https://doi.org/ 10.33472/AFJBS.6.9.2024.3517-3528

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Isolation and characterization of lupeol from the leaf and flowers of *Carthamus tinctorius*

Manish Agrawal[1,2], Parul Mehta[2]*

[1]School of Pharmacy, LNCT University, Bhopal, Madhya Pradesh, India- 462042

[2]School of Pharmacy, LNCT University, Bhopal, Madhya Pradesh, India- 462042

***Corresponding Author**

Dr. Parul Mehta

School of Pharmacy, LNCT University, Bhopal, Madhya Pradesh, India- 462042

Email: manish.agrawal1000@gmail.com

Article History

Volume 6,Issue 9, 2024

Received: 28 Apr 2024

Accepted : 10 May 2024

doi: 10.33472/AFJBS.6.9.2024.3517-3528

Abstract

Background: *Carthamus tinctorius*, commonly known as safflower, possesses diverse pharmacological properties. This study aimed to isolate and characterize lupeol, a bioactive compound, from both the leaves and flowers of *Carthamus tinctorius*.

Results: Employing a combination of solvent extraction and chromatographic techniques, lupeol was successfully isolated. Structural elucidation was performed using Fourier Transform Infrared (FTIR) Spectroscopy, Proton nuclear magnetic resonance (^{1}H-NMR),Carbon-13 (C13) nuclear magnetic resonance (^{13}C-NMR) and mass spectrometry analyses. The finding confirms the isolation of lupeol from leaves and flowers of *Carthamus tinctorius*.

Conclusions: This investigation shades light on the potential therapeutic applications of lupeol as a natural product derived from *Carthamus tinctorius*

Keywords: *Carthamus tinctorius*, *S*afflower, Isolation, Lupeol, Natural product

1. BACKGROUND

Carthamus tinctorius, commonly referred to as safflower, is an annual herbaceous plant classified within the Asteraceae family. Originating from the Middle East, it has been cultivated for centuries due to its versatile applications, spanning from culinary uses to traditional medicinal practices. Notably, *Carthamus tinctorius* harbors a rich array of bioactive compounds like flavonoids, c-glycosides, triterpenes alcohols, essential oils, coumarins, fatty acids, steroids etc. Among which pentacyclic triterpenoids stands out for their noteworthy pharmacological potential (Pant et al., 2019; Rahul *et.al.* 2019)

Pentacyclic triterpenoids represent a class of naturally occurring molecules renowned for their multifaceted biological activities, encompassing anti-inflammatory, antioxidant, anticancer properties and antiandrogenic action. *Carthamus tinctorius* has emerged as a promising reservoir of these triterpenoids, offering an opportunity for their isolation and subsequent characterization to unveil their chemical composition and potential therapeutic roles to treat prostatic hyperplasia (Patocka, 2003; Prachayasittikul et al., 2010).

Recently, we reported significant pharmacologic activity in the methanolic extract and its n-hexane fraction of *Carthamus tinctorius*, showing inhibition of testosterone-induced prostatic hyperplasia in rats. To identify the effective compounds responsible for this pharmacologic activity, we isolated and characterized pentacyclic triterpenes from the n-hexane fraction of the methanolic extract of *Carthamus tinctorius*. Utilizing diverse extraction methods and analytical techniques, our objective is to identify and quantify these bioactive compounds, thereby elucidating their structural attributes and potential pharmacological benefits. The outcomes of this investigation are poised to enhance current knowledge on natural products with therapeutic implications, while also paving the way for further exploration into the medicinal applications of *Carthamus tinctorius* and its derivatives.

2. METHODS

2.1 Plant material

The complete plant along with its flowers of *Carthamus tinctorius* was collected from local fields and nearby forest areas in Nauradehi, located in the Bundelkhand region of Madhya Pradesh, India.

2.2 Preparation of Extracts

The flowers and leaves of *Carthamus tinctorius* were dried separately at 30°C in a shaded area. They were then coarsely powdered using an electric grinder and extracted with a 70% methanol/water mixture using a Soxhlet apparatus. The solvent was recovered under reduced pressure, resulting in a yield of 5.2% w/w, using a rotary vacuum evaporator, and further subjected to freeze-drying using a lyophilizer. The resulting freeze-dried methanolic extract was dissolved in 200 ml of water and partitioned with n-hexane (4x 200ml) using a separator funnel.

