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Antimicrobial Activities of Cold-Pressed Oils Obtained from Nettle Seeds

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النشاط المضاد للميكروبات للزيوت المستخلصة بالعصر على البارد من بذور نبات القراص

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Abstract:
This study aimed to investigate the effectiveness of using the GeoGebra program in teaching mathematics and its effect on the academic achievement of first-year secondary school students in the city of Nalut. Specifically, the study sought to answer the following question: What is the effect of using the GeoGebra program in teaching mathematics on students' academic achievement?
To answer the research question and test the hypothesis derived from it, the researchers employed a quasi-experimental approach. The study population consisted of all first-year secondary school students enrolled in the city of Nalut. The study was conducted on a sample of 39 female first-year secondary school students from Ibn Jeldasin Secondary School. The sample was divided into two groups: an experimental group, which studied the graphical solutions unit using the GeoGebra program, and a control group, which studied the same unit using the traditional teaching method. The study was conducted during the second semester of the 2020–2021 academic year. Two instruments were administered to the study sample: a pre-achievement test and a post-achievement test.
The results indicated the effectiveness of the program in improving students' academic achievement. Statistically significant differences were found between the mean scores of the experimental and control groups on the post-achievement test, in favor of the experimental group. The study recommended employing GeoGebra in teaching mathematics at different educational stages. It also recommended preparing and training teachers to use educational software that supports the teaching process, particularly the GeoGebra program.

Keywords : Effectiveness, GeoGebra, Academic Achievement.

الملخص:
تتطلب أزمة مقاومة مضادات الميكروبات (AMR) المتصاعدة استكشاف عوامل مضادة للميكروبات طبيعية وحديثة. تحقق هذه الدراسة في التركيب الكيميائي والفعالية المضادة للميكروبات للزيت المستخلص بالعصر على البارد من بذور نبات القراص (Urtica dioica L.). تم الحصول على الزيت عن طريق العصر الميكانيكي على البارد، وتم تحديد ملفه الكيميائي باستخدام تقنية مطيافية الكروماتوغرافيا الغازية-الطيف الكتلي (GC-MS). تم تقييم النشاط المضاد للميكروبات ضد بكتيريا ذات أهمية سريرية؛ موجبة الجرام) المكورة العنقودية الذهبية (Staphylococcus aureus) وسالبة الجرام) الإشريكية القولونية Escherichia coli (باستخدام اختبار انتشار الآجار واختبار التخفيف الدقيق في المرق لتحديد مناطق التثبيط (ZOI) وتركيزات التثبيط الدنيا (MIC). كشف تحليل GC-MS عن تركيب معقد غني بالأحماض الدهنية غير المشبعة المتعددة، بشكل أساسي حمض اللينوليك (~40-50%) وحمض الأوليك (~20-30%)، إلى جانب مكونات ثانوية مثل التوكوبهيرولات والفيوستيرولات. أظهر الزيت نشاطًا مضادًا للميكروبات كبيرًا ومعتمدًا على الجرعة، حيث بلغت مناطق التثبيط 14.5 ± 0.7 ملم و16.2 ± 0.9 ملم ضد بكتيريا الإشريكية القولونية والمكورة العنقودية الذهبية على التوالي. تم تحديد قيم التركيز المثبط الأدنى بـ 0.625 ملغم/مل للمكورة العنقودية الذهبية و1.25 ملغم/مل للإشريكية القولونية. تشير هذه النتائج إلى أن الآلية المضادة للميكروبات ترتبط على الأرجح باضطراب الأغشية الخلوية الميكروبية بواسطة الأحماض الدهنية والعمل التآزري للمركبات المضادة للأكسدة. تضع هذه النتائج زيت بذور القراص المعصور على البارد كمصدر طبيعي واعد لتطوير عوامل مكملة مضادة للميكروبات للتطبيقات الصيدلانية والتجميلية.

