⁹⁾
8	Hexadecanoic acid, methyl ester	linoleic acid, esters	20.4475	97	possessing anti-inflammatory, cancer-preventive, liver-protective, antiarthritic, and anti-coronary effects. ⁽²⁷⁾

9	Pentadecanoic acid	Saturated fatty acids	21.042	93	antioxidant activity. ⁽²⁵⁾
10	cis-Vaccenic acid	omega 7 fatty acids	22.274	90	anti-carcinogenic effect. ⁽³⁰⁾
11	9,12-Octa decanoic acid, methyl ester	fatty acid methyl ester of linoleic acid.	22.568	98	analgesic, anti-inflammatory, and ulcerogenic properties. ⁽²⁷⁾
12	9-Eicosyne	terminal alkynes.	22.706	90	Anti-microbial and cytotoxic properties ⁽³¹⁾
14	lupeol	triterpene	23.216	91	Anti-inflammatory, anticancer. ⁽²⁷⁾
17	Phytol	Diterpene	23.979	91	Use in vaccine formulae: anti-inflammatory, antioxidant, anticancer, diuretic. ⁽²⁷⁾
19	cis-9Hexadecenal	C16 monounsaturated fatty aldehydes	25.805	96	antimicrobial and anti-inflammatory properties. ⁽³²⁾
20	13-Octadecenal, (Z)-	Long-chain unsaturated aldehyde	26.774	90	antioxidant, anti-inflammatory. ⁽³³⁾
25	Oleic Acid	(Z)-9-Octadecenoic acid, Fatty acid	28.246	90	Anticancer, Anemiagenic, Insectifuge, Antiandrogenic, Dermatitigenic. ⁽²⁸⁾
26	9-Octadecenal(Z)	Aldehyde compound	28.557	95	Antimicrobial. ⁽³⁴⁾
29	Squalene	triterpene	29.830	93	antioxidant properties, oxygen-scavenging agent. ⁽³⁵⁾
31	GammaTocopherol	lipid-soluble (Vit. E)	33.275	98	Antioxidant. ⁽³⁵⁾

Isolation of proposed detected lupeol by Combiflash NEXTGEN column chromatography

Flash chromatography is a purification method that enables quick separation by using air pressure instead of slower and less effective gravity-fed chromatography. It deviates from the traditional column method by using somewhat smaller silica gel particles and pressured gas ranging from 50 to 200 psi, among the many benefits of this approach are its rapid isolation time, minimal solvent use, and excellent chemical yields. ⁽²⁰⁾ The tubes collected from the Automated flash chromatography system for the n-hexane

fraction of *P. grandiflora* after being eluted successively with an isocratic solvent, (hexane: ethyl acetate (90:10)). After spotting all tubes on TLC plates, the results showed that TLC plate no.2 (tubes numbered 20-30) gave a spot that was similar to that of the Lupeol standard when checked by TLC, as shown in Figure 4, and Table 4. A component of the entire plant of *P. grandiflora* was successfully isolated using a two-step Combiflash chromatographic technique (Figure 5). Then the Isolated (H1) was identified by using different Identification methods.

Table 4. The *R_f* values of the isolated compound (H1) from the n-hexane fraction of Iraqi *P. grandiflora*.

mobile phase	No. of tubes that contain isolated (H1)	<i>R_f</i> of isolated compound (H1)	<i>R_f</i> of lupeol standard
hexane: ethyl acetate (90:10)	Tube 20 to 30	0	0.589



Figure 4. The hexane fraction produced from Combi-flash column chromatography employing hexane: ethyl acetate (8:2) mobile phase was analyzed using thin-layer chromatography. The detection of the compounds in the hexane fraction was done using a 5% H_2SO_4 spray reagent, Subjected to a temperature of $110^\circ C$ for a duration of 10 minutes.

