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Fatty Acid Profile, Phytochemical Composition and Antimicrobial Effect of Supercritical CO_2 Extracts of *Papaver somniferum* L. Seeds

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HIGHLIGHTS

- Supercritical CO_2 extraction yielded high-quality seed oil
- Linoleic acid was the dominant fatty acid in the oil
- Moderate phenolic and flavonoid contents contributed to activity
- Strong antimicrobial effect against *B. cereus* and *C. albicans*

Abstract

The demand for natural bioactive compounds has increased interest in plant-derived oils obtained through green extraction techniques. *Papaver somniferum* L. seed oil is a promising source of fatty acids and phytochemicals with potential biological activities. This study aimed to evaluate the fatty acid composition, phytochemical content, and antimicrobial activity of *P. somniferum* seed oil obtained by supercritical CO_2 extraction. Oil extraction was performed under optimized conditions (36.75 MPa, 47 °C, CO_2 flow rate: 1 L min^{-1}). Fatty acid composition was analyzed by gas chromatography, while total phenolic and flavonoid contents were determined spectrophotometrically. Antimicrobial activity was assessed using the broth microdilution method against selected Gram-positive,Gram-negative bacteria, and yeast strains.The extraction yielded approximately 20% oil. The fatty acid profile was dominated by polyunsaturated fatty acids, with linoleic acid as the major component, followed by oleic and palmitic acids. The total phenolic and flavonoid contents were 73.70 mg GAE g^{-1} extract and 21.42 mg QE g^{-1} extract, respectively. The oil exhibited the strongest antimicrobial activity against *Bacillus cereus* (MIC: 0.375 mg mL^{-1}) and *Candida albicans* (MIC: 0.75 mg mL^{-1}), while Gram-negative bacteria were less sensitive. The findings indicate that *P. somniferum* seed oil possesses significant bioactive potential, likely due to synergistic interactions between fatty acids and phenolic compounds. Supercritical CO_2 extraction is an effective and environmentally friendly method for obtaining high-quality oil with promising applications in food and pharmaceutical industries.

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1. Introduction

P. somniferum L., belonging to the Papaveraceae family, is an agriculturally and pharmacologically important plant species widely cultivated across Europe, Asia, and the Mediterranean region (Ngernsaengsaruay et al., 2023). While the plant is primarily known for its alkaloid-rich capsules, its seeds have gained increasing attention due to their high nutritional value and functional properties (Hedayati-Moghadam et al., 2022). Poppy seeds are particularly rich in lipids (up to 50%), proteins, and essential fatty acids, making them a valuable source of edible oil (Butnariu et al., 2022). The fatty acid composition of *P. somniferum* seed oil is dominated by polyunsaturated fatty acids (PUFAs), especially linoleic acid (ω-6), followed by oleic and palmitic acids, which are known to play significant roles in cardiovascular health, lipid metabolism, and anti-inflammatory processes (Hlinková et al., 2012). The high PUFA content and favorable lipid profile contribute to the nutritional and therapeutic potential of the oil, particularly in reducing serum cholesterol levels and supporting metabolic health(Azcan et al., 2004).

In addition to its lipid composition, *P. somniferum* seeds contain a diverse range of bioactive phytochemicals, including phenolic compounds, flavonoids, and tocopherols. These compounds are well known for their antioxidant capacity and their ability to modulate biological systems. Phenolic and flavonoid constituents, in particular, have been reported to exert antimicrobial activity through multiple mechanisms, including disruption of microbial cell membranes, inhibition of enzymatic systems, and interference with nucleic acid synthesis (Tajkarimi et al., 2010). The antimicrobial efficacy of plant-derived oils is often closely linked to both their phenolic content and fatty acid composition, as unsaturated fatty acids can also contribute to membrane destabilization and increased permeability in microbial cells.

Recent studies have demonstrated that *P. somniferum* seed oil exhibits antimicrobial activity against a range of pathogenic microorganisms, including both Gram-positive and Gram-negative bacteria as well as fungi, although the degree of susceptibility may vary depending on cell wall structure and composition (Luhmer et al., 2021). Gram-positive bacteria are generally more sensitive due to the absence of an outer membrane, which facilitates the penetration of hydrophobic compounds such as fatty acids and phenolics. In contrast, Gram-negative bacteria possess an outer lipopolysaccharide layer that can act as a barrier, reducing the effectiveness of these bioactive compounds. Therefore, understanding the interplay between fatty acid composition, phytochemical content, and antimicrobial activity is crucial for evaluating the functional potential of plant-derived oils (Courcelle et al., 2001; Keskinkaya et al., 2020).

