



RESEARCH ARTICLE

Isolation, characterization of lignan compound Pinoresinol in Iraqi *Mesembryanthemum Cordifolium*

Hayder Imad Jabar^{1*} & Amjed Haseeb Khamees²

¹Department of Pharmaceutics, College of Pharmacy, Ahl Al-Bayt University, Karbala, Iraq

²Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, University of Baghdad, Baghdad, Iraq

*Correspondence email - hyderimad@gmail.com

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Abstract

Among the *Aizoaceae* family, *Mesembryanthemum cordifolium* remains significantly understudied regarding its therapeutic properties despite its abundance. This large family is mostly native to Southern Africa and counts at least 120 genera. *M. cordifolium*'s traditional anti-inflammatory and antidepressant uses are scientifically supported. Its antioxidant, analgesic and cytotoxic properties and promising antidepressant activity, highlight its significant pharmacological potential, warranting further research. This study addresses this research gap by conducting the first investigation into the phytochemical components of Iraqi *M. cordifolium* and the isolation of a key compound. For 24 h, the entire plant powder, 100 g, was immersed in 100 mL n-hexane to remove any fat. After that, the defatted plant material was extracted with eighty-five percent methanol using the Soxhlet apparatus until it was entirely exhausted. The extract undergoes sequential liquid-liquid fractionation using solvents of increasing polarity: petroleum ether, chloroform and ethyl acetate, using a separatory funnel. This process allows for the separation of compounds based on their differential solubility in immiscible solvents. The results of the phytochemical analysis show that the methanolic crude extract contains steroids, tannins, alkaloids, flavonoids, phenols and saponins. Pinoresinol compound was identified using the technique of high-performance liquid chromatography (HPLC). Pinoresinol was isolated using preparative layer chromatography (PLC) and its identification was confirmed using Fourier transform infrared (FTIR) and proton nuclear magnetic resonance (¹H NMR) and Liquid chromatography-mass spectrometry (LC-MS). Analyses revealed that the plant contains lignan compounds with useful medicinal properties, so additional research into the *M. cordifolium* plant is necessary because it contains a wealth of components with promising health benefits for humans.

Keywords: FTIR; ¹H NMR; HPLC; LC/MS/MS *Mesembryanthemum cordifolium*

Introduction

Traditional medicine has historically depended on medicinal plants to alleviate many symptoms (1). Plants have met all of humanity's basic needs, including housing, clothing, food, flavouring, perfume and an endless list of medicinal substances (2). Research into organic material synthesis has recently increased exponentially. Conversely, plants have long been considered an essential raw material for the pharmaceutical and chemical industries (3). The complicated process of synthesizing chemicals from active molecules or the high manufacturing costs may be ascribed to this. The composition and proportion of the constituents in the plant vary depending on the specific plant part and the extraction technique used (either hot or cold) (4). The *Aizoaceae* is the most extensive family of plants globally that can store water in their tissues, known as succulents (5). The *Aizooideae*, *Mesembryanthmoideae*, *Rushioideae*, *Sesuvioideae* and *Tetragonioidae* subfamilies are all acknowledged. They are found mainly in Africa's humid and dry areas; just a few are located in the middle of the Pacific Ocean, Asia and Australia. Most of these species are found only in southern Africa (96 %) (6). Economically, several species of the family are essential as decorations and are cultivated all over the world (7). Certain plants' therapeutic properties are particularly noteworthy; for example,

Tetragonia tetragonioides may offer relief for patients suffering from enteritis, digestive discomfort, ulcerations, stomach cancer and other gastrointestinal disorders (8). Despite being one of the most prominent and varied families, *Aizoaceae* has received the least attention when researching its therapeutic properties. This highlights a significant literature gap. This study specifically utilized *Mesembryanthemum cordifolium* (family *Aizoaceae*), cultivated in an Iraqi garden, ensuring precise identification and enabling future replication. The *Mesembryanthemum cordifolium* plant itself possesses numerous reported health benefits, including antioxidant, analgesic, anti-inflammatory, liver health, cytotoxic, antidepressant and antihyperglycemic activities (9). Therefore, more research into additional *Aizoaceae* species, particularly *M. cordifolium*, is necessary for potential future medicinal uses. Naturally occurring chemical compounds, containing predominantly basic nitrogen atoms, are known as alkaloids (10). Plants, bacteria, fungi and mammals produce various chemicals that may be used medicinally. New pharmacological effects and plant sources of these chemicals, particularly those from the *Aizoaceae* family (mesembrine-type alkaloid), seem to be the focus of intense competition (11). Polyphenols are an extensive class of naturally occurring bioactive chemicals that typically include at least two hydroxyl groups in their structure (12). This study is considered the first for

the detection of the components of the Iraqi *M. cordifolium* plant and the isolation of lignan compounds. The *M. cordifolium* plant is represented in Fig.1.