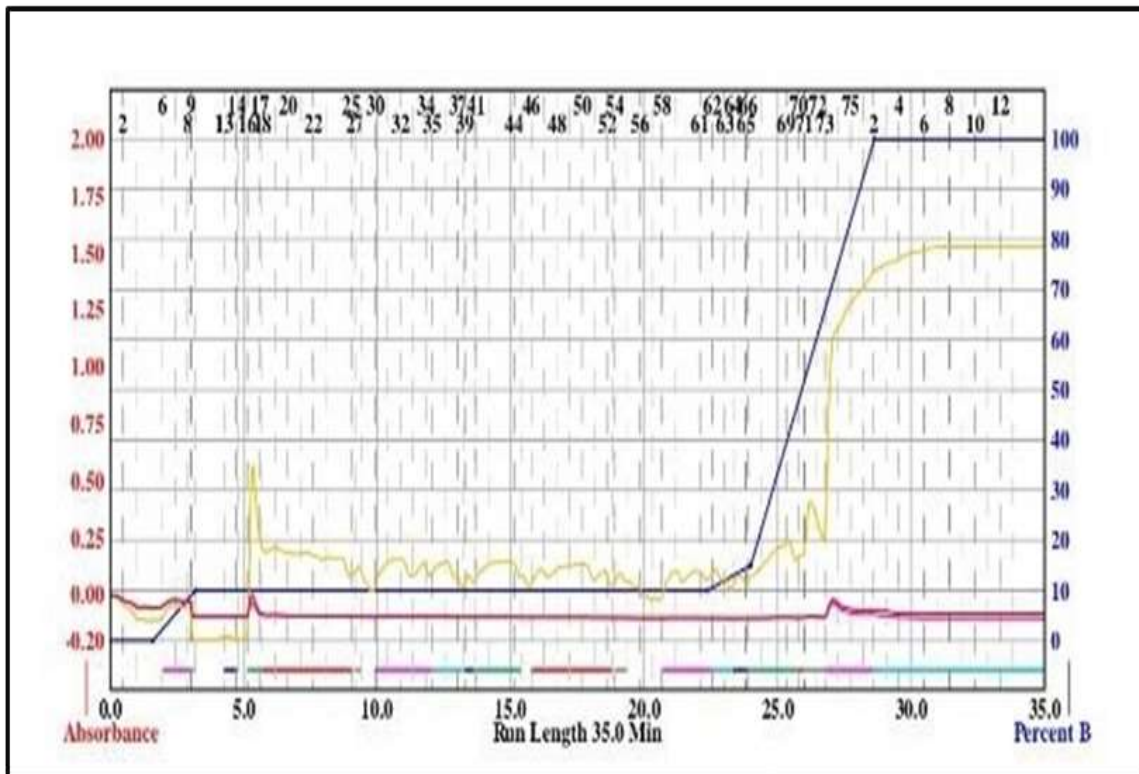


Figure 5. Combi-flash chromatogram of n-hexane fraction.

Identification and characterization of the isolated compound

Identification of isolated compound H1 and Spiking method by HPLC:

The qualitative identification was done by comparing the retention times obtained under

identical chromatographic conditions of the isolated (H1) and the authentic standard (lupeol) as in (Table 5). Then the isolated (H1) was spiked with lupeol standard the HPLC chromatogram showed a high and sharp peak as in (Figure 6).