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الكلمات المفتاحية: القراص، زيت بذور القراص، الاستخلاص بالعصر على البارد، النشاط المضاد للميكروبات، مطيافية الكروماتوغرافيا الغازية-الطيف الكتلي، الأحماض الدهنية، التركيز المثبط الأدنى .

1. Introduction

The relentless emergence of antimicrobial resistance (AMR) represents a catastrophic threat to global public health, rendering conventional antibiotics increasingly ineffective and necessitating the urgent discovery of alternative therapeutic agents (Fong, 2023, Hsin et al., 2025). The overuse and misuse of antibiotics have led to the proliferation of multidrug-resistant strains, such as methicillin-resistant *Staphylococcus aureus* (MRSA), severely limiting treatment options and escalating mortality rates (Moriel et al., 2024, Salem, 2025). In this context, plant-derived natural products have garnered significant scientific interest due to their complex phytochemistry, multi-target mechanisms of action, and potentially lower propensity for inducing resistance (Salem et al., 2025 ;Zhmool et al., 2026).

Plant essential oils and fixed oils, particularly those obtained through non-thermal methods like cold-pressing, are rich reservoirs of bioactive compounds, including fatty acids, terpenoids, and phenolics, which exhibit a broad spectrum of biological activities (Salem & Lakwani, 2024). For instance, studies on flaxseed (*Linum usitatissimum*) oil have identified compounds like 9,12-Octadecadienoic acid (linoleic acid) with demonstrated anti-inflammatory and anti-arthritic properties (Lalitharani et al., 2009; Alhijjaji & Arross, 2026). Similarly, the antimicrobial efficacy of plant extracts has been well-documented, as seen with methanol extracts of dandelion (*Taraxacum officinale*) showing potent activity against *E. coli* and *S. aureus* (Salem et al., 2025; Aloraibi et al., 2025).

Nettle (*Urtica dioica* L.) is a perennial herb renowned in traditional medicine for its diverse pharmacological properties. While research has often focused on its leaves and roots, the seeds remain a relatively under-explored resource. Nettle seeds are known to be rich in fixed oils, containing high levels of linoleic acid, oleic acid, and tocopherols, which contribute to their antioxidant and anti-inflammatory potential. However, a comprehensive analysis of the antimicrobial properties of cold-pressed nettle seed oil (CPSO) is lacking.

Building on the methodologies established in previous work on plant oils and extracts (Kadak & Salem, 2020; Salem & Lakwani, 2024), this study aims to:

1. Extract oil from nettle seeds using a cold-pressing technique.
2. Analyze the chemical composition of the extracted oil using GC-MS.
3. Evaluate its *in vitro* antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*.
4. Determine the Minimum Inhibitory Concentration (MIC) of the oil.

2. Materials and Methods

2.1. Plant Material and Oil Extraction

Mature seeds of *Urtica dioica* L. were harvested from wild populations in the Bani Waleed region, Libya, in the summer of 2024. A voucher specimen (UD-BWU-2024-001) was deposited at the Bani Waleed University Herbarium. The seeds were cleaned, shade-dried at room temperature ($\leq 40°C$) to a constant weight, and then subjected to mechanical cold-pressing using an oil press machine (as described by Salem & Lakwani, 2024). The crude oil was collected, allowed to settle for 5 days to separate any particulate matter, and then filtered through a 0.45 µm membrane filter. The clear oil was stored in amber glass vials at 4°C until analysis.

2.2. GC-MS Analysis

The chemical composition of CPSO was determined using a Shimadzu GCMS-QP2010 Ultra system, following the protocol outlined by Salem & Lakwani (2024). Briefly, the analysis was performed with an Rtx-5MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The injector

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temperature was set at 250°C. The oven temperature program was: initial temperature 90°C (held for 5 min), increased to 250°C at a rate of 4°C/min, and finally held at 250°C for 5 min. A 1 µL sample of oil, diluted in n-hexane (1:100, w/w), was injected in split mode (split ratio 10:1). The mass spectrometer operated in electron impact mode at 70 eV, with an ion source temperature of 200°C and a mass scan range of 40-650 m/z. Compound identification was achieved by comparing the mass spectra and relative retention times with those of authentic standards and the NIST/Wiley libraries.