Finally, the n-hexane extract was distilled off using a rotary evaporator and subsequently dried (Koriem, 2021).

2.3 Preliminary phytochemical analysis of extracts

Various qualitative chemical tests were conducted on the vacuum-dried n-hexane fraction of methanolic extracts from *Carthamus tinctorius* to detect the presence of different chemical constituents. Both the methanolic extract and its n-hexane fraction tested positive in the Liebermann-Burchard test, indicating the presence of terpenoids in the structure (Harborne, 1998).

2.4 Isolation of bioactive compounds

The dried residue from the n-hexane extract, obtained through fractionation of the methanolic extract of *Carthamus tinctorius*, underwent chromatography on a silica gel column (60-120 mesh) using the wet slurry method for column packing with n-hexane and silica gel. The column underwent gradient elution starting with 100% hexane, followed by 2% and 5% chloroform in hexane. Seven fractions (1-7) of 30 ml each were collected using 100% hexane; five fractions (8-12) of 30 ml each were collected for 2% chloroform, and three fractions (12-14) of 30 ml each were collected for 4% chloroform in hexane (Nasution et al., 2015).

Each collected eluate was continuously monitored by thin-layer chromatography (TLC) (Silica-gel G; petroleum ether: benzene (3:7)), visualized with anisaldehyde-sulfuric acid reagent. Elute 1 exhibited a single band just below the solvent front on the TLC, which turned pink and deep purple upon treatment with anisaldehyde-sulfuric acid followed by heating at 105°C. Upon concentration, it formed a thin film of white flakes on the watch glass (C-A; 1.8 mg)(Muktar et al., 2018).

Elutes 2, 3, and 4 contained a mixture of different compounds, while elutes 5 to 8 each exhibited three bands on the TLC plate, invisible under ultraviolet (UV) light, and turned pink with anisaldehyde-sulfuric acid. Upon concentration, elutes 5 to 8 formed cream-colored granular powders (Compound-B; 38 mg). Elutes 9 to 14 were mixtures of different compounds; they were combined and concentrated to obtain a cream-colored granular powder (200 mg).

Further purification of compound B (38 mg) involved subjecting it to extensive column chromatography. The sample was reapplied to an n-hexane and silica gel column. The column was eluted using 100% n-hexane, gradually increasing the ratio of hexane to ethyl acetate. Several elutes of 10 ml each were collected and monitored on TLC. Elutes (4-6) showing a single band were combined and evaporated under reduced pressure, leading to the isolation of the pure compound. The isolated compound underwent UV, IR, ^{1}H-NMR, and ^{13}C-NMR analysis to ascertain its chemical structure.

3. RESULTS

Isolated compound is a cream colored granular powder with melting point 212-213 ^{0}C. It was soluble in hexane, benzene and chloroform and gave a positive Liebermann Buchard test.

Spectroscopic methods are employed to gain further insight into the structure of isolated compounds. (Nasution*et al.* 2014).

3.1 Spectral analysis

3.1.1 FTIR: In IR spectroscopy, the sample is typically prepared using the potassium bromide (KBr) pellet method, involving mixing the sample with powdered and compressing it into a KBr pellet. A FTIR spectrometer, which utilizes an interferometer, is then used to measure the interference pattern generated as infrared radiation passes through the sample. The instrument is calibrated and adjusted for the desired measurement parameters, including wavelength range and resolution. Subsequently, the IR radiation is transmitted through the sample, and the absorption of infrared radiation is measured across the chosen wavelength range (Besbes et al., 2017).

Figure 1: Infra-red spectrum of lupeol isolated from the *Carthamus tinctorius*

The resulting IR spectrum is plotted as percent transmittance versus wave number (the frequency of radiation), revealing peaks and troughs corresponding to specific molecular vibrations. Analysis of the IR spectrum allows for the identification of absorption bands corresponding to functional groups present in the molecule. The position, intensity, and shape of these absorption bands provide valuable insights into the molecule's chemical environment and bonding. Finally, the IR spectrum is interpreted to determine the presence of specific functional groups and structural features within the sample. Results are typically presented in the form of a spectral table, along with an analysis of observed absorption bands and their assignments.

