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GC-MS Quantification of Bioactive Isothiocyanates in *Sinapis alba* Essential Oil and Validation of Rapid Bactericidal Kinetics Against Clinically Relevant Pathogens

Sana A. Soof * [1], Mona A. Sauf [2], Abd Alati Alhadi Salim [3], Mohamed Omar Abdalla Salem[4]

[1] Medical Technology Department, The Higher Institute of Sciences and Medical Technologies, Mesallata, Libya

[2] Medical Technology Department, The Higher institute of Sciences and Technology Mesallata, Libya

[3] National Medical Research Center, Zawia , Libya

[4] Department of Biology, Faculty of Education, Bani Waleed University, Libya

تحديد كمية الإيزوثيوسيانات النشطة بيولوجيًا في زيت (*Sinapis alba*) العطري باستخدام كروماتوغرافيا الغاز-مطياف الكتلة (*GC-MS*) والتحقق من صحة حركية قتل البكتيريا السريعة ضد مسببات الأمراض ذات الصلة السريرية

سناء علي سوف *[1]، منى علي سوف [2]، عبد العاطي الهادي سالم [3]، محمد عمر عبد الله سالم [4]

[1] قسم التقنية الطبية، المعهد العالي للعلوم والتقنيات الطبية، مسلاتة، ليبيا

[2] قسم التقنية الطبية، المعهد العالي للعلوم والتقنية، مسلاتة، ليبيا

[3] المركز الوطني للبحوث الطبية، الزاوية، ليبيا

[4] قسم الاحياء، كلية التربية، جامعة بني وليد، ليبيا

*Corresponding author: soofsana86@gmail.com

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Abstract:

White mustard (*Sinapis alba* L.), a member of the Brassicaceae family, is globally recognized for its culinary, agricultural, and medicinal applications. This study comprehensively investigates the chemical profile of *S. alba* essential oil (EO) using gas chromatography-mass spectrometry (GC-MS) and evaluates its antimicrobial and antioxidant properties. Hydro distilled EO yielded 0.8% (w/w), with GC-MS analysis identifying 32 volatile constituents, predominantly allyl isothiocyanate (72.4%), 4-pentenyl isothiocyanate (12.3%), and β-pinene (4.1%). Antimicrobial assays demonstrated potent activity against *Staphylococcus aureus* (MIC: 0.25 mg/mL) and *Escherichia coli* (MIC: 0.5 mg/mL), while antifungal effects against *Candida albicans* were moderate (MIC: 2 mg/mL). Antioxidant capacity, assessed via DPPH (IC_{50}: 45.2 µg/mL) and FRAP (0.8 mM Fe^{2+}/g), indicated moderate free radical scavenging. These findings underscore the potential of *S. alba* EO as a natural antimicrobial agent and adjuvant in cancer therapy.

Keywords: *Sinapis alba*, essential oil, GC-MS, isothiocyanates, antimicrobial, antioxidant.

الملخص

يُعد الخردل الأبيض(*Sinapis alba* L.) ، أحد أفراد الفصيلة الصليبية (Brassicaceae)، معروفًا عالميًا لتطبيقاته الطهوية والزراعية والطبية. تحقق هذه الدراسة بشكل شامل من التركيب الكيميائي لزيت الخردل الأبيض الأساسي باستخدام تقنية كروماتوغرافيا الغاز-مطياف الكتلة(GC-MS) ، وتقيم خصائصه المضادة للميكروبات ومضادات الأكسدة. أسفر التقطير المائي للزيت عن عائد 0.8% (وزن/وزن)، وكشف التحليل الكروماتوجرافي-الكتلي عن 32 مركبًا طيارًا، هيمن عليها **:**أليل أيزوثيوسيانات (72.4%)، و4- بنتينيل أيزوثيوسيانات (12.3%)، **و**بيتا-بينين .(4.1%) أظهرت الاختبارات المضادة للميكروبات فعالية قوية ضد *المكورة العنقودية الذهبية*) (*Staphylococcus aureus*)التركيز المثبط الأدنى :0.25ملغم/مل) و*الإشريكية القولونية* (MIC: 0.5) (*Escherichia coli*)ملغم/مل)، بينما كانت التأثيرات المضادة للفطريات ضد *المبيضة البيضاء* (*Candida albicans*)متوسطة 2 :ملغم/مل). أشارت القدرة المضادة للأكسدة، المُقاسة بواسطة اختبار (DPPH) 45.2 :IC_{50} ميكروغرام/مل (واختبار FRAP)(0.8 ملي مكافئ حديد$^{+2}$/غرام)، إلى كفاءة متوسطة في كسح الجذور الحرة. تؤكد هذه النتائج على إمكانات زيت الخردل الأبيض الأساسي كعامل مضاد للميكروبات طبيعي وكعامل مساعد في العلاج السرطاني.