Alongside the growing interest in the biological properties of plant oils, there has been an increasing demand for sustainable and green extraction technologies. Conventional extraction methods, such as Soxhlet extraction, are often associated with the use of organic solvents, long processing times, and potential degradation of heat-sensitive compounds (Li et al., 2023). In contrast, supercritical carbon dioxide (SCO_2) extraction has emerged as an efficient and environmentally friendly alternative due to its non-toxic nature, recyclability, and ability to preserve bioactive compounds under mild conditions (Ahmad et al., 2019). The unique physicochemical properties of supercritical CO_2, including high diffusivity, low viscosity, and negligible surface tension, enable enhanced mass transfer and improved extraction efficiency (Yang & Hu, 2014). Moreover, the selectivity of SCO_2 can be tuned by adjusting pressure and temperature, allowing targeted extraction of specific lipid and phytochemical fractions (Chemat et al., 2014).

Despite the recognized importance of *P. somniferum* seed oil, studies integrating fatty acid composition, phytochemical characterization, and antimicrobial activity of oils obtained specifically by SCO_2 extraction remain limited. Most previous studies have focused either on conventional extraction techniques or on individual aspects of oil composition without establishing a comprehensive relationship between extraction

method, chemical profile, and biological activity (Dąbrowski et al., 2020; Luhmer et al., 2021). This gap highlights the need for a holistic approach to better understand the functional properties of SCO_2-derived poppy seed oil.

Therefore, the present study aims to comprehensively evaluate the fatty acid profile, total phenolic and flavonoid contents, and antimicrobial activity of *P. somniferum* L. seed oil obtained via supercritical CO_2 extraction under optimized conditions. Additionally, extraction efficiency and process parameters were assessed to elucidate the potential of SCO_2 technology in producing high-quality, nutritionally valuable, and biologically active plant oils. By integrating compositional and functional analyses, this study provides novel insights into the bioactive potential of SCO_2-derived *P. somniferum* seed oil and supports its applications in food, nutraceutical, and pharmaceutical industries.

2. Materials and Methods

2.1. Plant Material and Chemicals

P. somniferum L. seeds were purchased from a local herbal market in Konya, Türkiye. According to the supplier, the seeds originated from Afyonkarahisar, Türkiye, and were harvested in 2025. Since the seeds were commercially obtained, detailed information regarding the cultivar/genotype was not available. All chemicals and reagents used in the study were of analytical grade. Carbon dioxide (CO_2, 99% purity) was supplied by Doğanoksijen (Türkiye) and used as the extraction solvent without further purification.

2.2. Supercritical CO_2 Extraction

Supercritical CO_2 extraction (SCO_2) of *P. somniferum* seeds was performed using a P-25 (35 L) supercritical fluid extraction system (Nantong Borisbang Industrial Technology Co., Ltd., China). The system consisted of a high-pressure extraction vessel (25 L, operating up to 300 bar at 45 °C) and a cyclonic separator (8 L). Extraction was carried out under optimized conditions of 36.75 MPa pressure, 47 °C temperature, and a CO_2 flow rate of 1 L min^{-1}. The extraction process was conducted in a semi-continuous mode, and different fractions were collected at regular intervals. Extraction kinetics were evaluated using a fixed-bed model approach to assess mass transfer behavior and process efficiency. The overall extraction yield was determined to be approximately 20%, and the obtained oil extracts were stored in sealed containers at 4 °C until further analysis (Herrero, 2024).

2.3. GC–MS Analysis and Fatty Acid Composition

The fatty acid composition of *P. somniferum* L. seed oil was evaluated based on chromatographic analysis (Asil & Konuşkan, 2021). The fatty acid composition and phytochemical profile of *P. somniferum* L. seed oil were evaluated by gas chromatography–mass spectrometry (GC–MS) analysis according to the method described by Asil and Konuşkan (2021) with minor modifications. Analyses were performed using an Agilent 7890B GC–MS system (Agilent Technologies, Santa Clara, CA, USA) equipped with an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Agilent 19091S-433). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The injector temperature was maintained at 220 °C, and samples were injected in split mode with a split ratio of 50:1. The oven temperature program was set as follows: initially held at 60 °C for 5 min, then increased to 240 °C at a rate of 4 °C/min and held for 10 min. The transfer line temperature was maintained at 280 °C. Mass spectra were acquired in scan mode using electron ionization (EI) at 70 eV. Compound identification was achieved by comparing the obtained mass spectra with those available in the NIST Mass Spectral Library. The relative abundance of the detected compounds was calculated based on peak area normalization and expressed as percentage composition.

2.4. Determination of Total Phenolic Content (TPC)

Total phenolic content (TPC) of the extracts was determined using the Folin–Ciocalteu method, with gallic acid as the standard (Abdel-Hameed et al., 2012). Briefly, 1 mg of sample was mixed with Folin–Ciocalteu reagent and ultrapure water, followed by the addition of 2% Na_2CO_3 solution. After incubation for 2 hours, absorbance was measured at 760 nm using a UV–Vis spectrophotometer. Results were expressed as mg gallic

acid equivalents (GAE)/g extract based on the calibration curve (R^2 = 0.9931). All measurements were performed in triplicate, and the results were expressed as mean ± SD.

