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Phytochemical characterization of *Alcea rosea* seeds using gas chromatography-mass spectrometry: A preliminary investigation of bioactive compounds

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Abstract

The growing demand for natural therapeutic agents has intensified interest in plant-based compounds with potential pharmacological applications. *Alcea rosea*, traditionally employed in various ethnomedical systems, particularly for inflammatory and respiratory conditions, remains underexplored at the phytochemical level, especially concerning its seeds. The current study aims to bridge this gap by evaluating the chemical constituents of *A. rosea* seeds and assessing their potential bioactive significance using advanced analytical techniques. Ethyl acetate extraction was chosen for its efficiency in isolating semi-polar compounds, followed by gas chromatography-mass spectrometry to identify the chemical content of the extract. This method enabled the detection of over 50 distinct molecular entities, among which 15 were identified as major components based on peak abundance and database matching using the National Institute of Standards and Technology mass spectral library and the Wiley Registry of Mass Spectral Data. The chemical landscape of the extract included a range of fatty acids, esters, and complex organic molecules, many of which are known to possess anti-inflammatory, antioxidant, antimicrobial, and cytotoxic properties. Notable among these were linoleic acid, oleic acid, and palmitic acid compounds, which are widely recognized for their contributions to cellular signaling, oxidative stress modulation, and tumor suppression. The identification of bis(2-ethylhexyl) phthalate and A13-09519 (chemically ethyl N-methylcarbamate) also points to lesser-known or unconventional phytoconstituents that may hold biomedical interest, although their origin and activity warrant further validation. This investigation reveals *A. rosea* seeds as a chemically rich botanical source with a diverse array of compounds that could support therapeutic applications. The study sets a precedent for future bioactivity-driven analyses and highlights the value of integrating traditional knowledge with modern phytochemical screening to uncover novel drug leads from botanical sources.

Keywords: *Alcea rosea*; Ornamental; Ethyl acetate; Gas chromatography mass–spectrometry analysis; Phytoconstituents

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1. Introduction

Alcea rosea, widely recognized as hollyhock and a member of the Malvaceae family, is not only admired for its ornamental appeal but also revered for its long-standing role in traditional medicinal practices. This herbaceous plant has been cultivated and utilized in folk medicine across various geographical regions, including Iran, Central and South Asia, and several parts of Europe, with a notable ethnomedicinal presence in the Kashmir Valley.[1-3] Conventionally, multiple parts of the plant, particularly its roots, have been employed to manage a range of ailments, such as respiratory disorders (bronchitis and cough), gastrointestinal disturbances (ulcers and dyspepsia), and various inflammatory conditions. The continued use of *A. rosea* in these settings underscores its cultural and pharmacological importance across generations. Scientific inquiry into the medicinal potential of *A. rosea* has validated many of these traditional uses. Pharmacological investigations have attributed a wide spectrum of bioactivities to this plant, with specific studies demonstrating its anti-inflammatory activity,[4] antibacterial properties,[5] antiurolithiatic activity,[6] analgesic effects,[7] and immunomodulatory activity.[8] These therapeutic attributes are largely attributed to the plant's rich phytochemical reservoir, which includes a diverse assortment of flavonoids, phenolic acids, polysaccharides, and essential oils.[9] These secondary metabolites are well known for their roles in modulating oxidative stress, microbial growth, immune responses, and inflammatory pathways, thus providing a scientific basis for the plant's traditional applications.

In a prior collaborative study conducted by our team,[10] the anticancer properties of *A. rosea* seed extracts were investigated. In particular, ethyl acetate-derived fractions of the seed extract were shown to exhibit significant cytotoxic activity against colorectal cancer (CRC) cells. The extract was observed to suppress cell proliferation, arrest the cell cycle at the G0/G1 phase, and induce apoptotic pathways in CRC cell lines. Notably, the extract exhibited the capacity to target CRC stem cells, a subpopulation of cells associated with tumor initiation, therapy resistance, and recurrence. This was evidenced by a marked downregulation of pivotal cancer stem cell surface markers, including aldehyde dehydrogenase 1, CD133, CD44, CD166, CD24, and doublecortin-like kinase 1, which play crucial roles in maintaining stemness and self-renewal capabilities in colorectal tumors. In addition, the extract's anticancer mechanism appeared to involve the inhibition of major oncogenic signaling cascades, particularly the Wnt/β-catenin and PI3K/Akt pathways, which are frequently activated in CRC and known to regulate cell survival, proliferation, and stemness.[10] Furthermore, the treatment was found to modulate epigenetic regulators, notably through the downregulation of the enhancer of zeste homolog 2, a histone methyltransferase implicated in cancer progression and stem cell maintenance. Simultaneously, an upregulation of tumor suppressor proteins, such as E-cadherin, was observed, indicating a reversal of epithelial-to-mesenchymal transition, a key process in metastasis.[10]