Fig. 1. *M. cordifolium* plant.

Materials and Methods

Tools and equipment

Rotatory evaporator (Buchi rotatory evaporator attached to a vacuum pump), oven (Memmert 854) and an electronic sensitive balance (Germany) were utilized. Fourier transform infrared spectra (FT-IR) were recorded using a Shimadzu, Japan, instrument located in Baghdad, Adhamiya, near Al-Nu'man Teaching Hospital. HPLC analysis was performed using Cecil Technology of the United Kingdom equipment at the Scientific Centre for Chemical Analysis in Baghdad, Iraq. Proton nuclear magnetic resonance (^1H NMR) The analysis was conducted at Iraq, the University of Basra, College of Education for Pure Sciences, Department of Chemistry. Liquid chromatography-mass spectrometry (LC-MS/MS) data were collected at Jordan University of Science and Technology in Irbid, Jordan, using a Shimadzu, Japan LC/MS-8040 series system with an electrospray interface (ESI)

Chemicals and reagents

All solvents used, including n-hexane, 85 % methanol, petroleum ether (60 °C- 80 °C), chloroform, ethyl acetate, toluene and formic acid, were of analytical grade. Anhydrous sodium sulfate was used for drying extracts. Silica gel GF 254 was utilized as the stationary phase for preparative layer chromatography (PLC). For NMR analysis, deuterated chloroform (CDCl_3) and Dimethyl Sulfoxide (DMSO) were used as solvents.

Collection of plant materials

The taxonomic identification of the plant was carried out by Prof. Assist. Dr. Israa Abdul Razzaq Majeed of the Department of Biology/College of Science at the University of Baghdad, Iraq. The plant sample was obtained in the summer of 2023 from Al Diwaniyah City. The process begins with washing the whole plant part under running water (13). Then, sixteen consecutive days of open-air drying in the shade, followed by grinding with an electric blender, calculating the weight and finally, extraction (14).

Extraction

Extraction is the first and most important stage in the analysis process to separate and identify the specific chemical components of medicinal plants(15, 16). A two hundred grams of the dried herb

has been ground into a powder after macerating the powder with n -hexane for a day to eliminate any extra oil and plant debris. Next, it was processed using a Soxhlet instrument, then extracted with 85 % methanol (17). The resultant liquid was passed through Whatman no. one filter paper to get a clean extract. Since raw extract contains a wide variety of chemical components with different polarities, it is recommended to fractionate the extract before chromatographic analysis to isolate the main classes of plant components based on their solubility and polarity differences. This will allow for a comprehensive screening of the plant's phytochemical profile (18). Solvents of different polarities, the crude extract, petroleum ether (60 °C-80 °C), chloroform and ethyl acetate, were used in a separatory funnel. Each fraction was obtained using 250 mL of solvent. The process was iterated several times. Then, the three parts were dried with anhydrous sodium sulfate, filtered and evaporated using a rotary evaporator to eliminate any remaining moisture. After that, the leftover material was measured and set aside for further examination (19) (Fig. 2).

Preliminary phytochemical screening of *M. cordifolium*

The first step in phytochemical screening was conducting a series of assays, each suited to a specific target, on the plant extracts and determining by using standard procedures (17, 18).

Alkaloids

Extract with acid, then precipitate with Mayer's and Wagner's reagent; observe for turbidity/precipitate.

Flavonoids

Add magnesium and HCl; observe for red/orange/pink colour (Shinoda test).

Phenols

Add ferric chloride solution; observe for blue, green, or black colour.

Saponins

Shake extract vigorously with water; observe for persistent foam (froth test).

Steroids

Extract with chloroform, add acetic anhydride and conc. H_2SO_4 , observe for colour changes (Salkowski/Liebermann-Burchard test).

Lignan

ferric chloride test: Observe for characteristic red or colour changes, respectively.

Identification of Pinoresinol in *M. cordifolium* ethyl acetate plant extracts by using HPLC

The system was operated at a constant flow rate of 1 mL/min and the column oven was maintained at 25 °C. The injection volume for all samples was 20 μL and UV detection was performed at a wavelength of 280 nm. The dried petroleum ether fraction was accurately weighed and dissolved in HPLC conditions for the ethyl acetate fraction (Mobile phase: methanol -water 1:1 formic acid 5). The solution was sonicated for 10 minutes and filtered through a 0.45 μm syringe filter before injection. An authentic standard of Pinoresinol was prepared in the same manner and at the same concentration. Using Column, C18 with a stationary phase (5 μm particle size, 250 $\times$ 4.6).