2.5. Determination of Total Flavonoid Content (TFC)

Total flavonoid content (TFC) was determined using the aluminum nitrate colorimetric method with quercetin as the reference compound (Moreno et al., 2000). Sample solutions were mixed with ethanol, potassium acetate, and aluminum nitrate, followed by incubation at room temperature for 40 minutes. Absorbance was measured at 415 nm.Results were expressed as mg quercetin equivalents (QE)/g extract (R^2 = 0.9985). All measurements were performed in triplicate, and the results were expressed as mean ± SD.

2.6. Antimicrobial Activity

The antimicrobial activity of *P. somniferum* seed oil was evaluated using the broth microdilution method according to (Keskinkaya et al., 2020). Keskinkaya et al. (2020). The assays were performed against the following standard microbial strains: *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Klebsiella pneumoniae* ATCC 700603, *Staphylococcus aureus* ATCC 6538, *Salmonella enteritidis* ATCC 13076, *Sarcina lutea* ATCC 9341, *Bacillus cereus* ATCC 11778, and *Candida albicans* ATCC 10231. Microbial suspensions were adjusted to 0.5 McFarland standard (approximately 1×10^8 CFU/mL) and subsequently diluted to obtain the working inoculum concentration. Serial two-fold dilutions of the oil extract (6.25–0.003 mg mL^{-1}) were prepared in sterile broth medium using 96-well microplates. All antimicrobial assays were performed in triplicate. Gentamicin (10 mg mL^{-1} stock solution) was used as the positive control, whereas DMSO served as the negative control. Following incubation at 37 °C for 24–48 h, 2,3,5-triphenyltetrazolium chloride (TTC) was added as a growth indicator, and minimum inhibitory concentration (MIC) values were determined based on the absence of visible color change. Samples showing no inhibitory effect within the tested concentration range were considered inactive and reported as NA (not active). All antimicrobial assays were performed in triplicate, and MIC values were recorded as the lowest concentration inhibiting visible microbial growth.

2.7. Statistical Analysis

All experiments were performed in triplicate, and the results are expressed as mean ± standard deviation (SD). Statistical comparisons between total phenolic content (TPC) and total flavonoid content (TFC) values were performed using Student's *t*-test. Differences were considered statistically significant at $p < 0.05$. Standard deviation values and statistical analyses were calculated using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA).

3. Results

3.1. Total Phenolic and Flavonoid Contents

The total phenolic content (TPC) and total flavonoid content (TFC) of *P. somniferum* L. seed oil obtained via supercritical CO_2 extraction are presented in Table 1. The TPC was determined as 73.70 ± 0.22 mg GAE/g extract, while the TFC was 21.42 ± 0.33 mg QE/g extract. For comparative purposes, the phytochemical contents of turmeric and beet reported in the literature were considered. Turmeric exhibited significantly higher TPC (202.79 mg GAE/g) and TFC (72.73 mg QE/g), whereas beet showed lower values (13.36 mg GAE/g and 7.21 mg QE/g, respectively). The comparative distribution of TPC and TFC values is illustrated in Figure 1, which presents a logarithmic heatmap visualization of the relative concentrations.

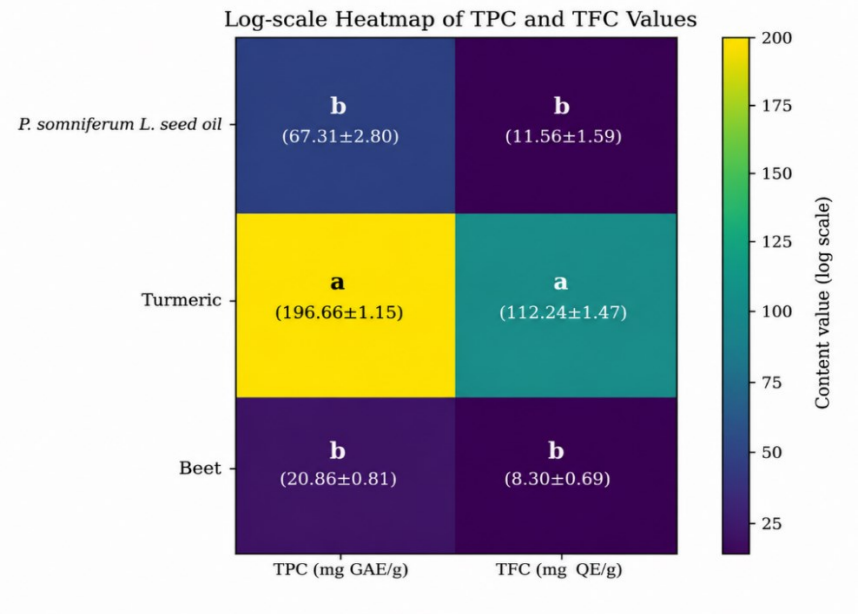

Figure 1. Heatmap showing comparative TPC and TFC values. Values are presented as mean ± SD (n = 3). Different letters within the same column indicate statistically significant differences according to Student's *t*-test ($p < 0.05$).

