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Extraction, Separataion, And Chemical Characterisation Of Salvadora Persica Using Gc-Ms, Nmr, And Ftir

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Abstract *Salvadora persica L. (Miswak/Siwak) has been used for oral hygiene for over 7,000 years, particularly in Islamic countries. This study investigates the phytochemical profile and therapeutic potential of S. persica (roots/stems), focusing on its applications for oral and microbial diseases. Successive Soxhlet extractions with petroleum ether, ethyl acetate, and ethanol were employed to isolate bioactive compounds. Functional groups and chemical constituents were characterised via FTIR, GC-MS, and NMR (1H/13C). Key findings include benzyl nitrile, dibutyl phthalate, and cis-10-heptadecenoic acid, the last of which was the most abundant. These compounds correlate with the plant's antimicrobial, antifungal, and anti-inflammatory properties. These results underscore S. persica's potential as a sustainable resource for biomedical applications.*
Keywords: *Trifluoroacetic acid, pentadactyl ester, natural products, phytochemicals, Salvadora persica*

INTRODUCTION

Salvadora persica L. is a tree native to Saudi Arabia, Iran, India, and parts of Africa, and it is widely used in Islamic countries (Aljarbou et al., 2022). The World Health Organization (WHO) recognises it as a safe instrument for oral hygiene. (Azizan et al., 2023). The plant has various names, including siwak, sewak, and miswak, and its chewing sticks are referred to differently in Arabic, Latin, and Japanese (Hunaydi et al., 2023). Miswak has been used for over 7,000 years by civilisations such as the Babylonians, Romans, Greeks, and ancient Egyptians, as well as by millions of Muslims worldwide (Jassim et al., 2021). The active components in Miswak vary in chemical formula, structure, concentration, and biological action (Khojasteh-Taheri et al., 2023). Its extracts contain important medicinal compounds, such as benzyl isothiocyanate, alkaloids, tannins, glycosides, terpenes, flavonoids, fatty acids, and minerals (e.g., silicon, potassium, sodium, fluorine) (Hunaydi et al., 2023). Aqueous extracts from its root's stems, or leaves exhibit antibacterial effects against specific pathogens, with low to moderate antimicrobial activity attributed to organic and inorganic constituents (Ameen, 2024). In this study, fresh Miswak material was collected, and components were extracted using solvents of varying polarities. Essential oils were isolated via column chromatography, and the structures of purified compounds were determined using FTIR spectroscopy, GC-MS, and NMR (1H and 13C). All isolated compounds were tested for antibacterial activity against pathogenic bacteria.

MATERIALS AND METHODS

Plant

Salvadora persica, the medicinal plant employed in this investigation, was procured from Saudi Arabia.

Plant extracts

A Soxhlet apparatus was employed for sequential extraction using solvents of varying polarity: petroleum ether, ethyl acetate, and ethanol.

Procedure

One hundred grams of Salvadora persica powder were extracted with 500 mL of each solvent in the Soxhlet apparatus for 8 hours daily until the solvent became colourless. The extracts were concentrated using a rotary evaporator at 40°C and stored in opaque glass bottles at 4°C for further analysis.

Analytical Techniques

NMR-based spectra (^{1}H and ^{13}C NMR) for all extracted compounds were recorded in the Islamic Republic of Iran. Infrared (I.R.) spectra were recorded using a Nicolet 100 infrared spectrometer in the laboratories of the College of Science – Chemistry Division, University of Kirkuk,. Gas Chromatography–Mass Spectrometry analysis was conducted utilizing the Shimadzu GC-MS-QP2010 Plus system (Japan) at the University of Samarra.the purification process involved Column

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chromatography using a silica gel beads as the stationary phase, with differential partitioning between the stationary and mobile phases for separation.

RESULTS AND DISCUSSION

Salvadora persica L. was collected from Saudi Arabia, and sequential Soxhlet extraction was performed using ethanol, petroleum ether, and ethyl acetate. Three crude extracts were obtained, and solvents were evaporated using rotary evaporation at 40°C. Each crude extract was subjected to column chromatography for compound isolation.

Petroleum ether crude

The crude extract was separated via "column chromatography" using a 2:1 hexane: ethyl acetate eluent. GC-MS analysis (Figure 1) revealed compounds including N, N-dimethylcyclohexanamine (7.06%), benzyl nitrile (2.14%), *cis*-vaccenic acid (11.97%, highest concentration), heptadecane (4.14%), and 9,12-octadecadienoic acid (3.94%). Peaks at retention times 5.222–14.230 minutes confirmed volatile organic compounds.