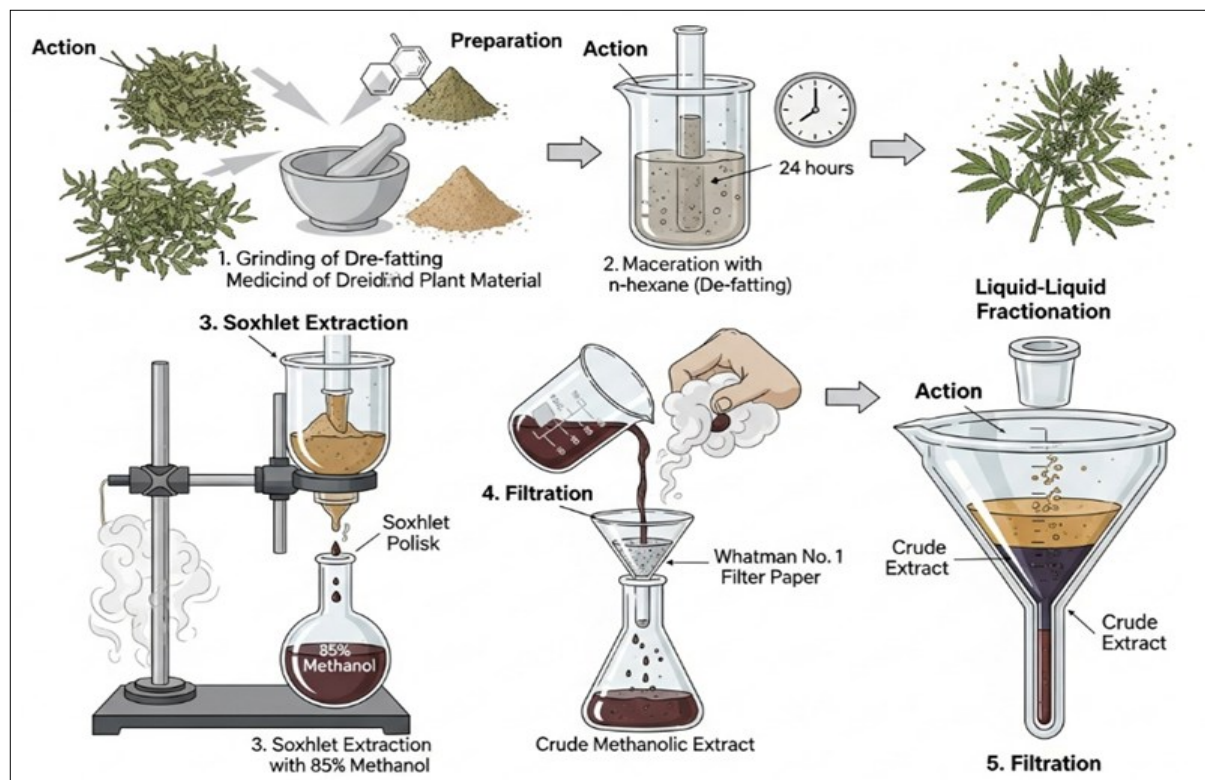


Fig. 2. Extraction of *Mesembryanthemum Cordifolium* by using Soxhlet.

Isolation of Pinoresinol by preparative layer chromatography (PLC)

PLC involved applying the ethyl acetate fraction onto silica gel GF 254 plates. A mobile phase of toluene: ethyl acetate: formic acid: methanol (55:30:10:5) was developed to 14 cm. Using twenty plates and the Pinoresinol band (R_f 0.652) was then scraped, eluted with methanol, filtered and subjected to FTIR, ^1H NMR and LC-MS for comprehensive identification.

Identification of the isolated Pinoresinol

Fourier transform infrared spectroscopy (FTIR)

The isolated compound was characterized by FTIR spectroscopy (Shimadzu). A KBr pellet of approximately 2 mg of the compound was scanned from 4000 to 400 cm^{-1} . Spectral bands were then compared against Pinoresinol known data and functional group frequencies for identification.

Observed chemical bands in molecules.

Proton nuclear magnetic resonance (^1H NMR)

For ^1H NMR analysis, the isolated compound was accurately weighed and dissolved in deuterated chloroform (CDCl_3). The ^1H NMR spectrum was then recorded on a Euro-vector EA 3000A NMR spectrometer (BRUKER, Germany) operating at 400 MHz. Chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard.

Liquid chromatography-mass spectrometry (LC-MS)

LC-MS/MS analysis was performed using a Shimadzu LC/MS-8040 series system (Shimadzu, Japan), equipped with an electrospray ionization (ESI) interface. Data acquisition occurred at Jordan University of Science and Technology in Irbid, Jordan. Specific chromatographic and mass spectrometric parameters (e.g. column, mobile phases, flow rate, injection volume, ion modes, scan range) will be detailed as per the analytical method developed for the target compounds.