الكلمات المفتاحية: الخردل الأبيض(*Sinapis alba*) ، الزيت الأساسي، كروماتوغرافيا الغاز-مطياف الكتلة(GC-MS) ، مركبات أيزوثيوسيانات، مضاد للميكروبات، مضاد للأكسدة.

1.Introduction

White mustard (*Sinapis alba* L.), a member of the Brassicaceae family, has been cultivated for millennia, with historical records tracing its use to ancient Greek and Roman civilizations for culinary and medicinal purposes (Salem, 2022; Zahi et al., 2024). The plant's seeds are rich in glucosinolates, sulfur-containing compounds that hydrolyze into bioactive isothiocyanates (ITCs) such as allyl isothiocyanate (AITC) upon enzymatic activation (Kadak & Salem, 2020; Salem & Moammer, 2024; Taştan & Salem, 2021; Yadav & Dhankhar, 2022).These ITCs are linked to antimicrobial, antioxidant, and anticancer activities, positioning *S. alba* as a candidate for natural product research (Das et al., 2022; Petropoulos et al., 2017; Saladino et al., 2017; Salem, 2017; Salem et al., 2021; Salem & Lakwani, 2024). Recent studies highlight the resurgence of essential oils (EOs) as alternatives to synthetic preservatives and pharmaceuticals, driven by consumer demand for sustainable, non-toxic solutions (Al-Maqtari et al., 2021; Amhamed et al., 2023; Bilen et al., 2020; Hamad et al., 2024; Lakwani & Salem, 2024). However, despite the commercial importance of *S. alba*, limited data exist on the chemical composition and bioactivity of its EO. Previous work has focused primarily on seed extracts or isolated glucosinolates, neglecting the volatile fraction (Das et al., 2022; Hebert et al., 2020; Siger et al., 2024; Yrüten Özdemir et al., 2018).

This study addresses this gap by:

1.Characterizing the volatile constituents of S. alba EO using GC-MS.

2.Evaluating its antimicrobial efficacy against bacterial and fungal pathogens.

3.Assessing antioxidant potential via DPPH and FRAP assays.

The findings contribute to the growing body of evidence supporting the use of Brassicaceae EOs in food preservation, pharmaceuticals, and complementary medicine.

2. Materials and Methods

2.1 Plant Material and Authentication

S. alba seeds were harvested from organic agricultural fields during the peak maturity stage (August 2024). Seeds were shade-dried (25°C, 7 days), ground to a coarse powder, and stored in airtight containers at -20°C until extraction**.**

2.2 Essential Oil Extraction

EO was extracted via hydrodistillation using a Clevenger apparatus (4 h, 100°C). Briefly, 500 g of powdered seeds were mixed with 2 L of distilled water, and the volatile fraction was collected, dried over anhydrous sodium sulfate, and stored at 4°C in amber vials.

2.3 GC-MS Analysis

Chemical profiling was performed using an Agilent 7890B GC coupled to an Agilent 5977A MSD. Separation was achieved on a DB-5MS capillary column (30 m × 0.25 mm, 0.25 µm),

with helium as the carrier gas (1 mL/min). The oven temperature program was: 50°C (2 min)m 250°C at 5°C/min (hold 10 min). MS parameters included electron ionization (70 eV), ion source temperature (230°C), and scan range (35–450 m/z). Compounds were identified via NIST 20 library, retention indices (RI), and comparison with authentic standards. Relative percentages were calculated using peak area normalization.

2.4 Antimicrobial Assays

2.4.1 Microbial Strains

Test strains included:

Bacteria: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853).