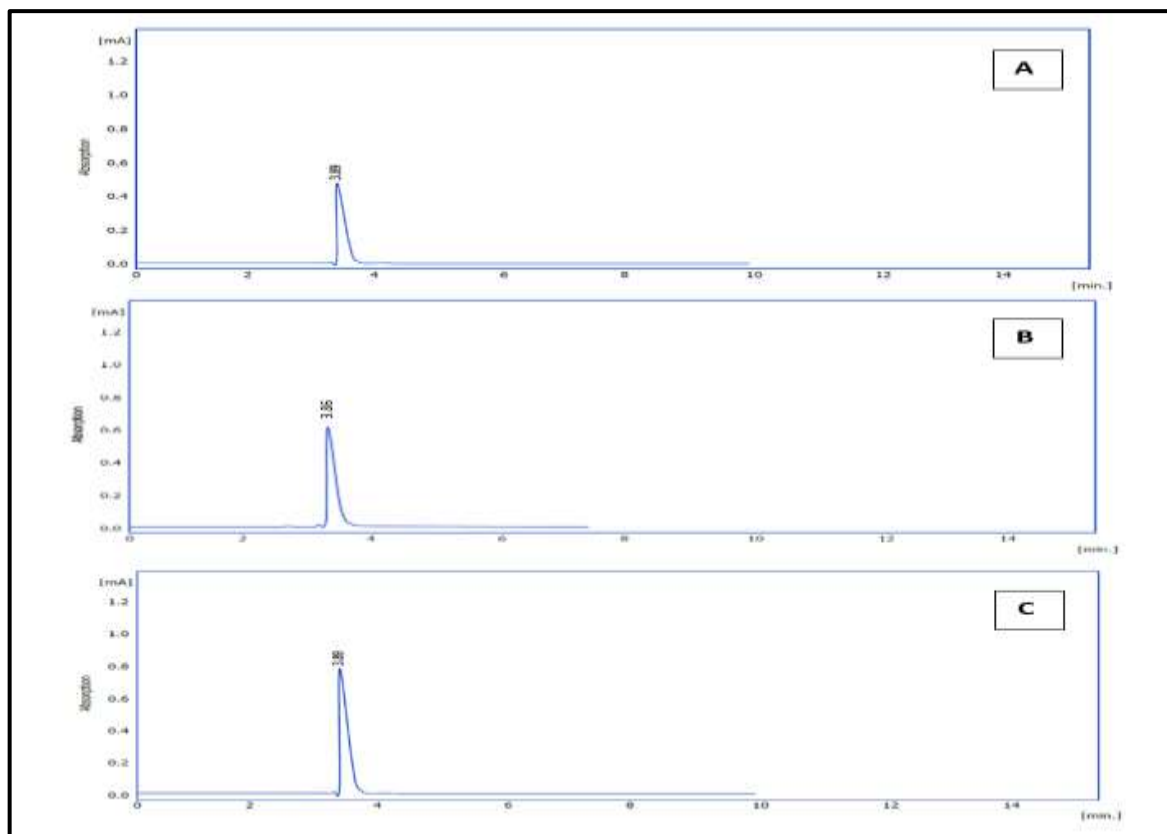


Figure 6. HPLC chromatogram of (A) isolated (H1), (B) Lupeol standard, (C) isolated H1 spiked by Lupeol standard.

Thin layer chromatography

The *R_f* value was compared with its standard (St) after a TLC chromatogram was

prepared for an isolated chemical. Figure 7 shows that the results demonstrated a matching between both, and their *R_f* values are identical at 0.589.



Figure 7. Thin layer chromatogram for isolated compound (H1), with lupeol std. Standard and the Corresponding Detected Compound H1.

Table 5. Retention Times (in minutes) of Lupeol

Standard used	Rt of Standards peaks (min)	Rt of isolates H1(min)
Lupeol	3.86	3.89

Melting point (M.P.)

An important physical characteristic that provides information about the sample and its purity is its melting point. Standard Lupeol had a melting point of (215-216) °C, whereas the isolated component had a value of (214-215) °C. ⁽³⁶⁾

FT-IR spectra for the isolated compound H1

It is most widely used in phytochemical investigations as a fingerprinting tool for contrasting the artificial reference standard with a

natural FT-IR spectroscopy. Figure (8) and Table(6) demonstrate that when compared to conventional lupeol ⁽³⁷⁾, the IR spectra and distinctive IR absorption bands of the separated compounds yielded equivalent findings.