Results

Extraction

The Soxhlet device is used for hot extraction. Dry plant material (200 g) was defatted with n-hexane and dried before being extracted with 85 % methanol in a thimble. The extract is evaporated at 40 $^{\circ}\text{C}$ using a rotary evaporator. It yields 42.5 g of dark sticky crude extract with a percent of yield (21.25 %). To dissolve the product, it was mixed with 250 mL of distilled water (DW) and mixed until fully dissolved. Water extract is fractionated with equal volumes of petroleum ether, chloroform and ethyl acetate. After drying, each portion weighed (1.66, 0.6 and 0.3) grams with % of yield (0.83 %, 0.30 % and 0.15 %).

Preliminary phytochemical screening of *M. cordifolium*

The results of the phytochemical analysis are given in Table 1.

Table 1. Preliminary phytochemical test of *Mesembryanthemum cordifolium*

Phytochemical	observations
Alkaloids	++
Flavonoid's	+++
phenols	++
saponin	++
steroid	+++
Lignan	+++

+++ very highly, ++ highly, +low

Identification of Pinoresinol in *M. cordifolium* ethyl acetate plant extracts by using HPLC

HPLC chromatogram for ethyl acetate fraction and standards, Pinoresinol, as shown in Fig. 3. Retention time of Pinoresinol and ethyl acetate fraction is shown in Table 2

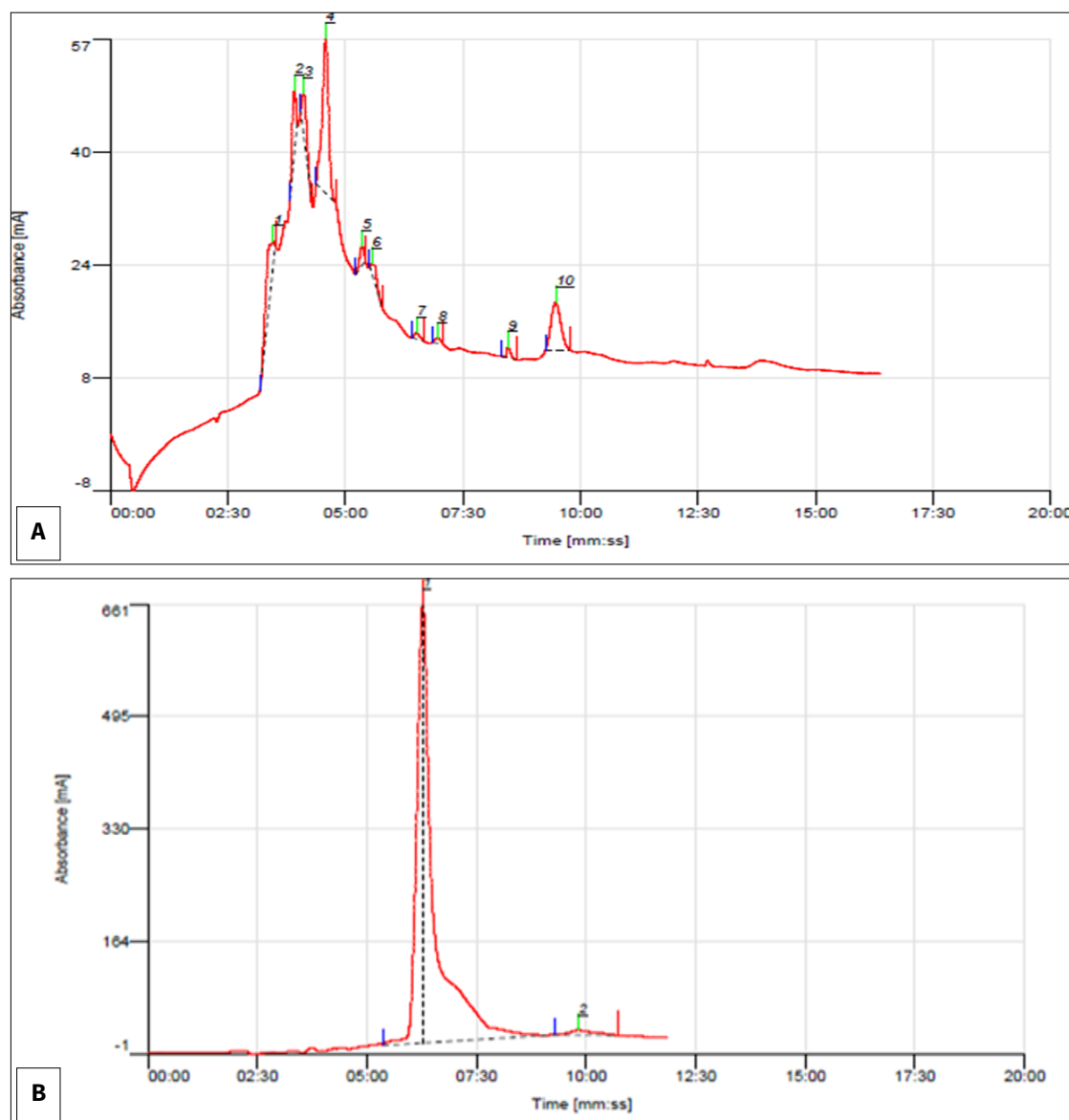


Fig. 3. HPLC chromatogram for ethyl acetate **A.** fraction and **B.** standards Pinoresinol.