2.3. Antimicrobial Assays

2.3.1. Microbial Strains and Culture Conditions

Reference strains of *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were obtained from the Libyan National Center for Disease Control. The strains were cultured in Mueller-Hinton Broth (MHB) and maintained on Mueller-Hinton Agar (MHA) plates at 37°C.

2.3.2. Agar Well Diffusion Assay

The antibacterial activity was screened using the agar well diffusion method (Bauer, 1966). Briefly, sterile MHA plates were inoculated with a standardized bacterial suspension (0.5 McFarland standard, ~1.5 × 10^8 CFU/mL). Wells (6 mm in diameter) were punched into the agar and filled with 100 µL of CPSO at different concentrations (100%, 50%, and 25% v/v, prepared in 10% DMSO). Tetracycline (30 µg/mL) and 10% DMSO were used as positive and negative controls, respectively. The plates were incubated at 37°C for 24 hours, after which the diameters of the zones of inhibition (ZOI) were measured in millimeters using a digital caliper. All tests were performed in triplicate.

2.3.3. Determination of Minimum Inhibitory Concentration (MIC)

The MIC of CPSO was determined using the broth microdilution method in 96-well microtiter plates (Salem et al., 2025). Two-fold serial dilutions of the oil were prepared in MHB, resulting in a concentration range from 10 mg/mL to 0.078 mg/mL. Each well was inoculated with a standardized bacterial suspension to achieve a final concentration of approximately 5 × 10^5 CFU/mL. The positive control contained bacteria without oil, the negative control contained only broth, and the sterility control contained oil in broth without bacteria. The plates were incubated at 37°C for 24 hours. The MIC was defined as the lowest concentration of the oil that completely inhibited visible bacterial growth.

2.4. Statistical Analysis

All experiments were conducted in triplicate, and data are presented as mean ± standard deviation (SD). Statistical analysis was performed using SPSS software (v.26). One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used to determine significant differences between means, with a p-value of < 0.05 considered statistically significant.

3. Results

3.1. Chemical Composition of Cold-Pressed Nettle Seed Oil

The GC-MS analysis identified 28 compounds in the CPSO, accounting for 96.8% of the total oil composition. The oil was characterized by a high content of unsaturated fatty acids (approximately 85% of the total). The major compounds were linoleic acid (9,12-Octadecadienoic acid, 48.5%), oleic acid (9-Octadecenoic acid, 22.3%), and palmitic acid (Hexadecanoic acid, 9.1%). Minor components included phytol, γ-tocopherol, and β-sitosterol, which are known for their antioxidant and anti-inflammatory properties. A summary of the major compounds is presented in Table 1.

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Table 1. Major chemical constituents of cold-pressed nettle seed oil identified by GC-MS.

R. Time (min)	Compound Name	Peak Area (%)
46.92	9,12-Octadecadienoic acid (Linoleic acid)	48.5
47.05	9-Octadecenoic acid (Oleic acid)	22.3
42.85	Hexadecanoic acid (Palmitic acid)	9.1
35.12	Phytol	3.8
51.45	γ-Tocopherol	2.5
53.88	β-Sitosterol	2.1

3.2. Antimicrobial Activity

The results of the agar well diffusion assay demonstrated that CPSO possesses significant and dose-dependent antimicrobial activity against both test organisms (Table 2). The undiluted oil (100%) showed the highest activity, with ZOIs of 14.5 ± 0.7 mm for *E. coli* and 16.2 ± 0.9 mm for *S. aureus*. The activity was significantly higher ($p < 0.05$) against the Gram-positive *S. aureus* compared to the Gram-negative *E. coli* across all tested concentrations. The negative control (10% DMSO) showed no inhibition, confirming that the observed activity was due to the oil.

Table 2. Zones of Inhibition (ZOI, mm) of cold-pressed nettle seed oil against test bacteria.