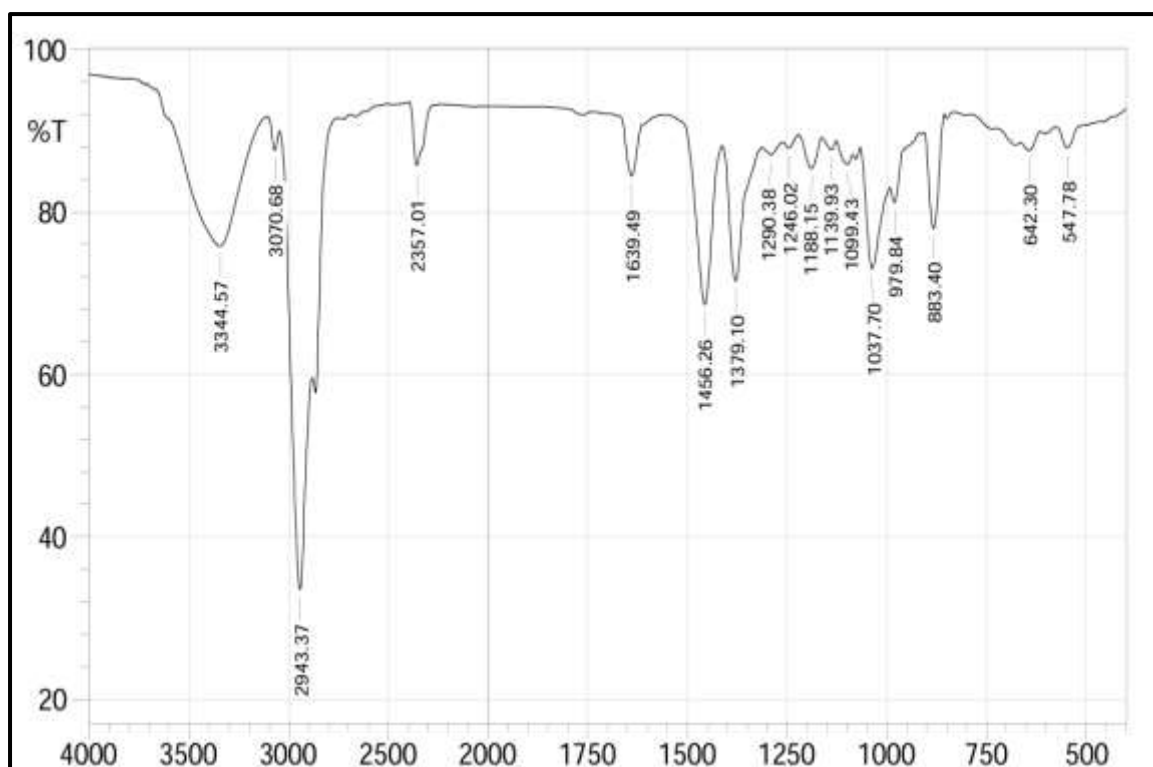


Figure 8. FTIR spectra of isolated compound H1

Table 6. Characteristic FTIR absorption bands (cm-1) of the isolated compound and Lupeol standard. ⁽³⁷⁾

Functional Group	Frequency Wave number(cm-1)	Main attributed
O-H	3344.57	stretching vibration of alcoholic O-H
C-H	3070.68	stretching vibration of C-H alkene
C-H	2943.37	stretching vibration of C-H alkane
C-H	2900, 2845	stretching vibration of C-H alkane
C=C	1639.49	stretching vibration of C=C alkene
C-H	1456.26, 1379.84	bending vibration of C-H alkane
C-O	1290.38, 1246.02, 1099.43	stretching vibration of alcoholic C-O

UV/Vis-spectra

The isolated ingredient, when separated, exhibited a spectrum that was identical to the

lupeol standard, with both having the same lambda max of 210 nm. This similarity was seen under the same conditions ⁽³⁸⁾, as shown in Figure 9.