Table 2. Retention time of Pinoresinol and ethyl acetate fraction

No. of peak	compound	The retention time of the standard	The retention time of the sample
8	Pinoresinol	06:15.9	06:57.9

Isolation of Pinoresinol by Preparative Layer Chromatography PLC from the chloroform fraction

The chemical analysis of the ethyl acetate fraction obtained by the hot method indicated the presence of Pinoresinol. Several mobile phases are used to identify compounds; however, the best one, resulting in a visible spot, is a mixture of 55:30:10:5, toluene:ethyl acetate:Formic acid:methanol. The solvent is allowed to rise to 14 cm. The R_f value is (0.652). The band was determined and scratched. As the mobile phase is polar, the collected silica is macerated with methanol. Afterwards, the solution is filtered using double filter paper. To fully remove the chemical from the silica, this procedure is performed three times (Fig. 4).

Fourier transform infrared spectroscopy (FTIR)

For unidentified substances in plant extracts, FTIR has been an invaluable technique for identifying and structurally elucidating components or functional groups. In most cases, the FTIR

spectra of isolated components are as distinctive as chemical fingerprints as shown in Fig. 5 (20). The characteristic IR

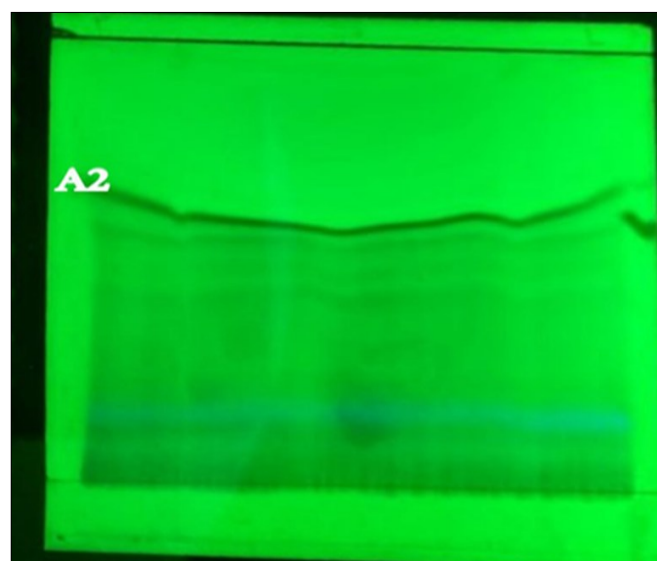


Fig. 4. Preparative layer chromatography was used on the ethyl acetate fraction using a solvent mixture of 55:30:10:5, toluene: ethyl acetate: Formic acid: methanol, under UV light 254 nm, A2 is Pinoresinol.