3.2. Proximate Composition

The proximate composition and nutritional characteristics of *P. somniferum* seed oil are presented in Tables 1a, 1b, and 1c. The results revealed that the oil possessed a high lipid content, with total fat accounting for **96.65 ± 0.54%**, confirming the suitability of *P. somniferum* seeds as an important source of edible oil. The calculated energy value of the oil was **895.05 ± 0.37 kcal/100 g** (3744.88 ± 0.71 kJ/100 g), reflecting its high caloric density, which is characteristic of vegetable oils rich in fatty acids.

No detectable amounts of protein, carbohydrates, dietary fiber, cholesterol, sodium salt, or trans fatty acids were observed in the analyzed oil sample. The absence of cholesterol is particularly noteworthy, as plant-derived oils are generally considered cholesterol-free and may therefore represent a healthier alternative to animal fats. Similarly, the absence of trans fatty acids enhances the nutritional quality of the oil, since dietary trans fats have been associated with adverse cardiovascular effects.

Table 1. (a) Nutritional composition, (b) fatty acid profile, and (c) fatty acid class distribution of *P. somniferum* seed oil.

Table 1.a. Nutritional composition (per 100 g)

Parameter	Content (%)
Energy(kcal/100 g)	895.05 ±0.37
Energy (kJ/100 g)	3744.88±0.71
Total fat	96.65±0.54
Trans fatty acids	0.00
Protein	0.00
Cholesterol	0.00
Carbohydrate	0.00
Fiber	0.00
Sodium salt	0.00

Table 1b. Fatty acid composition

Fatty Acid	Common name	Content (%)
C14:0	Myristic acid	0.05±0.54
C16:0	Palmitic acid	25.99±0.33
C16:1	Palmitoleic acid	0.23±0.81
C17:0	Margaric acid	0.05±0.28
C17:1	Heptadecenoic acid	0.10±0.97
C18:0	Stearic acid	1.82±0.96
C18:1	Oleic acid	19.14±0.41
C18:2	Linoleic acid	48.16±0.83
C18:3	Linolenic acid	0.80±0.74
C20:0	Arachidic acid	0.13±0.22
C20:1	Gadoleic acid	0.14±0.66
C22:0	Behenic acid	0.07±0.48
C24:1	Nervonic acid	0.20±0.96

Table 1c. Fatty acid classes and omega balance

Parameter	Content (%)
Σ SFA	28.11 ±0.53
Σ MUFA	19.81±0.76
Σ PUFA	48.96±0.15
Omega-6	48.16 ± 0.83
Omega-3	0.80 ± 0.74
ω6/ω3 ratio	60.20± 0.65

Σ SFA: *sum of saturated fatty acids;* ***Σ MUFA:*** *sum of monounsaturated fatty acids;* ***Σ PUFA:*** *sum of polyunsaturated fatty acids. Values are expressed as percentage (%) of total fatty acids.*

The fatty acid profile of *P. somniferum* seed oil was characterized by a predominance of unsaturated fatty acids. Polyunsaturated fatty acids (PUFAs) constituted the largest fraction (**48.96 ± 0.15%**), followed by monounsaturated fatty acids (MUFAs, **19.81 ± 0.76%**) and saturated fatty acids (SFAs, **28.11 ± 0.53%**). Linoleic acid (C18:2) was identified as the major fatty acid (**48.16 ± 0.83%**), followed by palmitic acid (C16:0; **25.99 ± 0.33%**) and oleic acid (C18:1; **19.14 ± 0.41%**). The predominance of unsaturated fatty acids, particularly linoleic acid, highlights the nutritional significance of the oil.

Furthermore, the oil exhibited a high omega-6 content (**48.16 ± 0.83%**) and a relatively low omega-3 content (**0.80 ± 0.74%**), resulting in an ω6/ω3 ratio of **60.20 ± 0.65**. Although linoleic acid is an essential fatty acid required for normal physiological functions, the relatively high ω6/ω3 ratio suggests that the oil should preferably be consumed as part of a balanced diet. The high lipid content confirms that *P. somniferum* seeds are a valuable oilseed source, suitable for both nutritional and industrial applications.