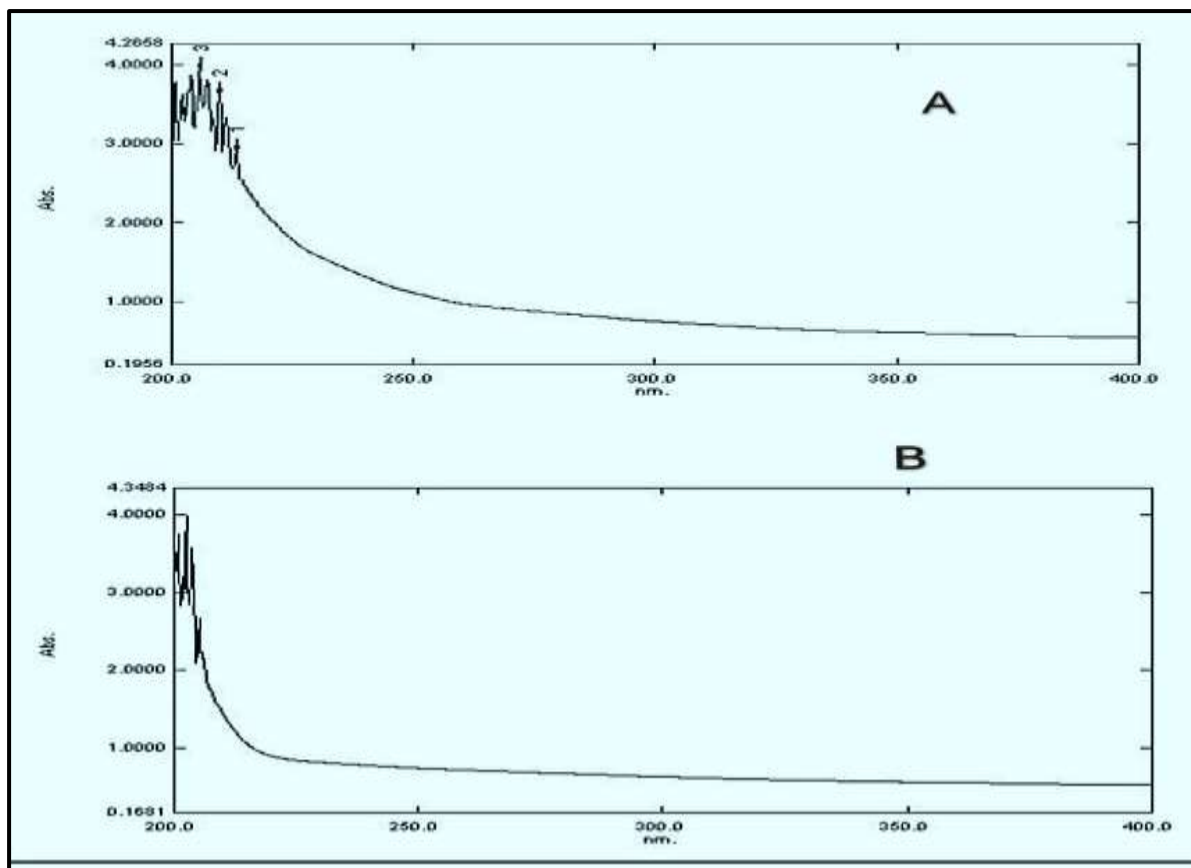


Figure 9. UV spectra of A= isolated H1, and B= Lupeol standard

All the above-mentioned data from several methods of analysis revealed that the isolated compound had coincidental features that exactly meet those for the lupeol standard, which supports the isolated compound might be lupeol.

Conclusion

Lupeol had little toxicity on healthy cells and showed synergistic effects when used in combination treatments. This highlights its potential for usage as a standalone treatment or as an adjunct to existing therapeutic approaches. An uncomplicated and straightforward technique for extraction and identification (GC/MS), together with a quick and efficient approach for isolating and purifying the primary plant terpene from *P. grandiflora*, was executed. This approach has the potential to provide chemicals that may be used as references for future investigations on the chemical properties of plants. As far as we know, this is the first documentation of the isolation of Lupeol from *P. grandiflora* using the flash column technique in Iraq. The separated ingredient was satisfactorily characterized, and its structure was elucidated using methods such as HPLC, spiking method by HPLC, melting point analysis, UV spectroscopy, TLC, and FTIR spectroscopy.