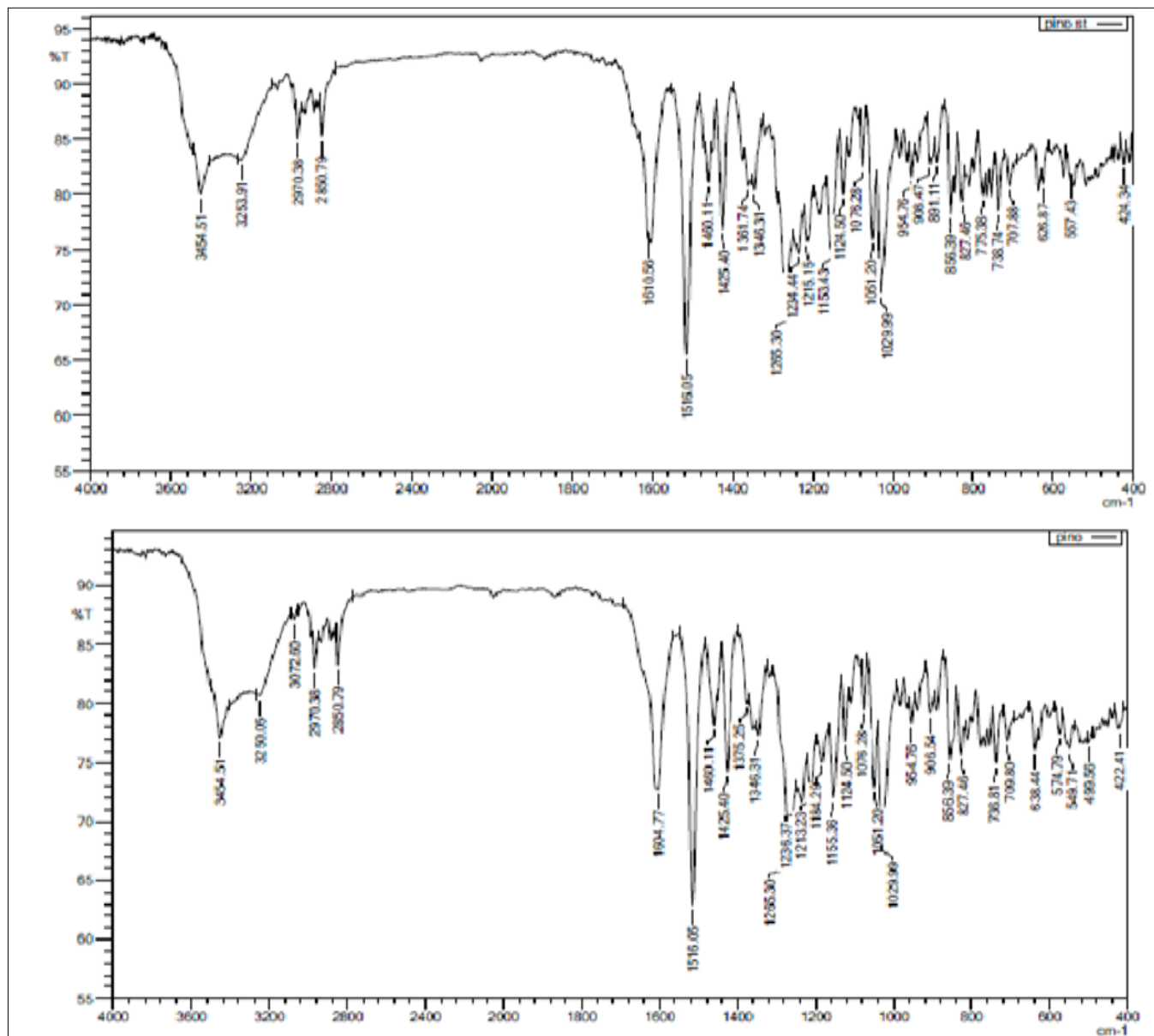
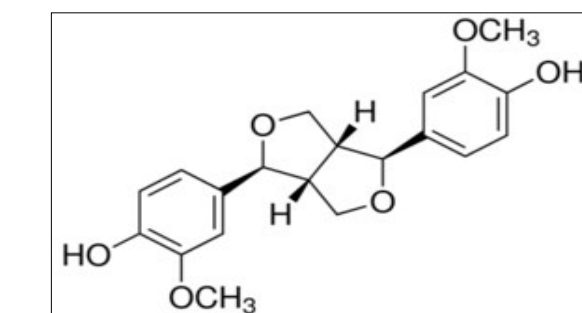


Fig. 5. FTIR spectra of the isolated compound and Pinoresinol standard.

Table 3. Characteristic FTIR absorption bands (cm⁻¹) of the isolated compound

Functional group	Group frequency wave number (in cm ⁻¹)		Main attributed
Isolated compound	Pinoresinol standard		
O-H	3354.51	3454.51	O-H stretching vibration
C-H	3072.6	3052.51	aromatic C-H stretching
C-H	2970.38, 2850.79	2970.38, 2850.79	C-H of CH ₂ & CH ₃ stretching vibration
C=C	1604.77, 1516.05	1610.56, 1516.05	Aromatic C=C stretching
C-H	1460.11	1460.11	Bending frequencies cyclic (CH ₂)
C-O-C	1265.30, 1236.370, 1213,23	1265.30, 1234.440, 1215.15	Ether stretching vibration



(21)

absorption bands of isolated compounds are listed in Table 3.

Proton Nuclear magnetic resonance (^1H NMR)

No reference was available for the isolated compound (unknown) that was separated by PLC; thus, nuclear magnetic resonance (NMR) spectrum measurements were undertaken utilizing the Bruker AVANCE-NEO technology. An NMR spectrometer was used to run the sample after dissolving it in Dimethyl Sulfoxide (DMSO), as shown in Fig. 6 and Table 4.

Liquid chromatography-mass spectrometry (LC-MS)

Each component's molecular masses were estimated using an

LC-MS-MS spectrum. In order to determine the molecular mass, only the molecular ion peak from each chromatogram was examined. When trying to identify components by their molecular masses, mass spectra may be a helpful tool. A compound's structure may be determined from the pattern of fragmentation of its different groups, as shown in Fig. 7 and Table 5. The full scan mass spectra of A2 showed that the $[\text{M}+\text{H}]^+$ ion with a mass-to-charge ratio (m/z) of 359.2 was chosen as the molecular ion. Table 5 illustrates that the most abundant fragment closely matched what was published in the literature for Pinoresinol (23).