3.3. Fatty Acid Composition

The fatty acid composition of *P. somniferum* seed oil is presented in Table 1b. The nutritional analysis revealed that the oil contained a high total fat content (**96.65 ± 0.54%**), confirming its suitability as a rich source of edible oil. The oil was predominantly composed of unsaturated fatty acids, with linoleic acid (C18:2) identified as the major constituent (**48.16 ± 0.83%**), followed by palmitic acid (C16:0; **25.99 ± 0.33%**) and oleic acid (C18:1; **19.14 ± 0.41%**). Minor fatty acids, including stearic acid (C18:0; **1.82 ± 0.96%**), linolenic acid (C18:3; **0.80 ± 0.74%**), eicosenoic acid (C20:1; **0.14 ± 0.66%**), arachidic acid (C20:0; **0.13 ± 0.22%**), and nervonic acid (C24:1; **0.20 ± 0.96%**), were also detected in lower proportions. The distribution of fatty acid classes revealed that polyunsaturated fatty acids (PUFAs) constituted the largest fraction (**48.96 ± 0.15%**), followed by saturated fatty acids (SFAs; **28.11 ± 0.53%**) and monounsaturated fatty acids (MUFAs; **19.81 ± 0.76%**). In addition, the oil exhibited a high omega-6 content (**48.16 ± 0.83%**) and a relatively low omega-3 content (**0.80 ± 0.74%**), resulting in an ω6/ω3 ratio of **60.20 ± 0.65**. The predominance of unsaturated fatty acids highlights the considerable nutritional potential of *P. somniferum* seed oil as a valuable source of essential fatty acids. The GC–MS chromatogram corresponding to the fatty acid profile is presented in Figure 2, while the molecular structures of the major fatty acids identified in the oil are illustrated in Figure 3, providing structural insight into the lipid composition.

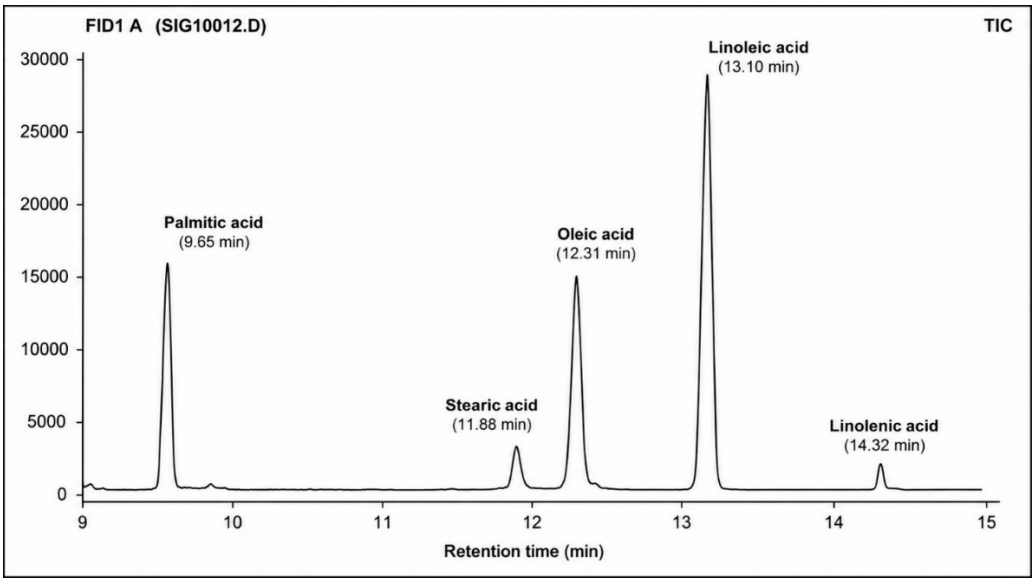

Figure 2. GC–MS chromatogram of *P.somniferum* L. seed oil illustrating the separation and identification of major fatty acids, with linoleic acid as the predominant component.

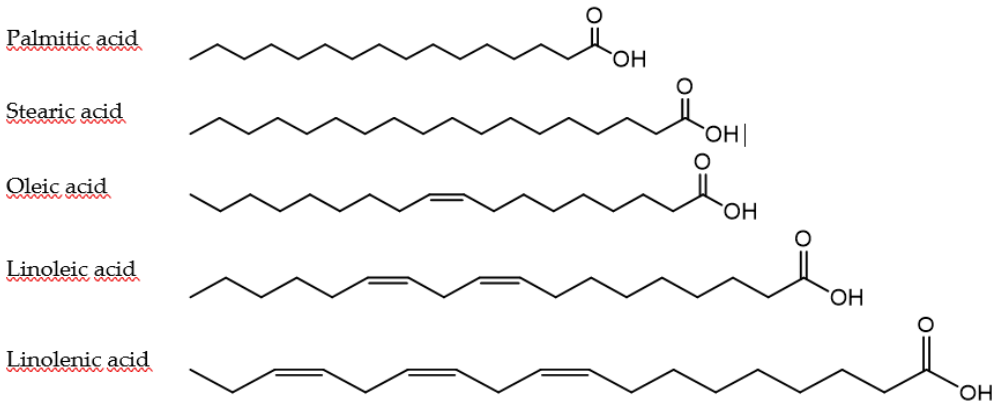

Figure 3. Molecular structures of the major fatty acids identified in *P. somniferum* L. seed oil, including palmitic, stearic, oleic, linoleic, and linolenic acids.