Table1: **Interpretation of FTIR spectra of test compound**

S.No.	Peak(cm^{-1})	Interpretation
1.	**3406.1**	Peak corresponds to the stretching vibration of O-H groups present

		in hydroxyl (OH) functional groups .**Confirms one or more hydroxyl group in the structure**.
2.	**2937.4, 2923.2 2854.5,2862.8**	Peak is associated with the stretching vibrations of C-H bonds in aliphatic chains. **Peak intensity confirms the presence of several aliphatic chains in the Structure**
3.	**1460.3, 1467.8**	Peaks are associated with bending vibrations of C-H bonds in methyl group. **Structure contain contains several methyl groups**
4.	**1632.4, 1648.3 1611.2,1624.5**	Stretching vibrations due to conjugated double bonds, **confirms multiple double bonds in its structure**
5.	**1050.3, 1063.7 1071.9**	Stretching vibration of C-O bonds in secondary alcohols or ethers, c**onfirms secondary alcoholic group**
6.	**1387.5 ,1392.3**	With bending vibrations of C-H bonds in methylene (-CH_2) groups, **confirms the presence =CH_2 group**

3.1.2 ^{1}H-NMR: The proton nuclear magnetic resonance spectra of test compound (Figure 2) were recorded using a Bruker Avance II 400 NMR spectrometer. For NMR spectroscopy, the compound solution was analyzed in deuterated dimethyl sulfoxide (DMSO-d6), ideally containing deuterium to provide a sharp reference signal (usually tetramethylsilane, TMS) for chemical shift referencing. The sample solution was then transferred into an NMR tube, and the NMR instrument was set up to adjust parameters such as pulse sequence, acquisition time, relaxation delay, and temperature control. Shimming was performed to optimize magnetic field homogeneity and achieve sharp, well-defined peaks. The NMR experiment was initiated by applying a radiofrequency pulse to the sample. These signals were detected by the NMR spectrometer and processed to generate the NMR spectrum. The final spectrum was generated by baseline correction against the TMS peak at 0 ppm.

The spectrum was analyzed to identify peaks corresponding to different proton environments within the molecule. The observed chemical shifts (in ppm), peak integrations (relative proton ratios), and peak multiplicities (singlet, doublet, triplet, etc.) were found as follow

0.70 (1H,m,C-5 aH),0.76 (3H,s,C-23H),0.79(3H,s,C-28H),0.83(3H,s,C-25H),0.95 (3H,s, C-27H),0.98 (3H,s,C-24H),1.026 (3H,s,C-26H),1.22(1H,m,C-21 H),1.25 (1H,m,C-9 aH),1.35 (1H,m,C-18 aH),1.38 (2H , m 7H),1.5 (2H, m C- 2H), 1.66 (5H , m C-1H,C-12H, C-13βH),2.45 (1H, m,C-19βH),3.2 (1H,dd,C-3βH),4.6 (1H, spl.d,C-29H).