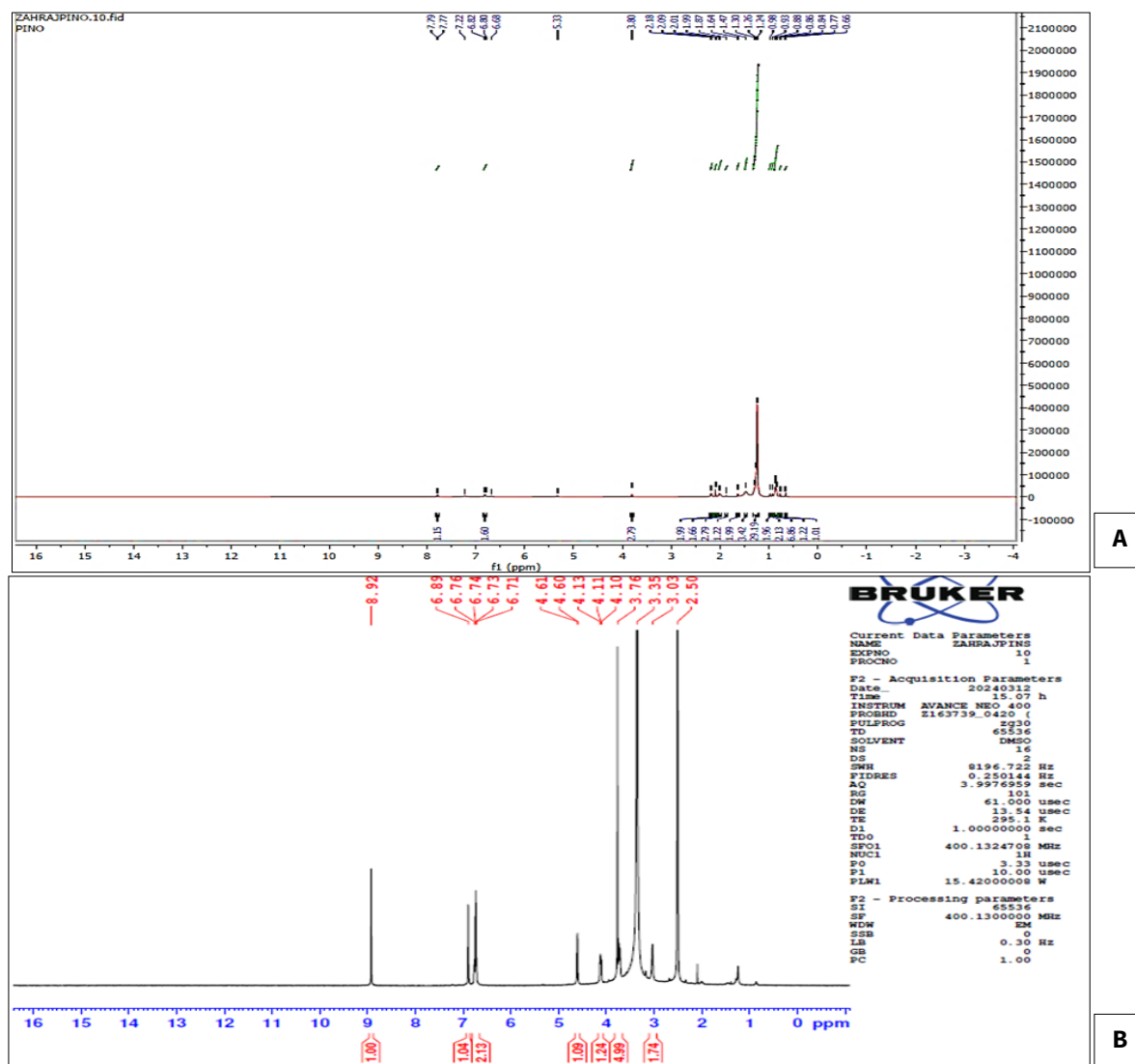
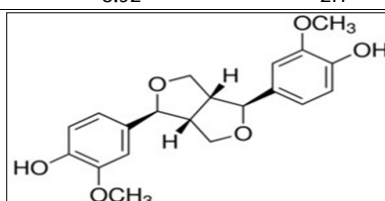


Fig. 6. ^1H NMR of the **A.** isolated compound and **B.** Pinoresinol standard (B).