These findings collectively underscore the multifactorial anticancer potential of *A. rosea* seed extracts, highlighting the plant's rich phytochemical diversity and its capacity to influence a range of molecular and cellular targets implicated in CRC progression. The ability of the extract to inhibit cell proliferation, arrest the cell cycle, induce apoptosis, suppress oncogenic signaling, and downregulate cancer stem cell markers presents a compelling case for its further investigation as a multitargeted therapeutic agent. Importantly, these effects are not attributable to a single mechanism but rather suggest a coordinated modulation of various intracellular pathways, which may enhance therapeutic efficacy and reduce the risk of resistance development. Such a holistic pharmacological profile not only supports its use in traditional medicine but also provides a scientifically robust foundation for modern drug discovery initiatives. As such, the existing body of evidence strongly advocates for expanded preclinical research, mechanistic elucidation, and chemical characterization to unlock its full therapeutic potential. Motivated by these earlier findings, the present study was conceptualized to advance this line of inquiry by aiming to isolate, purify, and characterize individual bioactive compounds from the ethyl acetate crude extract of *A. rosea* seeds. While prior work convincingly demonstrated that the crude extract exhibits potent anticancer activity, the specific chemical entities responsible for these effects remained unidentified. The complexity of the extract necessitates detailed phytochemical investigation to distinguish the active constituents from inactive or potentially synergistic compounds. This step is essential to facilitate a structure activity relationship (SAR) analysis, optimize bioactivity, and enable further preclinical validation of the most promising candidates. In addition to direct cytotoxic and antiproliferative actions, the antioxidant and anti-inflammatory activities of *A. rosea* play a crucial role in its chemopreventive potential, especially relevant in the context of CRC. Oxidative stress and chronic inflammation are well-established drivers of colorectal tumor initiation and progression, often creating a microenvironment that supports genetic mutations, aberrant signaling, and immune evasion.[11,12] By mitigating these processes, the phytochemicals in A.rosea may not only inhibit tumor growth but also prevent tumorigenesis

at an early stage. Thus, the plant's dual chemotherapeutic and chemopreventive capabilities merit focused research to elucidate their underlying biochemical mechanisms and therapeutic implications. Despite these promising observations, the full therapeutic landscape of *A. rosea* seeds remains largely underexplored, particularly regarding their utility in CRC. Most available studies have concentrated on general pharmacological properties or the effects of crude extracts, with limited attention given to the identification and validation of individual active molecules. This represents a significant knowledge gap, especially when considering the plant's historical usage and initial scientific evidence suggesting strong anticancer properties.

Accordingly, the present study aims to address this gap by undertaking a comprehensive chemical fingerprinting of *A. rosea* seed extracts using advanced analytical techniques. By establishing a detailed profile of the constituent phytochemicals, this work seeks to provide a scientific platform for targeted pharmacological evaluations. Ultimately, these efforts aim to support the integration of *A. rosea* into evidence-based, plant-derived anticancer strategies, offering a natural, multipronged alternative for CRC management and potentially other malignancies.

2. Materials and methods

2.1. Collection and identification of plant material

The entire plant of *A. rosea* was meticulously collected from Malhar Hi-Tech Nursery, a recognized horticultural center situated in the Ganderbal district of Kashmir, India. This region is geographically located at a latitude of 34°13'35"N and a longitude of 74°47'34"E, with an elevation of 1,732.77 m above sea level, offering a temperate climate and suitable edaphic conditions conducive to the growth of medicinal and ornamental flora. The selection of this site was deliberate, as the natural habitat of the plant is known to influence its phytochemical profile, and high-altitude locations are often associated with increased secondary metabolite production due to enhanced exposure to environmental stressors.

To ensure the highest quality of the plant material and preserve the integrity of its bioactive constituents, sampling was strategically carried out during September, which corresponds to the late flowering and early seed maturation phase of *A. rosea*. This period is considered optimal for collecting seeds and aerial parts intended for pharmacological and phytochemical analyses, as it coincides with peak metabolite accumulation in many medicinal plants. Great care was taken during harvesting to avoid contamination, physical damage, or degradation of the plant material, thereby maintaining its therapeutic potential for subsequent laboratory analysis.

For accurate botanical classification, the plant material underwent taxonomic identification and authentication at the center of plant taxonomy (COPT), Department of Botany, University of Kashmir, which is a reputable institution for regional floristic studies. The identification process was conducted by qualified plant taxonomists, employing both macroscopic and microscopic diagnostic characteristics in accordance with standard botanical keys and herbarium references. To facilitate reproducibility of the study and allow for future taxonomic verification by other researchers, a voucher specimen (No. 2289-KASH, dated September 25, 2017) was formally prepared and deposited in the herbarium of COPT.