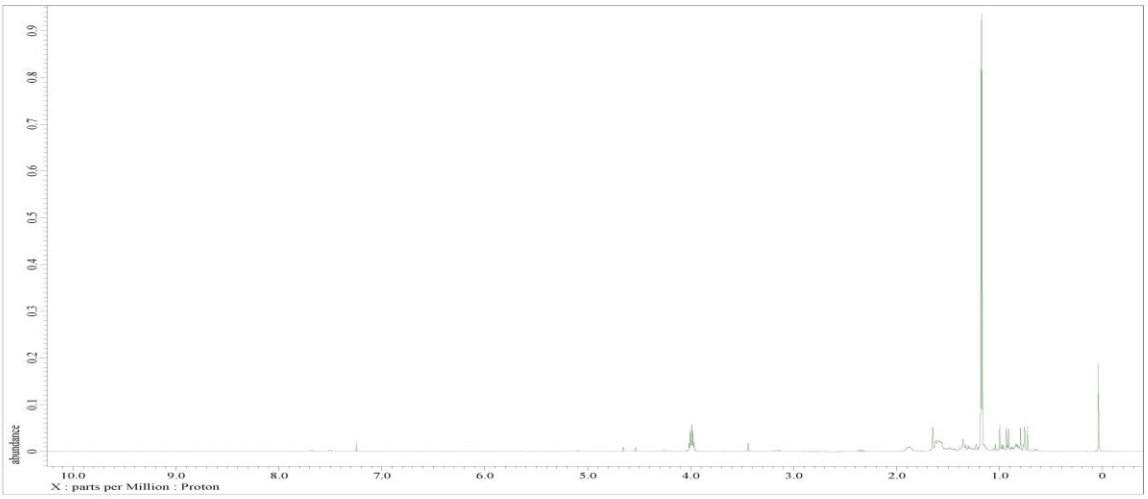


Figure 2: ^{1}H- NMR spectrum of lupeol isolated from the *Carthamus tinctorius*

The ^{1}H-NMR spectrum suggests that the compound is a triterpenoids. Due to the hyperfine splitting of 20 protons in the cyclohexane ring and mixed coupling constants, no singlet, doublet, or triplet can be seen in that region. Therefore, several multiplets corresponding to cyclohexane protons are observed in the spectra. Downfield signals at δ4.82 and 4.67 indicate the presence of a terminal double bond, while the signal at δ3.36 indicates the presence of a hydroxyl group. Several singlet peaks at δ1.01, 1.08, 1.13, 1.25, 1.27, 1.29, and 1.77 strongly suggest the presence of a pentacyclic ring in the structure. The proton signals obtained were compared with earlier reported values (Mimaki et al., 2004; Xie et al., 2006).

3.1.3 ^{13}C NMR : ^{13}C NMR relies on analyzing chemical shifts, along with peak intensities and multiplicities, for the elucidation of a molecule's structure. It typically reveals the environment of carbon atoms present in the structure. Sample preparation, instrument setup, and shimming for ^{13C} NMR are generally performed similarly to proton NMR spectroscopy. Carbon-13 nuclei have low natural abundance and a low gyromagnetic ratio compared to hydrogen nuclei, necessitating longer acquisition times to achieve satisfactory signal-to-noise ratios. The resulting free induction decay (FID) signal is detected by the NMR spectrometer and processed to generate the NMR spectrum. Finally, the resulting spectrum is analyzed to identify peaks corresponding to different carbon environments within the molecule.

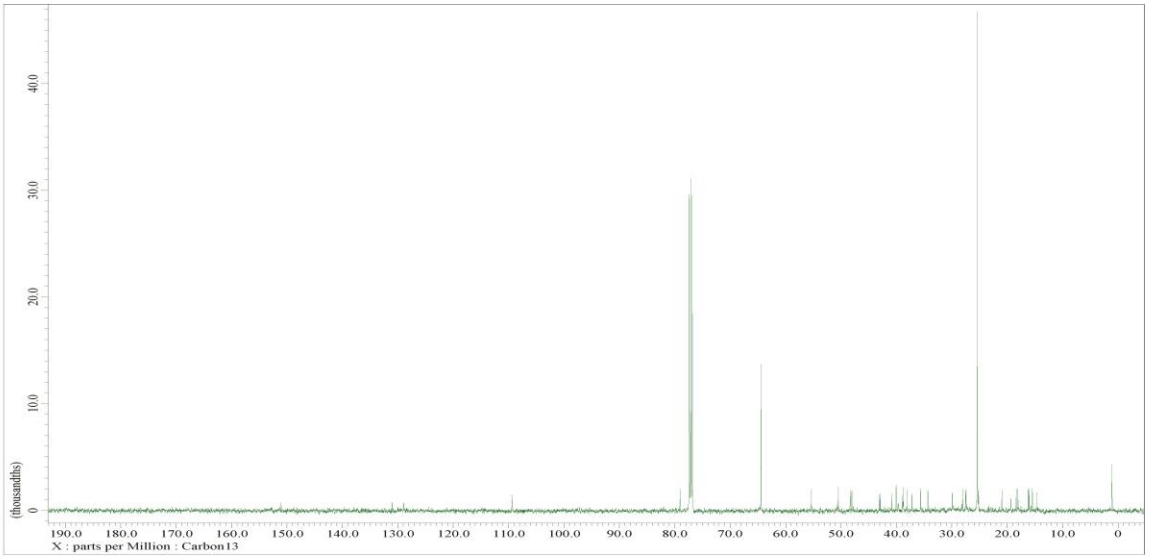


Figure 3: ^{13}C - NMR spectrum of lupeol isolated from the *Carthamus tinctorius*