The high proportion of linoleic acid highlights the nutritional and functional significance of the oil, as this essential fatty acid plays a key role in maintaining cell membrane integrity and regulating inflammatory responses. The predominance of unsaturated fatty acids, particularly PUFAs, also contributes to the fluidity and bioactivity of the oil, which may be associated with its observed biological properties, including antioxidant and antimicrobial effects. In addition, the relatively low SFA content suggests a favorable lipid profile from a health perspective. Variations in fatty acid composition may arise from factors such as geographical origin, climatic conditions, and extraction method, which should be considered when comparing results across different studies. Overall, the compositional profile supports the potential use of *P. somniferum* seed oil as a valuable source of bioactive lipids for applications in food, pharmaceutical, and cosmetic industries.

3.4. Antimicrobial Activity

The antimicrobial activity of *P. somniferum* seed oil is summarized in Table 2. The oil exhibited notable inhibitory activity against both bacterial and fungal strains. The lowest minimum inhibitory concentration (MIC) value was observed against *B. cereus* (0.375 mg mL^{-1}), followed by *C. albicans* (0.75 mg mL^{-1}). Moderate activity was detected against *P. aeruginosa* (1.2 mg mL^{-1}) and *S. enteritidis* (1.2 mg mL^{-1}) while the highest MIC value was observed for *E. coli* (2.5 mg mL^{-1}). Overall, the results indicate that the oil demonstrates higher antimicrobial efficacy against Gram-positive bacteria and fungi compared to Gram-negative bacteria.

Table 2. Antimicrobial activity of *P. somniferum* L. seed oil expressed as minimum inhibitory concentration (MIC, mg mL^{-1}) against selected bacterial and fungal strains.

		MIC (mg mL^{-1})	
Test Microorganism	*P. somniferum* SCO_2 extract	Gentamycin	DMSO
Escherichia coli	2.5	<0.02	12.5%
Pseudomonas aeruginosa	1.2	<0.02	12.5%
Klebsiella pneumoniae	NA*	0.78	12.5%
Staphylococcus aureus	NA*	<0.02	25%
Salmonella enteritidis	1.2	0.04	12.5%
Sarcina lutea	NA*	<0.02	12.5%
Bacillus cereus	0.375	<0.02	12.5%
Candida albicans	0.75	<0.02	12.5%

*NA: No antimicrobial activity was detected within the tested concentration range.

This differential sensitivity may be attributed to structural differences in the cell envelope, particularly the presence of an outer membrane in Gram-negative bacteria, which can act as a permeability barrier limiting the penetration of bioactive compounds. The observed antimicrobial activity of the oil may also be associated with its rich composition of unsaturated fatty acids and phenolic constituents, which are known to disrupt microbial cell membranes, alter membrane permeability, and interfere with essential metabolic processes. Furthermore, the relatively strong antifungal activity against *C. albicans* suggests that the oil could serve as a promising natural antifungal agent. These findings support the potential application of *P. somniferum* seed oil as a bioactive ingredient in pharmaceutical, nutraceutical, and food preservation systems, although further studies on mechanism of action, toxicity, and in vivo efficacy are required to validate its practical use.

4. Discussion

The present study provides a comprehensive evaluation of the chemical composition and biological activity of *P. somniferum* L. seed oil obtained via supercritical CO_2 extraction, emphasizing the relationship between fatty acid profile, phytochemical content, and antimicrobial efficacy.

The fatty acid composition of the oil was dominated by polyunsaturated fatty acids (PUFAs), particularly linoleic acid, followed by oleic and palmitic acids. This profile is consistent with previous reports on poppy seed oil (Dąbrowski et al., 2020; Hlinková et al., 2012), confirming the characteristic lipid composition of this species. In the present study, linoleic acid was identified as the predominant fatty acid, accounting for 48.16 ± 0.83% of the total fatty acids, followed by palmitic acid (25.99 ± 0.33%) and oleic acid (19.14 ± 0.41%). Although the linoleic acid content was lower than the values reported by (Dąbrowski et al., 2020), (65.8–74.2%) and (Hlinková et al. (2012) (62.4–74.8%), the oleic acid content observed in the present study was within the range previously reported for different poppy genotypes. Moreover, the predominance of polyunsaturated fatty acids (48.96 ± 0.15%) confirms that *P. somniferum* seed oil is an important source of essential fatty acids. The observed variations in fatty acid composition may be attributed to differences in genotype, geographical origin, environmental conditions, and extraction techniques. Nevertheless, the results indicate that supercritical CO_2 extraction efficiently preserves the characteristic fatty acid profile of poppy seed oil while maintaining its nutritional quality.However, the preservation of unsaturated fatty acids observed in this study may be attributed to the use of supercritical CO_2 extraction, which operates under mild conditions and minimizes oxidative degradation (Ahmad et al., 2019; Papamichail et al., 2000). From a nutritional perspective, the high PUFA content contributes to membrane fluidity, regulation of lipid metabolism, and reduction of cardiovascular risk factors. Nevertheless, it is important to consider that high PUFA levels may also increase susceptibility to lipid peroxidation, highlighting the need for appropriate storage conditions and potential antioxidant stabilization strategies (Ghafoor et al., 2019).