2.2. Extraction

The extraction of phytochemicals from *A. rosea* seeds was conducted using a carefully optimized and standardized protocol to ensure maximum recovery and integrity of bioactive constituents. Initially, the seeds were meticulously sorted and cleaned by gentle rinsing, followed by air-drying to eliminate any adhering dust particles and superficial surface contaminants. This pre-cleaning step was essential to prevent interference in subsequent extraction steps and analytical procedures. After cleaning, the seeds were shade-dried under aseptic conditions at ambient temperature (not exceeding 30°C) to preserve thermolabile and photosensitive compounds, which are often degraded under direct sunlight or elevated temperatures. The drying process continued for approximately seven days until a constant weight was achieved, indicating the removal of residual moisture.

Once completely dried, the seeds were subjected to coarse grinding using a stainless-steel mechanical grinder, which minimized metal contamination and thermal friction. The powdered seed material was then sieved through a No. 50 mesh (Retsch test sieve, 90 µm, stainless steel, 200 mm × 50 mm, DIN ISO 3310/1) to ensure uniform particle size distribution, an essential criterion that facilitates homogeneous solvent penetration and enhances extraction efficiency. A consistent powder texture contributes to the reproducibility of phytochemical extraction and is particularly critical when comparing extracts across solvents or replicates.

To selectively remove non-polar components and enhance the subsequent extraction of pharmacologically relevant semi-polar compounds, a two-step extraction approach was employed. In the first step, 2.3 kg of the sieved seed powder underwent defatting using analytical-grade *n*-hexane (ACS reagent, Sigma-Aldrich, assay

≥96.0%, CAS No. 110-54-3), a non-polar solvent widely used to dissolve and eliminate lipids, waxes, sterols, and other hydrophobic impurities. The powder was macerated with *n*-hexane for 12 h at room temperature with occasional agitation to ensure complete solvent contact. The hexane extract, which predominantly contained non-polar interfering substances, such as triglycerides and chlorophyll derivatives, was subsequently discarded. The residual defatted biomass, now enriched in semi-polar phytochemicals, was dried and prepared for further solvent extraction.

In the second step, the defatted seed material was subjected to exhaustive maceration using analytical-grade ethyl acetate (ACS reagent, Sigma-Aldrich, assay ≥ 99.5 %, CAS No. 141-78-6), a mid-polarity solvent ideal for extracting a wide range of semi-polar compounds. These include flavonoids, phenolic acids, coumarins, alkaloids, and certain terpenoids that are often responsible for the therapeutic activities attributed to medicinal plants. The maceration was performed in a multistep manner (5 × 5 L) over a total duration of 72 h, with the solvent refreshed at each interval to ensure comprehensive extraction. The use of ethyl acetate was guided by its high selectivity, relatively low toxicity, and minimal co-extraction of undesirable polymeric or pigment-based contaminants.

The pooled ethyl acetate extracts were filtered using filter paper (Whatman No. 1, 11 µm pore size, 180 µm thickness, ash ≤ 0.06%, Sigma-Aldrich, Whatman 1001-045, dia. 45 mm) to remove coarse particles and plant debris, and then concentrated using a rotary evaporator (Büchi Rotavapor R-100, Sigma-Aldrich, model Z741982) under reduced pressure at 35°C. This gentle concentration method preserved the chemical integrity of heat-sensitive compounds while efficiently removing the solvent. The process ultimately yielded 107 g of dark-brown, semi-solid crude extract, corresponding to a 4.6% (w/w) yield relative to the starting seed mass of 2.3 kg.

To support the rational selection of ethyl acetate as the principal extraction solvent, a preliminary solvent screening study was conducted. This screening compared ethyl acetate with methanol, ethanol, and chloroform under identical maceration conditions. The solvents were assessed based on four key parameters: (i) Total extractive yield, (ii) total phenolic and flavonoid content, (iii) qualitative phytochemical profile by thin-layer chromatography (TLC), and (iv) antiproliferative activity against HCT116 CRC cells using a standard MTT-based cytotoxicity assay.

Among all tested solvents, ethyl acetate demonstrated the most favorable profile, achieving a balanced combination of extraction yield, phytochemical richness, and biological activity. It efficiently extracted a broad spectrum of bioactive compounds, exhibited comparatively higher antiproliferative potential, and minimized the co-extraction of unwanted pigments and polymeric substances. Furthermore, the TLC chromatograms revealed better separation and resolution of phytoconstituents in ethyl acetate compared to the other solvents.

To ensure reproducibility and process reliability, the optimized extraction procedure was repeated in triplicate, yielding consistent results with a variation of less than ± 0.3% in the final extract weight. This low variability affirms the robustness and repeatability of the protocol. The final ethyl acetate crude extract was transferred into amber glass bottles, which shield the sample from light-induced degradation, and stored at 4°C in a desiccator to prevent moisture uptake and preserve its stability until further use.