Table 2: Interpretation of ^{13}C - NMR spectra of test compound

Carbon No.	^{13}C NMR signal	Carbon No.	^{13}C NMR signal	Carbon No.	^{13}C NMR signal
1.	40.1	11	21.3	21	30.2
2.	27.8	12	28.3	22	39.8
3.	78.3	13	38.2	23	28.7
4.	39.6	14	43.2	24	16.2
5.	56.1	15	29.8	25	15.4
6.	18.9	16	35.2	26	14.7
7.	34.6	17	42.1	27	16.2
8.	41.2	18	48.5	28	18.3
9.	50.9	19	48.3	29	19.6
10.	37.2	20	150.8	30	110.5

The ^{13}C NMR spectra of the compound indicate the presence of 30 carbons, further confirming the pentacyclic structure. Higher peak signals at δ150.8 and 110.5 ppm indicate the presence of quaternary carbons, while the signal at δ78.3 confirms the presence of a hydroxylated carbon. Multiple peaks (Figure 3) in the range of δ10-45 ppm (δ27.8, 18.9, 21.3, 28.3, 16.2, 14.7, 15.4) suggest the presence of a large number of aliphatic carbons (bonded to CH, CH_2, CH_3) in the structure. All peaks match previously reported reference spectra of lupeol and lupeol acetate, as evidenced by other reported values from various sources(Furukawa et al., 2002; Qiao et al., 2007).

3.1.4 Mass spectroscopy: Mass spectra provide valuable information through the typical characteristic fragmentation pathways of molecular structures. In mass spectrometry, the sample is introduced into an inlet chamber and volatilized. It is then subsequently introduced into the high vacuum chamber where the neutral molecule M passes through a beam of electrons, producing the molecular ions M+ after ionization. With higher bombardment energies (60-70 eV), fragmentation of the molecular ions occurs. By varying the strength of the magnetic field, ions are separated based on their m/z values.

The highest peak at m/z 426.37 (mass-to-charge ratio) (Figure 4) represents the molecular mass fragment of its parent ion, corresponding to the basic pentacyclic triterpenoids ring, which also matches the molecular mass of lupeol ($C_{30}H_{50}O$). The peak at m/z 409.38 indicates fragmentation to its basic pentacyclic structure after the removal of a hydroxyl group. Other peaks at m/z 338.33, 288.28, 217.01, and 158.96 occur due to the formation of different smaller fragments resulting from the loss of H_2O, CH_3-, or other groups (ToMosAkA et al., 2001). Smaller peaks at m/z 441.36 and m/z 468.32 indicate the presence of lupeol esters and its derivatives like lupeol acetate. Comparison with reference spectra and base peaks of fragments confirms the structure of lupeol and its derivatives, such as lupeol acetate, in the isolate(Sparkman et al., 2011).