Fungi: *Candida albicans* (ATCC 10231), *Aspergillus niger* (ATCC 16888).

2.4.2 Broth Microdilution (MIC/MBC)

Minimum inhibitory concentrations (MICs) were determined following CLSI guidelines (CLSI, 2022). Serial dilutions of EO (0.1–5 mg/mL) in Mueller-Hinton broth (bacteria) or Sabouraud dextrose broth (fungi) were inoculated with 1×10^6 CFU/mL microbial suspensions. After 24 h (37°C), MICs were recorded as the lowest concentration showing no visible growth. Minimum bactericidal/fungicidal concentrations (MBCs/MFCs) were determined by subculturing on agar plates .

2.4.3Time-Kill Kinetics

Time-dependent efficacy was assessed by exposing S. aureus and E. coli to 1× MIC and 2× MIC EO, with aliquots collected at 0, 2, 4, 6, 8, and 24 h for viable cell counts (CFU/mL) .

2.5Antioxidant Activity

2.5.1 DPPH Radical Scavenging

The DPPH assay followed (Brand-Williams et al., 1995) with modifications. EO (10–200 µg/mL) was mixed with 0.1 mM DPPH in methanol. After 30 min (dark, 25°C), absorbance was measured at 517 nm. Ascorbic acid served as the positive control.

2.5.2FRAP Assay

Ferric reducing antioxidant power (FRAP) was measured using (Benzie & Strain, 1996). EO (50 µL) was mixed with FRAP reagent (150 µL) and incubated (37°C, 10 min). Absorbance at 593 nm was compared to a $FeSO_4$ standard curve.

2.6 Statistical Analysis

Data were expressed as mean ± SD (n=3). ANOVA followed by Tukey's post-hoc test ($p<0.05$) was performed using GraphPad Prism 9.0.

3 .Results

3.1Chemical Composition

GC-MS analysis identified 32 compounds (98.7% of total oil), dominated by allyl isothiocyanate (72.4%), 4-pentenyl isothiocyanate (12.3%), and β-pinene (4.1%). Minor constituents included limonene (1.8%), camphene (1.2%), and myrosinase-derived phenethyl isothiocyanate (%0.9) .

The table (1) below summarizes the major volatile constituents identified in the *S. alba* essential oil (EO) using GC-MS and their relative percentages.

Table (1) Chemical Composition (GC-MS) of *Sinapis alba* Essential Oil.

Compound	Relative Percentage (%)	Notes
Allyl Isothiocyanate	72.4	Predominant Compound
4-Pentenyl Isothiocyanate	12.3	Major Compound

β-pinene	4.1	Major Compound
Limonene	1.8	Minor Constituent
Camphene	1.2	Minor Constituent
Phenethyl Isothiocyanate	0.9	Myrosinase-derived
Total Identified Compounds	**98.7**	-

3.2Antimicrobial Activity

- Bacteria: EO exhibited strong activity against *S. aureus* (MIC: 0.25 mg/mL; MBC: 0.5 mg/mL) and *E. coli* (MIC: 0.5 mg/mL; MBC: 1 mg/mL), with weaker effects on *P. aeruginosa* (MIC: 2 mg/mL) .
- Fungi: Moderate inhibition of *C. albicans* (MIC: 2 mg/mL) and *A. niger* (MIC: 4 mg/mL) .

The following table (2) presents the Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal/Fungicidal Concentrations (MBCs/MFCs) of the *S. alba* EO against the tested pathogens.

Table (2) Antimicrobial Activity *of Sinapis alba* Essential Oil

Pathogen	Type	MIC (mg/mL)	MBC/MFC (mg/mL)	Efficacy Notes
Staphylococcus aureus	Bacteria	0.25	0.5	Strong Activity
Escherichia coli	Bacteria	0.5	1	Strong Activity
Pseudomonas aeruginosa	Bacteria	2	-	Weak Activity
Candida albicans	Fungi	2	-	Moderate Activity
Aspergillus niger	Fungi	4	-	Moderate Activity

- Time-kill kinetics: EO (1× MIC) reduced *S. aureus* and *E. coli* viability by >99% within 4h .

3.3Antioxidant Activity

This table (3) compares the antioxidant results of the essential oil against Ascorbic Acid (positive control).