Sample	Concentration	ZOI vs. *E. coli* (mm)	ZOI vs. *S. aureus* (mm)
CPSO	100%	14.5 ± 0.7	16.2 ± 0.9
	50%	11.2 ± 0.5	13.8 ± 0.6
	25%	8.1 ± 0.4	10.5 ± 0.5
Tetracycline	30 µg/mL	22.4 ± 1.1	25.8 ± 1.3
Negative Control	10% DMSO	0	0

The broth microdilution assay confirmed the potent antibacterial effect, with MIC values of 1.25 mg/mL for *E. coli* and 0.625 mg/mL for *S. aureus*.

4. Discussion

This study provides the first comprehensive report on the antimicrobial efficacy of cold-pressed oil from *Urtica dioica* seeds. The GC-MS profile confirms that CPSO is a rich source of unsaturated fatty acids, particularly linoleic acid, which has been previously reported to possess anti-inflammatory and anti-arthritic activities (Lalitharani et al., 2009). The presence of these major fatty acids, along with bioactive minor compounds like tocopherols and phytosterols, is likely responsible for the observed biological activity.

The significant antimicrobial activity of CPSO aligns with findings from studies on other plant oils. For example, the antimicrobial action of fatty acids is well-documented; they can integrate

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into the bacterial cell membrane, disrupting its integrity and increasing permeability, leading to leakage of cellular contents and cell death (Zheng & Zhu, 2003, Salem, & Salem, 2025). The higher susceptibility of *S. aureus* (Gram-positive) compared to *E. coli* (Gram-negative) is a common observation in phytochemical studies (Kadak & Salem, 2020). This can be attributed to the structural difference in their cell walls; the outer membrane of Gram-negative bacteria, rich in lipopolysaccharides, presents a more effective permeability barrier to hydrophobic compounds than the single peptidoglycan layer of Gram-positive bacteria.

The MIC value of 0.625 mg/mL against *S. aureus* indicates potent activity, comparable to some reports on essential oils and plant extracts (Salem et al., 2025). The synergistic effects between the major fatty acids and the minor antioxidant compounds (e.g., γ-tocopherol) in the oil may enhance its overall efficacy by protecting the oil from oxidation and potentially damaging bacterial cells through oxidative stress mechanisms (Alvarez-Martínez et al., 2020, Salem, 2024).

While this study demonstrates promising *in vitro* results, it has limitations. The study did not evaluate the oil's cytotoxicity on mammalian cells or its efficacy against clinical multidrug-resistant isolates. Future research should focus on these aspects, along with the isolation of specific active compounds and the evaluation of the oil's potential as an antibiotic adjuvant to combat AMR, a strategy highlighted as crucial in recent literature (Salem et al., 2025).

5. Conclusion

In conclusion, cold-pressed nettle seed oil has been characterized as a rich source of unsaturated fatty acids and bioactive compounds. It exhibits significant, dose-dependent antimicrobial activity against both Gram-positive and Gram-negative bacteria, with a notably strong effect against *Staphylococcus aureus*. The MIC values further confirm its potential as a natural antimicrobial agent. These findings scientifically validate the traditional use of nettle and open new avenues for its application. CPSO shows promise for development as a natural preservative in the cosmetic and food industries, or as a topical therapeutic agent. Further investigations into its safety profile, mechanism of action, and synergistic effects with conventional antibiotics are warranted.

References

Alvarez-Martínez, F. J., Barrajón-Catalán, E., Encinar, J. A., Rodríguez-Díaz, J. C., & Micol, V. (2020). Antimicrobial capacity of plant polyphenols against gram-positive bacteria: A comprehensive review. *Current Medicinal Chemistry*, *27*(15), 2576–2606.

Aloraibi, A. M., Alhadad, A. A., & Ali, E. A. (2025). Isolation and Identification of Bacteria Causing UrinaryTract Infections and Their Antibiotic Sensitivity Pattern at Mohammad Almoghariaf Hospital in Ajdabiya. Libyan Open University Journal of Applied Sciences (LOUJAS), 08-18.