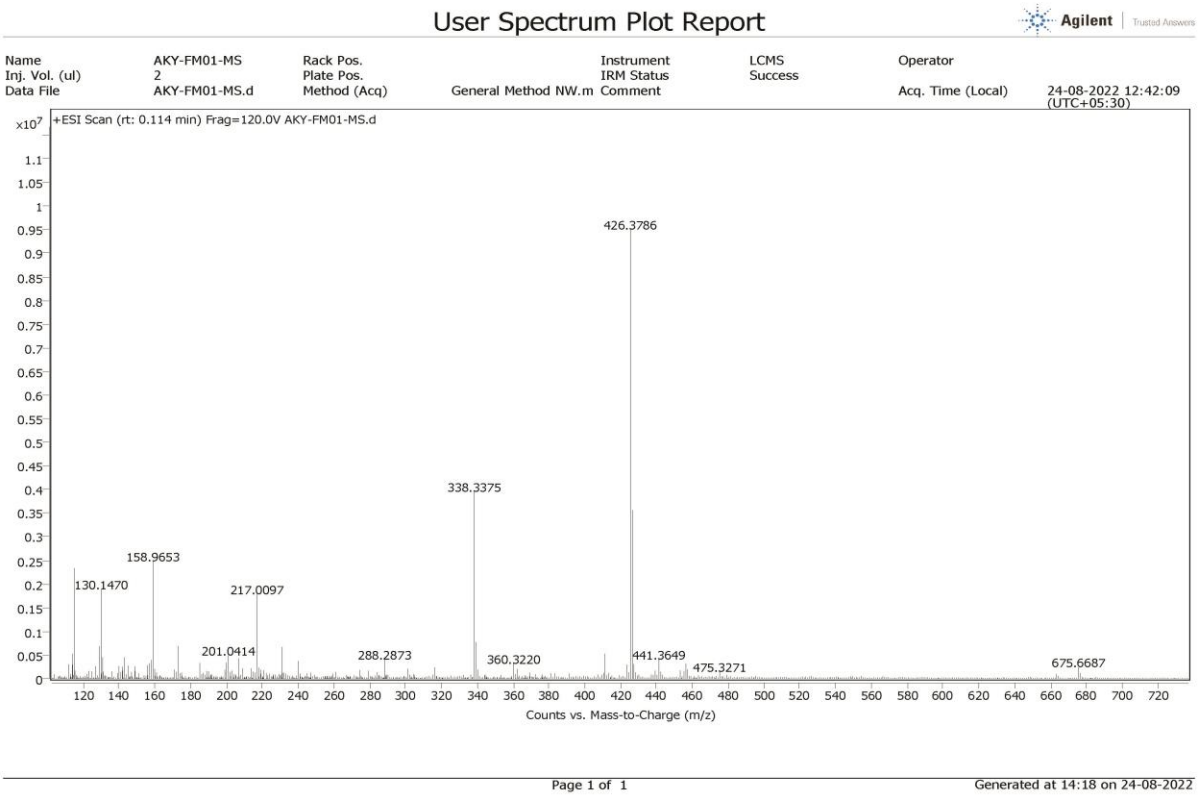


Figure 4: Mass spectrum of lupeol isolated from the *Carthamus tinctorius*

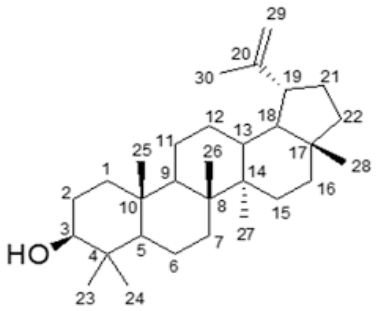


Figure 5: Structure of Lupeol

Lupeol is a pentacyclic triterpenoid comprising 30 carbon atoms skeleton of five rings.this compound displays promising therapeutic attributes,indicating its potential as a natural pharmaceutical ingredient.Overall our study enhances the understyanding of *Carthamus tinctorius* chemical composition and medicinal properties,paving the way to further exploration of its therapeutic potential.

4. Discussion

The comprehensive analysis of the isolated compound, integrating data from various spectroscopic and spectrometric techniques, provides compelling evidence for its structural elucidation as a pentacyclic triterpenoid compound lupeol.

Firstly, the physical characteristics, such as its cream-colored granular powder form and narrow melting point range of 212°C to 213°C, indicate a high degree of purity and consistency in composition, supporting the reliability of subsequent analyses. The positive Liebermann Buchard test suggests the presence of characteristic functional groups common in steroidal and triterpenoid compounds.

Moreover, the compound's solubility behavior, being soluble in hexane, benzene, and chloroform, indicates nonpolar or weakly polar characteristics, hinting at the presence of hydrophobic groups or regions within its structure.

The spectroscopic analysis revealed significant features. Infrared spectroscopy highlighted characteristic peaks corresponding to hydroxyl functional groups, aliphatic chains, methyl groups, and conjugated double bonds, all of which are consistent with triterpenoid structures.

The proton nuclear magnetic resonance (1H-NMR) spectrum further supported the identification of the compound as a triterpenoid, with characteristic signals indicating the presence of cyclohexane rings, a terminal double bond, and hydroxyl groups. Additionally, the presence of singlet peaks strongly suggested a pentacyclic ring system.