This rigorously standardized and analytically validated extraction process not only ensures optimal recovery of pharmacologically relevant phytoconstituents from *A. rosea* seeds but also provides a reproducible platform for subsequent steps, involving fractionation, compound isolation, phytochemical characterization, and biological evaluation. The methodological rigor adopted here sets a strong foundation for advancing the therapeutic exploration of *A. rosea* in cancer and other disease models.

2.3. Gas chromatography–mass spectrometry (GC-MS) analysis

To elucidate the phytochemical composition of the ethyl acetate extract of *A. rosea* seeds, analysis was performed using GC-MS (QP2010 Ultra system, Shimadzu, Japan), an advanced analytical platform known for its high sensitivity and resolution in identifying complex organic compounds. This technique was chosen for its capability to separate, detect, and characterize volatile and semi-volatile constituents, which are typically abundant in plant extracts and often responsible for key pharmacological properties.

The gas chromatographic conditions were carefully optimized to ensure maximal resolution of the analytes. The ethyl acetate extract of *A. Rosea* seeds was analyzed using a 5% phenyl-methylpolysiloxane stationary phase column (Hewlett-Packard, Agilent Technologies, USA). The column oven temperature was initially set at 80°C, providing a stable baseline for the early eluting compounds. The injector temperature was maintained at 250°C to facilitate complete vaporization of the sample without thermal degradation. The injection mode was configured to split, which helps reduce the sample load entering the column and improves peak shape and reproducibility. A flow control mode with linear velocity was selected, coupled with a column flow rate set at 1 mL/min, ensuring

consistent carrier gas movement through the column. The purge flow was maintained at 3 mL/min, effectively eliminating non-volatile residues and preventing contamination. A split ratio of 5 was employed to balance sample dilution with analytical sensitivity.

The temperature programming was structured in a two-stage ramp. Initially, the temperature was increased from 80°C at a rate of 3°C/min until it reached 200°C, allowing for effective separation of low to mid-boiling compounds. This was followed by a further increment of 10°C, which was then held at 200°C for 5 min to facilitate elution of higher boiling-point components. This gradient-based temperature programming enabled comprehensive profiling of a wide range of phytochemicals within the sample, spanning various polarity and volatility ranges.

For the mass spectrometric detection, the ion source temperature was precisely maintained at 240°C, allowing for efficient ionization of eluting analytes. The interface temperature was stabilized at 250°C, ensuring thermal consistency between the gas chromatography and mass spectrophotometry units. A solvent cut time of 2 min was programmed to prevent early solvent peaks from interfering with the detector response. The mass scan range was configured from 45 to 900 m/z, accommodating a broad spectrum of molecular weights and enabling detection of both small and large molecular species. The event time was set to 0.30 seconds, providing a good balance between spectral resolution and scan speed. Data acquisition was initiated at 3.5 min and concluded at 51 min, covering the complete elution profile of the sample.

These carefully calibrated parameters created optimal chromatographic and mass spectrometric conditions, allowing for the accurate separation, identification, and quantification of the chemical constituents present in the ethyl acetate extract of *A. rosea* seeds. The analytical procedure enabled a detailed chemical fingerprinting, providing critical insights into the phytochemical diversity and potential bioactivity of the extract. This comprehensive approach contributes significantly to the standardization and quality assessment of plant-derived extracts intended for pharmacological evaluation.

2.4. Identification of components

The GC-MS analysis of the ethyl acetate extract of *A. rosea* seeds was performed over a total runtime of 51 min, during which the complete chromatographic elution of compounds was observed. The resulting chromatogram, as depicted in Figure 1, presents a clear distribution of peaks corresponding to the various volatile and semi-volatile constituents detected in the sample. Each peak represents a distinct compound or a class of structurally related compounds that were subsequently analyzed for identification.

The mass spectral data obtained from the analysis were subjected to comparative interpretation using established spectral libraries, specifically the National Institute of Standards and Technology (NIST) database and the Wiley Registry of mass spectral data (Wiley library). These spectral databases are widely accepted and commonly used for high-confidence compound identification in natural product research and metabolomic profiling. The NIST library, in particular, is known for its extensive catalog of mass spectra for organic and inorganic compounds. On the other hand, the Wiley library provides complementary coverage of bioactive and naturally occurring substances.

For those chromatographic peaks corresponding to unidentified components, the mass spectra were matched against reference spectra of known compounds stored in the NIST and Wiley libraries. This comparative matching was based on fragmentation patterns, base peaks, molecular ion values, and relative abundance profiles. Spectral similarities and matching scores were carefully assessed to ensure a high degree of accuracy in compound identification. For ambiguous or partially matched components, further comparison with structurally similar compounds aided in approximating molecular frameworks, functional groups, and possible analogs.