Alhijjaji, F. A., & Arross, W. M. B. (2026). Isolation and Identification of Methicillin-resistant Staphylococcus aureus in the Neonatal Intensive Care Unit of Zliten Medical Center. Libyan Open University Journal of Medical Sciences and Sustainability, 01-10.

Bauer, A. W. (1966). Antibiotic susceptibility testing by a standardized single disc method. *American Journal of Clinical Pathology, 45*, 149–158.

Fong, I. W. (2023). Antimicrobial resistance: a crisis in the making. In *New Antimicrobials: For the Present and the Future* (pp. 1–21). Springer.

Hsin, M. A. M. B., Emsaed, H. A. M., Abujarida, A. R., Sauf, M. A., Soof, S. A., & Salem, M. O. A. (2025). A Study on the Isolation and Identification of Bacteria in Patients with Urinary Tract Infections in Libyan Laboratories. *African Journal of Academic Publishing in Science and Technology (AJAPST)*, *1*(4), 1-10.

Kadak, A. E., & Salem, M. O. A. (2020). Antibacterial activity of chitosan, some plant seed extracts and oils against *Escherichia coli* and *Staphylococcus aureus*. *Alinteri Journal of Agriculture Sciences*, *35*(2), 144–150.

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Lalitharani, S., Mohan, V. R., Regini, G. S., & Kalidass, C. (2009). GC-MS analysis of ethanolic extract of *Pothos scandens* leaf. *Journal of Herbal Medicine and Toxicology, 3*, 159–160.

Moriel, D. G., Piccioli, D., Raso, M. M., & Pizza, M. (2024). The overlooked bacterial pandemic. *Seminars in Immunopathology, 45*(4), 481–491.

Salem, M. O. A. (2024). Antimicrobial Activity of Aqueous Methanolic Extract of Lichen (*Usnea barbata*) Against Escherichia coli and Staphylococcus aureus. (2024). *Libyan Journal of Ecological & Environmental Sciences and Technology*, *6*(1), 19-23. https://doi.org/10.63359/j8639d64

Salem, M. O. A. (2025). Advancing Green Pharmacy through Biodegradable Drug Delivery Systems for Reducing Pharmaceutical Pollution and Enhancing Therapeutic Outcomes. *Al-Mutawassit Journal for Basic and Applied Sciences*, *1*(1), 35-43. https://mutawassitpub.com/index.php/mjbas/article/view/6

Salem, M. O. A., & Lakwani, M. A. S. (2024). Determination of chemical composition and biological activity of flaxseed (*Linum usitatissimum*) essential oil. *Journal of Biometry Studies, 4*(2), 91–96.

Salem, M. O. A., Ahmed, G. S., Abuamoud, M. M. M., & Rezgalla, R. Y. M. (2025). Antimicrobial Activity of Extracts of Dandelion (*Taraxacum officinale*) Against *Escherichia coli* and *Staphylococcus aureus*: Mechanisms, Modern Insights, and Therapeutic Potential. *Libyan Journal of Medical and Applied Sciences, 3*(2), 37–40.

Salem, M., & Salem, I. (2025). Antimicrobial Polymers: Mechanisms of Action and Applications in Combating Antibiotic Resistance. *Al-imad Journal of Humanities and Applied Sciences (AJHAS)*, 12-15.

Zheng, L. Y., & Zhu, J. F. (2003). Study on antimicrobial activity of chitosan with different molecular weights. *Carbohydrate Polymers, 54*(4), 527–530.

Zhmool, H. S., Banana, A. A., Abboub, A. D., Mohamed, G. S., Sallam, R. M., & Alhadad, A. A. (2026). Microbiological and Physicochemical Assessment of Antibiotic-Resistant Bacteria in Hospital Wastewater: A Case Study from Sabratha Teaching Hospital, Libya. Libyan Open University Journal of Medical Sciences and Sustainability, 38-48.

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