The carbon-13 nuclear magnetic resonance (13C NMR) spectrum corroborated the pentacyclic structure, with signals indicating quaternary carbons, hydroxylated carbons, and aliphatic carbons, consistent with pentacyclic triterpenoid frameworks.

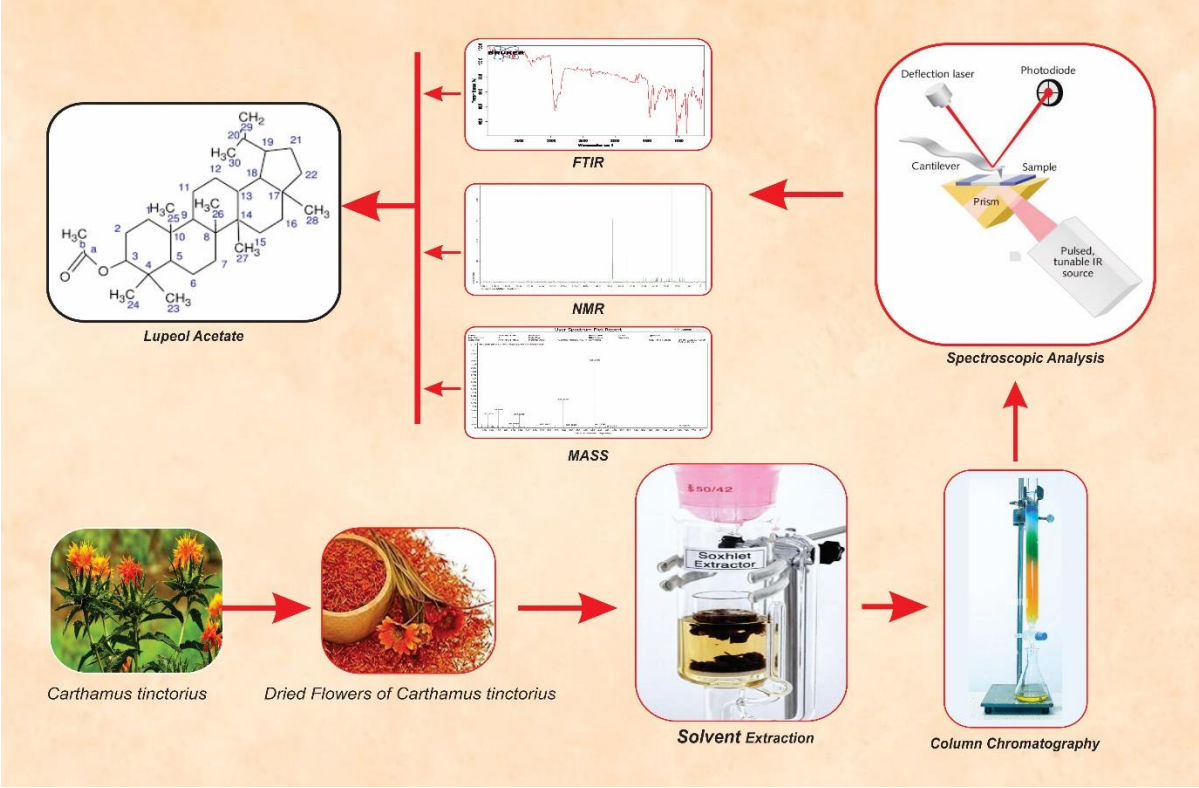
Furthermore, mass spectrometry provided crucial molecular composition data, with peaks corresponding to the molecular ion and fragmentation patterns characteristic of pentacyclic triterpenoids lupeol, reinforcing the structural assignment.

Overall, the convergence of data from multiple analytical techniques supports the confident identification of the compound as a pentacyclic triterpenoid lupeol. This comprehensive characterization lays the groundwork for further investigations into its biological activities and potential applications.

5. Conclusion

In summary, our investigation successfully isolated and characterized lupeol from both the leaves and flowers of *Carthamus tinctorius*. The use of solvent extraction and chromatographic methods facilitated the effective extraction of lupeol. Phytochemical screening of the extract confirmed the presence of terpenoids in both the extract and its n-hexane fraction. The results of IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectroscopy confirm that the isolated compound is lupeol.