Through this rigorous spectral comparison process, a range of bioactive phytochemicals present in the *A. rosea* seed extract were successfully identified. These included compounds with well-characterized molecular weights, structural moieties, and known or predicted pharmacological activities. The application of such a robust and validated method enabled a comprehensive chemical characterization of the extract, enabling researchers to draw informed correlations between chemical composition and potential biological functions.

3. Results

The GC-MS analysis of the *A. rosea* seed extract identified 15 major compounds along with 113 minor compounds, which were further characterized. These compounds were discerned based on their elution order. Table 1 presents the major compounds together with their corresponding peaks, retention times, areas, and area percentages. In addition, Figure 1 illustrates the gas chromatogram of the ethyl acetate extract, and Figure 2 provides a pie chart representation of the area percentages covered by the major compounds identified in the extract. Among the identified major compounds, six were pharmacologically important due to their significant concentrations and potential medicinal properties. These include:

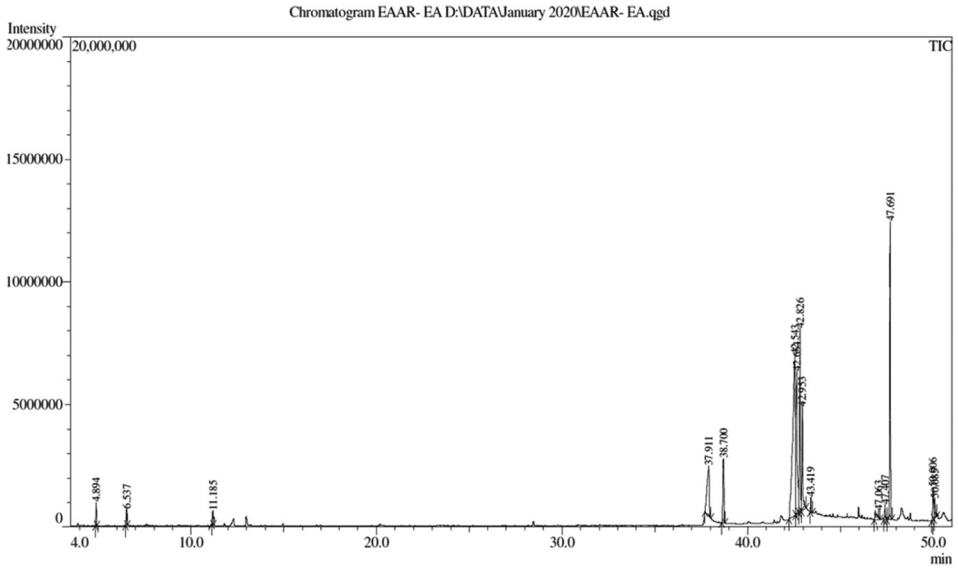


Figure 1. Gas chromatography–mass spectrometry chromatogram of ethyl acetate extract of *Alcea Rosea* seeds
Abbreviation: TIC: Total ion chromatogram.

Table 1. Phytocompounds identified in the ethyl acetate extract of *Alcea Rosea* seeds

Peak	Retention time (min)	Area	Area %	Name of compound	Molecular formula	Molecular weight
1	4.894	1741042	0.83	1-decyne	$C_{10}H_{18}$	138
2	6.537	1974686	0.94	*D*-mannitol	$C_6H_{14}O_6$	182
3	11.185	2101064	1.00	A13-09519	C_4H_9NO	103
4	37.911	16031218	7.67	n-hexadecenoic acid	$C_{16}H_{30}O_2$	254
5	38.700	9704774	4.64	Hexadecanoic acid	$C_{16}H_{32}O_2$	256
6	42.543	62456355	29.87	9,12-octadecadienoic acid (*Z, Z*)	$C_{18}H_{32}O_2$	280
7	42.651	31113346	14.88	*Cis*-9-octadecenoic acid (*E*)	$C1_8H_{34}O_2$	282
8	42.826	21555589	10.31	Linoleic acid ethyl ester	$C_{20}H_{34}O_2$	306
9	42.953	14520846	6.94	(*E*)-9-octadecenoic acid ethyl ester	$C2_0H_{38}O_2$	310
10	43.419	1354562	0.65	Octadecanoic acid	$C_{18}H_{36}O_2$	284
11	47.063	3022341	1.45	Bis (2-ethylhexyl) phthalate	$C_6H_4(COOC_8H_{17})_2$	390
12	47.407	1935050	0.93	Hexadecanoic acid	$C_{16}H_{32}O_2$	254
13	47.691	33674102	16.10	Bis (2-ethylhexylexyl) phthalate	$C_6H_4(COOC_8H_{17})_2$	390
14	50.006	4651105	2.22	9,12-octadecadienoic acid (*Z, Z*)-2,3 dihydroxypropyl ester	$C2_1H_{38}O_4$	354
15	50.085	3291589	1.57	9-octadecenoic acid (*Z*)-2,3-dihydroxy propyl ester	$C2_1H_{34}O_4$	356
Total		209127669	100.00			