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Conflicts of Interest

There is no conflict of interest regarding the publication of my manuscript.

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Ethics Statements

The manuscript did not include human and/or animal studies, so ethical approval is unnecessary for this research.

Author Contribution

The authors conceived and planned the experiments and carried out the sample preparation, extraction process, identification, isolation, and Structure elucidation. The authors also wrote the manuscript.

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استخلاص اللوبيول ترايترين من النبات العراقي بورتولاكا جرانديفلورا واجراء الفحص الكيميائي بواسطة كروماتوغرافيا الغاز/قياس الطيف الكتلي وفصله بواسطة الفلاش كروماتوغرافي وتوضيح تركيبه

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الخلاصة

ينتمي النبات المعروف باسم (بورتولاكا جرانديفلورا) إلى عائلة النباتات الزهرية المعروفة باسم (بورتولاكاسيا) . هو عبارة عن نبات مرين ينمو جيداً في البيئات القاسية ويعمل كعلاج تقليدي في مختلف الدول. ورد الصبح، ورد الحادي عشر، ورد الشمس، جادي روجا، نيلاكيرا ليست سوى عدد قليل من الأسماء الشائعة التي تستخدم للإشارة إلى هذا النبات في جميع أنحاء العالم. كان الاهتمام الرئيسي في البحث الحالي هو إثبات وجود اللوبيول كمستقلب جديد في نبات البرتولاكا جرانديفلورا العراقي، وهو ترايترين، وقد أظهر فعالية كبيرة كمضاد للالتهابات، ومضاد للسرطان، ومضاد للميكروبات، وخافض للكوليسترول، ومضاد لمرض السكر. ومخفف للجروح. تم إخضاع مائة جرام من النبات بأكمله لجهاز سوكلت (الطريقة الساخنة) لاستخلاص اللوبيول باستخدام محلول النورمال هكسان . وكان غرامان من المنتج هو العائد النهائي. تم التعرف على اللوبيول والمواد الكيميائية النباتية الأخرى من جزء الهكسان المستخلص من النبات باستخدام تقنية كروماتوغرافيا الغاز/قياس الطيف الكتلي. وشملت عملية عزل وتنقية اللوبيول باستخدام كومي فلاش نيكستجين حيث تم اخذ (٠,٢٥غم) من مستخلص الهكسان وخلطها مع (٤ غم) من السيليكا جل والتي تم وضعها في الانبوب المخصص في الجهاز. بعد ذلك، تم ربطه بالعمود الذي يحتوي خليط من مذيب ن-هكسان : اثيل استيت بنسبه (١:٩) . يعد جزء النورمال هكسان المستخلص من النبات مثمراً للغاية نظراً لوفرة المواد الكيميائية المتنوعة الموجودة في النباتات التي تنتمي إلى العديد من المجموعات، بما في ذلك الأحماض الدهنية والزيوت المتطايرة والفياتوستيروول والترايترينينات ومركبات الألدهيدات وكحولات التربين وأيزومرات فيتامين هـ والدايترينينات والألكينات، الألكينات. أظهرت نتائج خصائص نقطة الانصهار والأشعة فوق البنفسجية الطيفية للأشعة فوق البنفسجية والتي السي وتحويل فوربيه للأشعة تحت الحمراء للمادة المفصولة أنه مطابق لستاندر اللوبيول. إن اكتشاف نبات البرتولاكا جرانديفلورا العراقي كمصدر طبيعي جديد للوبيول فتح آفاقاً جديدة نحو هذا النبات الواعد للأنشطة الدوائية والتطبيقات الصيدلانية الكلمات المفتاحية : فلاش كروماتوغرافي، كروماتوغرافيا الغاز/قياس الطيف الكتلي، لوبيول، برتولاكا جرانديفلورا.