Graphical Abstract



Conflict of interests: None

List of Abbreviations

^{13}C-NMR: Carbon-13 (C13) nuclear magnetic resonance

^{1}H-NMR: Proton nuclear magnetic resonance,
DMSO-d6: Deuterated dimethyl sulfoxide
FID:Free induction decay
FTIR: Fourier Transform Infrared Spectroscopy,
KBr:Potassium bromide
TLC:Thin-layer chromatography
TMS: Tetramethylsilane
UV: Ultraviolet

References:

1. Besbes, M., Guérineau, V., and Touboul, D. (2017). Isolation and structure elucidation of acetylcholinesterase lipophilic lupeol derivatives inhibitors from the latex of the Tunisian Periploca laevigata. Arabian Journal of Chemistry *10*, S2767-S2772.
2. Furukawa, S., Takagi, N., Ikeda, T., Ono, M., Nafady, A. M., Nohara, T., Sugimoto, H., Doi, S., and Yamada, H. (2002). Two novel long-chain alkanoic acid esters of lupeol from alecrim-propolis. Chemical and pharmaceutical Bulletin *50*, 439-440.
3. Harborne, A. (1998). Phytochemical methods a guide to modern techniques of plant analysis: springer science & business media).
4. Koriem, K. M. M. (2021). Focus on Phytochemical Screening, Chemical Constituents, Pharmacological Effects and Medical Uses of Gummi myrrha. Biointerface Research in Applied Chemistry *12*, 5510-5522.
5. Mimaki, Y., Yokosuka, A., Hamanaka, M., Sakuma, C., Yamori, T., and Sashida, Y. (2004). Triterpene Saponins from the Roots of Clematis c hinensis. Journal of natural products *67*, 1511-1516.
6. Muktar, B., Bello, I., and Sallau, M. (2018). Isolation, characterization and antimicrobial study of lupeol acetate from the root bark of Fig-Mulberry Sycamore (Ficus sycomorus LINN). Journal of Applied Sciences and Environmental Management *22*, 1129-1133.
7. Nasution, R., Barus, T., Nasution, P., and Saidi, N. (2014). Isolation and structure elucidation of steroid from leaves of Artocarpus camansi (Kulu) as antidiabetic. International Journal of PharmTech Research *6*, 1279-1285.
8. Nasution, R., Marianne, M., and Nur, H. (2015). β-Amyrin acetate of ethyl acetate extract of the bark plant Artocarpus camansi and its antidiabetic activity.
9. Pant, R., Irchhaiya, R., and Rao, C. V. (2019). A research article phytochemical and pharmacological evaluation of Carthamus tinctorius leaves. Journal of Drug Delivery and Therapeutics *9*, 285-294.
10. Patocka, J. (2003). Biologically active pentacyclic triterpenes and their current medicine signification. J Appl Biomed *1*, 7-12.

11. Prachayasittikul, S., Saraban, P., Cherdtrakulkiat, R., Ruchirawat, S., and Prachayasittikul, V. (2010). New bioactive triterpenoids and antimalarial activity of Diospyros rubra Lec. Excli Journal *9*, 1.

12. Qiao, C. F., Han, Q. B., Song, J. Z., Mo, S. F., Kong, L. D., Kung, H. F., and Xu, H. X. (2007). Chemical fingerprint and quantitative analysis of Fructus Psoraleae by high-performance liquid chromatography. Journal of Separation Science *30*, 813-818.

13. Sparkman, O. D., Penton, Z., and Kitson, F. G. (2011). Gas chromatography and mass spectrometry: a practical guide: Academic press).

14. ToMosAkA, H., Koshino, H., Tajika, T., and OMATA, S. (2001). Lupeol esters from the twig bark of Japanese pear (Pyrus serotina Rehd.) cv. Shinko. Bioscience, biotechnology, and biochemistry *65*, 1198-1201.

15. Xie, P., Chen, S., Liang, Y.-z., Wang, X., Tian, R., and Upton, R. (2006). Chromatographic fingerprint analysis—a rational approach for quality assessment of traditional Chinese herbal medicine. Journal of chromatography A *1112*, 171-180.