The ω-6/ω-3 ratio observed in *P. somniferum* seed oil is relatively high, primarily due to the dominance of linoleic acid. In the present study, the ω-6/ω-3 ratio was determined as 60.20 ± 0.65, which is considerably higher than the ratio generally recommended for a balanced human diet. Similar high ω-6/ω-3 ratios have previously been reported for poppy seed oils, mainly as a consequence of their high linoleic acid content and

relatively low levels of α-linolenic acid (Hlinková et al. 2012; Dąbrowski et al., 2020). While ω-6 fatty acids are essential for human health, an imbalanced ratio may be associated with pro-inflammatory processes when consumed excessively (Abedi & Sahari, 2014). Therefore, although the oil demonstrates strong nutritional potential, its incorporation into balanced dietary systems should be considered to optimize health benefits. Nevertheless, the high proportion of polyunsaturated fatty acids (48.96 ± 0.15%) identified in the present study further highlights the nutritional value of *P. somniferum* seed oil as a rich source of essential fatty acids.

In addition to its lipid composition, the moderate levels of total phenolic and flavonoid compounds observed in this study further support the functional potential of the oil. Although the phenolic content is lower than that of highly enriched plant matrices such as turmeric, it falls within the typical range reported for edible oils and remains sufficient to exert biological effects (Butnariu et al., 2022). The total phenolic content determined in the present study (73.70 mg GAE g^{-1} extract) was considerably higher than that reported for beet extracts (13.36 mg GAE g^{-1} extract), but lower than that of turmeric (202.79 mg GAE g^{-1} extract). Likewise, the total flavonoid content (21.42 mg QE g^{-1} extract) exceeded the values reported for beet (7.21 mg QE g^{-1} extract), suggesting that *P. somniferum* seed oil represents a moderate yet significant source of bioactive phytochemicals. From a structure–activity relationship (SAR) perspective, phenolic compounds contribute to antioxidant activity through electron donation and radical scavenging mechanisms, while flavonoids may enhance antimicrobial activity by interacting with microbial proteins and membran. Importantly, the retention of these compounds in SFE-derived oil suggests that supercritical extraction preserves bioactive integrity more effectively than conventional solvent-based techniques.

The antimicrobial activity results revealed a clear pattern of selective efficacy, with Gram-positive bacteria (*B. cereus*) and fungi (*C. albicans*) being more susceptible than Gram-negative bacteria (*E. coli* and *P. aeruginosa*). This observation is consistent with previous studies (Luhmer et al., 2021) and can be explained by differences in cell envelope structure. Among the tested microorganisms, the strongest antimicrobial activity was observed against *B. cereus* (MIC: 0.375 mg mL^{-1}), followed by *C. albicans* (MIC: 0.75 mg mL^{-1}). In contrast, *E. coli* exhibited the lowest susceptibility (MIC: 2.5 mg mL^{-1}). These findings are in agreement with Dilek et al. (2018), who reported that Gram-positive bacteria are generally more susceptible to plant-derived bioactive compounds than Gram-negative bacteria due to the absence of an outer membrane barrier. Gram-negative bacteria possess an outer lipopolysaccharide membrane that acts as a permeability barrier, limiting the diffusion of hydrophobic compounds such as fatty acids and phenolics (Dilek et al., 2018; Lou et al., 2025).

Mechanistically, the antimicrobial activity of *P. somniferum* seed oil is likely driven by synergistic interactions between its lipid and phytochemical components. Unsaturated fatty acids, particularly linoleic and oleic acids, can integrate into microbial membranes, disrupting lipid packing and increasing membrane permeability. This results in leakage of intracellular components, ion imbalance, and eventual cell death (Gültepe et al., 2019). Concurrently, phenolic compounds can denature proteins, inhibit key metabolic enzymes, and interfere with nucleic acid synthesis (Tajkarimi et al., 2010). In the present study, the strong inhibitory activity observed against *B. cereus* (MIC: 0.375 mg mL^{-1}) and *C. albicans* (MIC: 0.75 mg mL^{-1}) may be attributed to the high proportion of unsaturated fatty acids, particularly linoleic acid (48.16 ± 0.83%) and oleic acid (19.14 ± 0.41%), together with the presence of phenolic and flavonoid compounds (73.70 mg GAE g^{-1} extract and 21.42 mg QE g^{-1} extract, respectively). The selective susceptibility of Gram-positive bacteria compared to Gram-negative bacteria observed in this study further supports the role of membrane-active phytochemicals in the antimicrobial mechanism.This dual mode of action enhances antimicrobial efficacy and reduces the likelihood of resistance development.