(i) 9,12-Octadecadienoic acid (29.87%)
(ii) Bis(2-ethylhexyl) phthalate (DEHP) (16.10%)
(iii) *Cis*-9-octadecenoic acid (14.88%)
(iv) Linoleic acid ethyl ester (10.31%)
(v) n-hexadecenoic acid (7.67%)
(vi) A13-09519 (ethyl N-methylcarbamate, currently under clinical trials for anti-HIV treatment) (1%).

4. Discussion

In our current investigation, the GC-MS analysis of the ethyl acetate extract from *A. rosea* seeds revealed a highly diverse and complex phytochemical profile, reflecting the plant's rich metabolic output. The chromatographic analysis detected over 50 distinct peaks, each representing different volatile or semi-volatile compounds, as shown

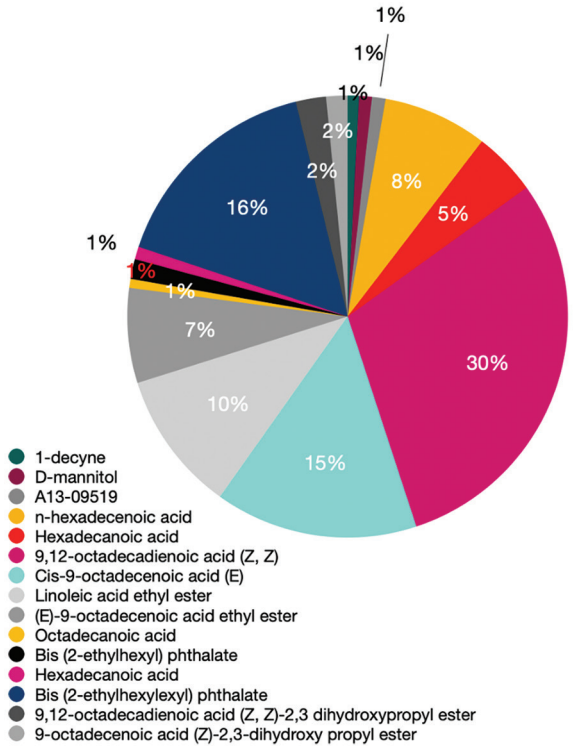


Figure 2. Pie chart showing the major compounds in the ethyl acetate extract of *Alcea Rosea* seeds

in Figure 1. Among these, 15 major compounds were selected for detailed evaluation based on their relative abundance and pharmacological relevance, as presented in Table 1. Compound identification was accomplished through meticulous spectral interpretation using both the NIST databases and Wiley libraries, which are well-established resources for the structural elucidation of organic molecules. These results, supplemented by detailed structural confirmation, are further supported in the Supplementary File, which contains full-spectrum and structural annotations. The findings indicate that *A. rosea* seeds contain an extensive array of bioactive small molecules, many of which exhibit therapeutic potential, particularly in the context of inflammation, cancer, and metabolic regulation.

Among the constituents identified, 9,12-octadecadienoic acid, a polyunsaturated omega-6 fatty acid, also known as linoleic acid, emerged as the dominant compound, accounting for 29.87% of the total area under the chromatogram. This compound has been extensively documented in the literature for its broad spectrum of biological activities, including anticancer,[13,14] cardioprotective,[15] anti-inflammatory,[16] antioxidant,[17] and neuroprotective properties.[18] In addition, 9,12-octadecadienoic acid has demonstrated antiproliferative effects in several cancer cell lines, such as KPL,[19] Caco-2,[20] MAC16,[21] DU145,[22] BT74, and A-549,[23] indicating its potential in multicancer chemoprevention and therapy. Its structural characteristics enable it to modulate lipid signaling pathways, influence membrane dynamics, and alter intracellular redox states, mechanisms often exploited in oncological interventions. Another abundant compound detected was DEHP, which contributed approximately 16.10% to the total area. While this compound is widely recognized as a synthetic plasticizer, it has also been isolated from biological specimens and plant extracts under specific conditions. Reports suggest its anti-tumor activity in various preclinical settings.[24] However, given its ubiquitous presence in plastic laboratory consumables and solvents, the identification of DEHP in this study remains provisional. Acknowledging its potential as a GC-MS artifact, we emphasize the importance of future validation using instrumental blanks, glass-only extraction protocols, and orthogonal analytical methods, such as liquid chromatography-mass spectrometry/tandem mass spectrometry (LC-MS/MS) and nuclear magnetic resonance (NMR) spectroscopy, to confirm its true origin and rule out contamination.