Table 4. ^1H NMR of the isolated compound and Pinoresinol standard

Carbon atom	Ppm Isolated compound	Ppm standard	Integration	Multiplicity	Assignment
C10, C12	0.66-1.64	0.86-1.29	4H	Multiplet	CH ₂
C8, C9	1.87-2.18	2-3.04	2H	Multiplet	CH
C5, C17	3.80	3.77	6H	Singlet	OCH ₃
C7, C14	5.33	4.61	2H	Singlet	CH
C2, C20	6.68	6.77	2H	Singlet	Aromatic ring
C1, C4	6.81	6.89	4H	Duplet	Aromatic ring
C16, C19	7.77	8.92	2H	Singlet	OH



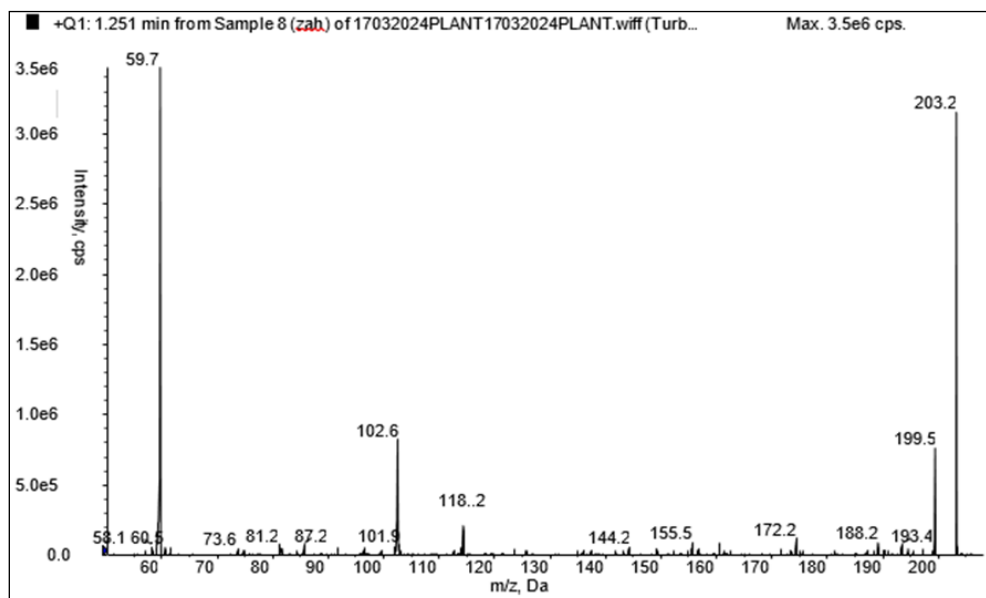


Fig. 7. Full scan product ion mass fragmentation spectra of isolated Pinoresinol.

Table 5. The mass fragments of A2 and the chemical structure

Fragment weight (m/z)	Chemical formula	Chemical structure
359.2 parent ion	M+H	
344.2	C ₁₉ H ₂₀ O ₆ ⁺	
328.2	C ₁₉ H ₂₀ O ₅ ⁺	
342.2	C ₁₉ H ₁₉ O ₄ ⁺	
236.2	C ₁₃ H ₁₅ O ₄ ⁺	
124.2	C ₇ H ₇ O ₂ ⁺	

Discussion

Pinoresinol, isolated and characterized from *M. cordifolium*, an Iraqi plant in the *Aizoaceae* family, is the focus of this work. The compound's first recorded existence in this species suggests a unique biosynthetic capacity in the plant that may contribute to its medicinal properties (24). *M. cordifolium* has a traditional use in herbal medicine and one possible explanation is that it contains the lignan Pinoresinol, which has antioxidant, anti-inflammatory and anticancer characteristics (25). Humans can benefit from the health advantages of the compound after consuming the plant, which indicates that the plant may utilize it as a chemical defence mechanism (26). Isolation using advanced chromatographic and spectroscopic techniques highlights the plant's rich phytochemical matrix, suggesting synergistic interactions that may increase Pinoresinol bioactivity. Studies on other plants in the *Aizoaceae* family have also found a lot of beneficial chemicals (10). Click or tap here to enter text. However, the fact that Pinoresinol was only found in *M. cordifolium* makes it different from other species in the same family, which suggests that it has unique phytochemicals. Click or tap here to enter text. The results of this study are different from those of others because the plant's biochemical processes are affected by its location and climate (11). The comparison also shows how little is known about this plant species and what it might add to the field of natural product medicine. The results of this research add to the huge database of medicinal plants throughout the world and improve the understanding of *M. cordifolium*.

Conclusion

This study successfully isolated and characterized Pinoresinol from Iraqi *M. cordifolium*, marking its first documented presence in this species. This discovery highlights the plant's unique biosynthetic capacity, likely influenced by local environment. The identification of Pinoresinol, a lignan with established antioxidant, anti-inflammatory and anticancer properties, scientifically supports the traditional medicinal uses of *M. cordifolium*. This research significantly enriches the understanding of *M. cordifolium*'s phytochemical profile, underscoring its potential as a valuable source of bioactive compounds in natural product medicine.

Authors' contributions

All authors contributed to the study conception and design, data acquisition and analysis, manuscript drafting and critical revision. 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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