The antifungal activity observed against *C. albicans* further suggests that the oil may target ergosterol-containing fungal membranes, leading to structural destabilization and impaired cellular function. In the present study, *P. somniferum* seed oil exhibited considerable antifungal activity against *C. albicans*, with an MIC value of 0.75 mg mL^{-1}. This inhibitory effect may be associated with the high content of unsaturated fatty acids, particularly linoleic acid (48.16 ± 0.83%), as well as the presence of phenolic and flavonoid compounds retained in the SCO_2-derived oil. This is particularly relevant given the increasing resistance of fungal pathogens to conventional antifungal drugs, highlighting the importance of exploring plant-derived alternatives.

From a technological perspective, supercritical CO_2 extraction plays a crucial role in determining the final bioactivity of the oil. Compared to conventional extraction methods, SFE enables selective extraction of bioactive fractions while preserving thermolabile compounds and preventing solvent contamination. The approximately 20% extraction yield obtained in the present study further demonstrates the efficiency of the supercritical CO_2 process for recovering bioactive oil fractions from *P. somniferum* seeds. Similar extraction efficiencies have previously been reported for other oil-rich plant materials processed under supercritical conditions (Papamichail et al., 2000; Herrero, 2024). Additionally, the tunable parameters of SFE (pressure, temperature, and flow rate) allow optimization of both yield and composition, making it a highly versatile technique for producing functional plant oils.

Beyond its compositional and biological properties, the findings of this study also have important industrial implications. The combination of a favorable fatty acid profile and antimicrobial activity suggests that *P. somniferum* seed oil may serve as a natural preservative or functional ingredient in food systems. Furthermore, its potential application in nutraceutical and pharmaceutical formulations highlights its value as a multifunctional bioactive compound.

Despite these promising findings, several limitations should be acknowledged. The antimicrobial activity was evaluated under in vitro conditions, which may not fully represent complex in vivo environments. In addition, the study focused on total phenolic and flavonoid contents rather than identifying individual compounds, limiting the ability to establish precise structure–activity relationships.

Future research should focus on detailed metabolomic profiling and in vivo studies to better understand the bioavailability, safety, and therapeutic efficacy of *P. somniferum* seed oil. Moreover, investigating synergistic interactions with other natural compounds and evaluating its stability in different formulation systems could further enhance its applicability.

Overall, this study demonstrates that the biological activity of *P. somniferum* seed oil is closely linked to its chemical composition and that supercritical CO_2 extraction is an effective approach for obtaining high-quality, bioactive plant oils. These findings provide novel insights into the bioactive potential of SCO_2-derived *Papaver somniferum* seed oil and support its promising applications in food, nutraceutical, and pharmaceutical industries.

5. Conclusions

This study provides a comprehensive evaluation of *P. somniferum* L. seed oil obtained via supercritical CO_2 extraction, demonstrating that the applied extraction approach plays a decisive role in preserving both the chemical integrity and biological functionality of the oil. The results revealed that the oil is characterized by a high proportion of polyunsaturated fatty acids, particularly linoleic acid, which is associated with important physiological functions such as regulation of lipid metabolism and support of cardiovascular health.

Beyond its nutritional profile, the presence of phenolic and flavonoid compounds contributes significantly to the functional properties of the oil. Although present at moderate levels, these bioactive compounds, in combination with unsaturated fatty acids, appear to enhance the overall biological activity. In particular, the observed antimicrobial efficacy especially against Gram-positive bacteria and fungal strains—highlights the potential of *P. somniferum* seed oil as a natural alternative to synthetic antimicrobial agents.

Importantly, the findings of this study underscore the synergistic relationship between fatty acid composition and phytochemical content in determining the bioactive performance of plant-derived oils. The ability of supercritical CO_2 extraction to selectively preserve these compounds without the use of organic solvents further strengthens its suitability as a green and sustainable technology for high-value bioactive products.

From an application perspective, the combined nutritional and antimicrobial properties of *P. somniferum* seed oil suggest its potential use not only as a functional food ingredient but also as a natural preservative and a candidate for incorporation into nutraceutical and pharmaceutical formulations. In this context, the oil may

contribute to the development of safer, plant-based alternatives in response to the growing demand for clean-label and sustainable products.

However, further investigations are required to fully elucidate the molecular mechanisms underlying its biological activity, including detailed characterization of individual bioactive compounds and their interactions. Additionally, in vivo studies and formulation-based research are necessary to validate its efficacy, stability, and safety in real-world applications.

In conclusion, this study provides novel and integrated insights into the bioactive potential of SFE-derived *P. somniferum* seed oil, positioning it as a promising multifunctional natural product with significant potential across food, nutraceutical, and pharmaceutical sectors.

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