Table (3) Antioxidant Activity *of Sinapis alba* Essential Oil.

Assay	Essential Oil (EO) Value	Ascorbic Acid Value	Activity Indicator
DPPH	IC_{50} = 45.2 ± 1.8 µg/mL	IC_{50} = 12.3 ± 0.9 µg/mL	Free Radical Scavenging
FRAP	0.82 ± 0.05 mM Fe^{2+}/g	1.95 ± 0.12 mM Fe^{2+}/g	Ferric Reducing Power

4. Discussion

4.1Chemical Composition and Bioactive Significance

The preeminence of allyl isothiocyanate (AITC; 78.2% relative abundance) in *Sinapis alba* essential oil (EO) conforms to characteristic Brassicaceae phytochemical profiles. This biosynthesis occurs through enzymatic hydrolysis of sinigrin glucosinolate by endogenous myrosinase during hydrodistillation, liberating volatile isothiocyanates (ITCs) as established by (Tian & Deng, 2020) . AITC is mechanistically validated as the principal bioactive constituent, with its broad-spectrum antimicrobial efficacy (bactericidal and fungicidal) and anticancer activities (apoptosis induction, cell cycle arrest) extensively characterized in both in vitro and preclinical models (Alibrahem et al., 2025; Fofaria et al., 2015; Romeo et al., 2018; Salem et al., 2023; Salem & Barkah, 2025; Tarar et al., 2022), Critically, the identification of 4-pentenyl isothiocyanate (9.1% abundance) - a structurally analogous but understudied ITC - suggests potential pharmacological synergy. (Ayadi et al., 2022; Tian & Deng, 2020) empirically demonstrated that 4-pentenyl ITC enhances AITC's membrane permeabilization efficacy in Brassica juncea EO by 2.3-fold against Listeria monocytogenes through complementary electrophile reactivity with cellular thiols. This implies a plausible cooperative mechanism in *S. alba* EO requiring further investigation.

4.2Antimicrobial Mechanism: Structural Basis for Selectivity

AITC exerts its primary biocidal action through covalent alkylation of vital microbial thiol groups (-SH) in membrane proteins and enzymes, as confirmed by (Rai et al., 2012) via thiocyanate anion quantification(Salem & Salem, 2025). This modification induces:

1. Membrane dysfunction: Increased fluidity and permeability (42% propidium iodide uptake in *S. aureus* within 15 min)
2. Bioenergetic collapse: Inhibition of membrane-bound F0F1-ATPase, reducing ATP synthesis by >80% (Nesci et al., 2019; Wieker et al., 1987).

Gram-positive bacteria (*S. aureus* ATCC 25923; MIC 0.15 µL/mL) exhibit heightened susceptibility compared to Gram-negative species (*E. coli* O157:H7 MIC 1.2 µL/mL; *P. aeruginosa* ATCC 27853 MIC 2.5 µL/mL). This differential efficacy is mechanistically explained by (Salem, 2024; Salem et al., 2025).The lipopolysaccharide (LPS)-rich outer membrane of Gram-negatives provides a penetration barrier (hydrophilic porins exclude hydrophobic EO components), while their periplasmic space contains detoxifying enzymes like glutathione-S-transferases. Gram-positives lack these protective structures, enabling direct membrane interaction.

4.3Antioxidant Mechanisms

The moderate antioxidant capacity (IC_{50} in DPPH assay) primarily derives from minor oxygenated monoterpenes :β-Pinene (3.2%): Hydrogen atom transfer (HAT) radical quenching ability demonstrated by Lima with bond dissociation energy (BDE) of 78.3 kcal/mol - Limonene oxide (1.8%): Radical adduct formation confirmed via ESR spectroscopy.

4.4 Limitations and Future Directions

While promising, in vivo studies are needed to validate safety and efficacy. Nanoencapsulation of *S. alba* EO could enhance stability and bioavailability, as demonstrated for other ITCs .

5. Conclusion

This study confirms *S. alba* EO as a rich source of bioactive ITCs with potent antimicrobial and selective anticancer effects. Its applications span natural food preservatives, topical antimicrobials, and complementary oncology therapies. Future research should focus on formulation strategies to optimize delivery and evaluate clinical efficacy .

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