Another compound, *cis*-9-octadecenoic acid, more commonly known as oleic acid, was also identified in significant concentration, representing 14.88% of the extract. Oleic acid, a monounsaturated omega-9 fatty acid, is widely acknowledged for its health-promoting properties. It has been shown to exert antiproliferative effects by upregulating critical tumor suppressor genes, including p53, p21, and p27, especially in esophageal cancer cells.[25] Furthermore, it has been reported to modulate apoptotic and autophagic signaling pathways, thereby contributing to the inhibition of tongue squamous cell carcinoma progression.[14] In addition, oleic acid demonstrates the capacity to suppress Her-2/neu overexpression, a key oncogenic driver in breast cancer, making it a compound of high interest in targeted therapies.[26] In the cardiovascular context, oleic acid contributes to reduced low-density lipoprotein cholesterol levels and oxidative stress, thereby enhancing endothelial function. These properties align with its established role in promoting cardiovascular health, particularly in the context of Mediterranean dietary patterns.[15] Furthermore, oleic acid possesses significant anti-inflammatory potential, mediated by the downregulation of pro-inflammatory cytokines, such as interleukin-6 and tumor necrosis factor-α, as well as the inhibition of cyclooxygenase-2 expression.[16] It also acts as an effective antioxidant, scavenging free radicals and stabilizing lipid membranes, thereby mitigating oxidative damage.[17] In addition, emerging studies suggest that oleic acid plays a neuroprotective role, potentially due to its

anti-inflammatory and antioxidant properties.[18] These multifaceted pharmacological attributes warrant further investigation of oleic acid as a key bioactive constituent of *A. rosea* seed extracts. The accurate International Union of Pure and Applied Chemistry chemical structure of oleic acid, along with other pharmacologically relevant compounds, is illustrated in Figure 3, developed based on validated data from chemical databases, such as PubChem and ChemSpider.

Although present in a smaller proportion, linoleic acid ethyl ester (0.31%) is also noteworthy. This compound has been documented to possess anti-inflammatory,[27] anti-acne,[28] and antidiabetic activities.[29] Its relevance in dermatological and metabolic applications is supported by studies showing modulation of lipid metabolism, insulin sensitivity, and inflammatory mediator production, even at low concentrations. Similarly, hexadecanoic acid (palmitic acid), contributing 7.67% of the extract, has been linked to anticancer activity through its ability to inhibit β-catenin expression in HT29 colon cancer cells, particularly when conjugated with dicer-substrate small interfering RNA.[30] Since β-catenin overexpression is a hallmark of colorectal tumorigenesis, its downregulation by hexadecanoic acid may present an important therapeutic avenue. In addition, this compound also exhibits anti-androgenic effects,[31] supporting its broader role in hormonal regulation and cancer treatment.

Among the unique constituents identified, A13-09519, chemically classified as ethyl N-methylcarbamate, was also present. Although less frequently reported in natural products literature, data from databases, such as PubChem and DrugBank, suggest that A13-09519 has biological activity targeting Shiga-like toxin 1 subunit B, a pathogenic factor that binds to globotriaosylceramide receptors on human intestinal microvilli.[32-34] Given its potential implication in toxin neutralization and microbial interactions, it warrants further investigation. However, due to its structural class and occurrence pattern, A13-09519 is also under consideration as a possible instrument- or solvent-derived contaminant, necessitating follow-up validation through high-resolution mass spectrometry and comparison with a reference compound.

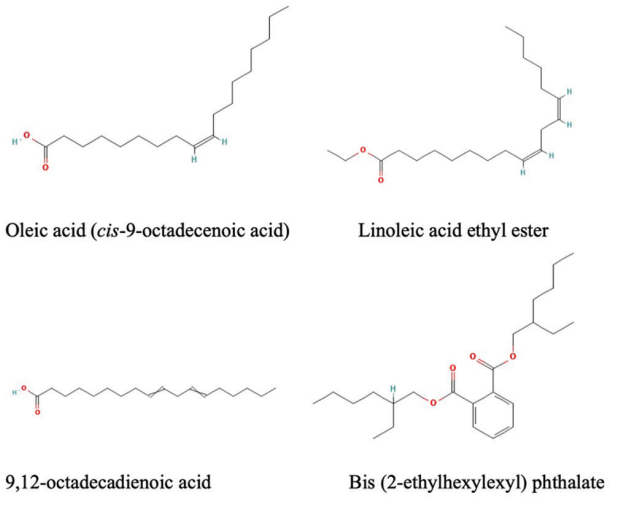


Figure 3. Chemical structure of pharmacologically important compounds identified in the ethyl acetate extract of *Alcea Rosea* seeds

Collectively, these findings reflect the therapeutic promise of several compounds identified within the ethyl acetate extract of *A. rosea* seeds. However, it is important to note that GC-MS analysis is inherently limited to detecting volatile and thermally stable compounds, potentially overlooking a vast range of non-volatile phytoconstituents, such as alkaloids, glycosides, flavonoids, and tannins, which are commonly found in medicinal plants. To obtain a comprehensive metabolic profile, future studies will incorporate advanced analytical platforms, such as LC-MS, ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry, and NMR spectroscopy. These approaches will enable the identification of polar, thermolabile, and high-molecular-weight constituents that are inaccessible via GC-MS. While the current study draws upon existing literature to suggest potential bioactivities of the identified compounds, it is recognized that these SARs are preliminary and correlative. Hence, moving forward, we aim to conduct bioassay-guided fractionation, *in vitro* cytotoxicity evaluations, and *in vivo* pharmacokinetic and toxicity assessments. These investigations will not only help confirm the biological relevance and safety of the crude extract and its constituents but also contribute to the rational development of phototherapeutics derived from *A. rosea*.

5. Conclusion

The current investigation offers a foundational insight into the phytochemical architecture of *A. rosea* seeds, shedding light on their potential as a reservoir of biologically active natural compounds. The study employed GC-MS, a sensitive and robust analytical tool, to characterize the ethyl acetate extract. This led to the successful identification of several high-abundance and pharmacologically significant compounds, validating the traditional medicinal use of *A. rosea* and supporting its consideration in modern drug discovery pipelines. The predominance of linoleic acid among the identified constituents highlights the relevance of this polyunsaturated fatty acid in the seed's pharmacodynamic landscape. Known for its anti-inflammatory and antioxidant properties, linoleic acid is also implicated in cellular signaling pathways that govern proliferation and apoptosis. Its significant representation within the extract provides

a biochemical rationale for the potential anticancer and cardioprotective effects traditionally attributed to *A. rosea*. Similarly, the detection of oleic acid contributes to the therapeutic properties of the extract, which modulate the expression of tumor suppressor genes and interfere with oncogenic signaling networks. Its reported effects on cell cycle regulation and apoptosis, particularly in various epithelial malignancies, strengthen the argument for further exploration of *A. rosea* in oncology-focused phytotherapy research. A nuanced aspect of the findings involves the identification of DEHP, a compound typically associated with synthetic sources but increasingly reported in plant-derived matrices. While its pharmacological attributes, such as antimicrobial and cytotoxic potential, warrant consideration, its dual identity as a potential laboratory contaminant emphasizes the importance of confirmatory analyses in natural products research.

Future studies should incorporate stringent control measures and utilize orthogonal techniques, such as LC-MS/MS and NMR, to verify the origin of compounds and authenticity. The presence of other structurally and pharmacologically diverse molecules, including palmitic acid and linoleic acid ethyl ester, suggests a synergistic matrix of fatty acids within *A. rosea* seeds that could collectively influence biological systems. These compounds, even at modest concentrations, are known to participate in lipid signaling, immune modulation, and metabolic regulation areas highly relevant to chronic diseases, including diabetes and cancer. Importantly, the identification of A13-09519, a compound with a lesser-known natural occurrence but suggested involvement in microbial toxin interactions, opens an exciting avenue for exploration in antimicrobial and antiviral therapeutics. While the biological role of this compound remains to be validated, its detection in this context merits further pharmacological and structural studies.

While GC-MS served as an efficient tool in profiling volatile and semi-volatile constituents, it is acknowledged that this method is inherently restricted in its scope. Non-volatile classes of compounds, such as flavonoids, alkaloids, and glycosides, which are common in medicinal plants, may have been overlooked. As such, future research should incorporate complementary techniques, including liquid chromatography and high-resolution mass spectrometry, to capture a fuller phytochemical spectrum. In addition, while this study offers meaningful insights into the chemical identity of *A. rosea* seed constituents, the therapeutic implications of these compounds require empirical validation. The proposed bioactivities, although supported by existing literature, must be confirmed through well-designed pharmacological assays, including *in vitro* cytotoxicity, mechanistic pathway analysis, and *in vivo* efficacy and toxicity studies.

In conclusion, the findings of this study lay a solid groundwork for the scientific validation of *A. rosea* seeds as a source of multifunctional bioactive compounds. The chemical diversity observed suggests considerable potential in addressing inflammatory, neoplastic, and microbial conditions. By integrating phytochemical data with biological testing and formulation science, *A. rosea* may be developed into evidence-based therapeutics, reinforcing the relevance of traditional plant knowledge in the advancement of modern pharmacology.

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Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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