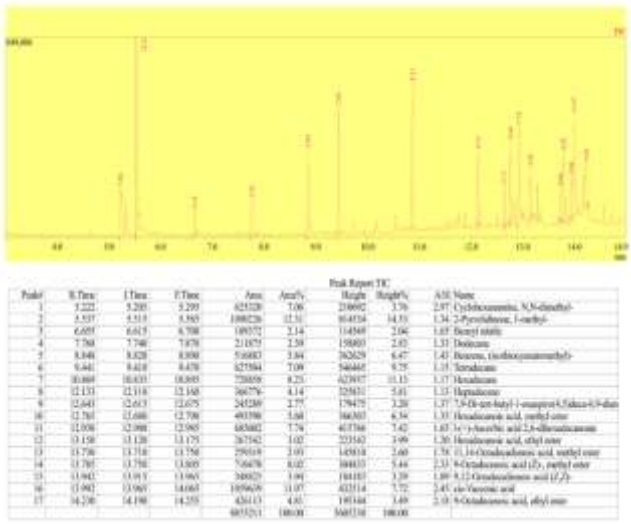

Figure 1. The GC-MS spectrum for the crude

^{1}H NMR (500 MHz, DMSO-d_6): δ 2.35 (s, 6H), 1.82–1.77 (m, 3H), 1.66–1.63 (m, 3H), 1.45 (dt, J = 7.7, 5.8 Hz, 2H), 1.36–1.32 (m, 3H)

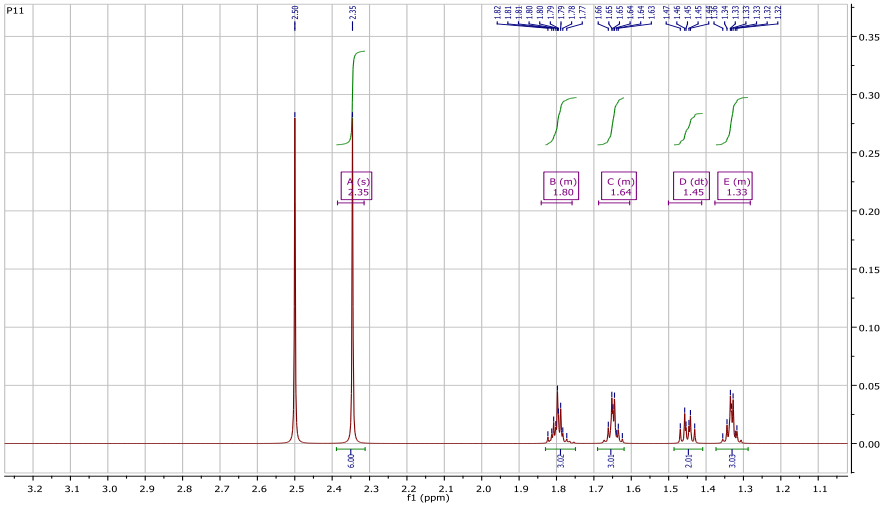

Figure 2. The ^{1}H NMR spectrum for the crude.

^{13}C NMR (125 MHz, DMSO-d_6): δ 62.92, 41.08, 28.72, 25.34, 24.18

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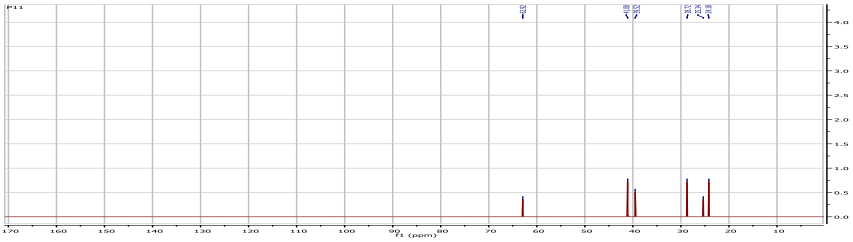

Figure 3. The ^{13}C NMR spectrum for the crude.

FTIR: Aliphatic C-H stretching (2863–2934 cm^{-1}), C-H bending (1463 cm^{-1}), and C-N stretching (1382 cm^{-1}) (Haj et al., 2020; Haj et al., 2023) (Figure 2). The isolated compound was identified as N, N-dimethylcyclohexanamine.

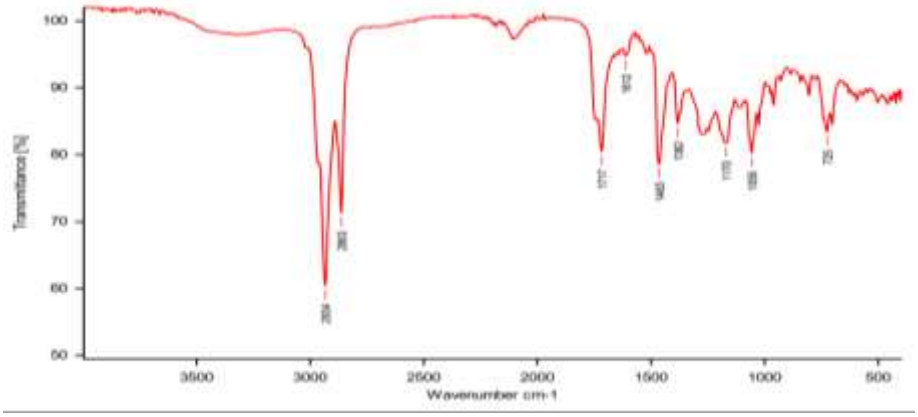

Figure 4. The IR spectrum for the isolated compound.

Ethyl acetate crude

GC-MS analysis (Figure 5) identified trifluoroacetic acid pentaacetyl ester (20.85%, highest), dibutyl phthalate (6.30%), and phenol derivatives.

^1H NMR (500 MHz, DMSO-d_6): δ 4.14 (t, *J* = 5.1 Hz, 2H), 1.64 (p, *J* = 5.5 Hz, 2H), 1.31–1.35 (m, 23H), 0.99 (t, *J* = 6.4 Hz, 3H).

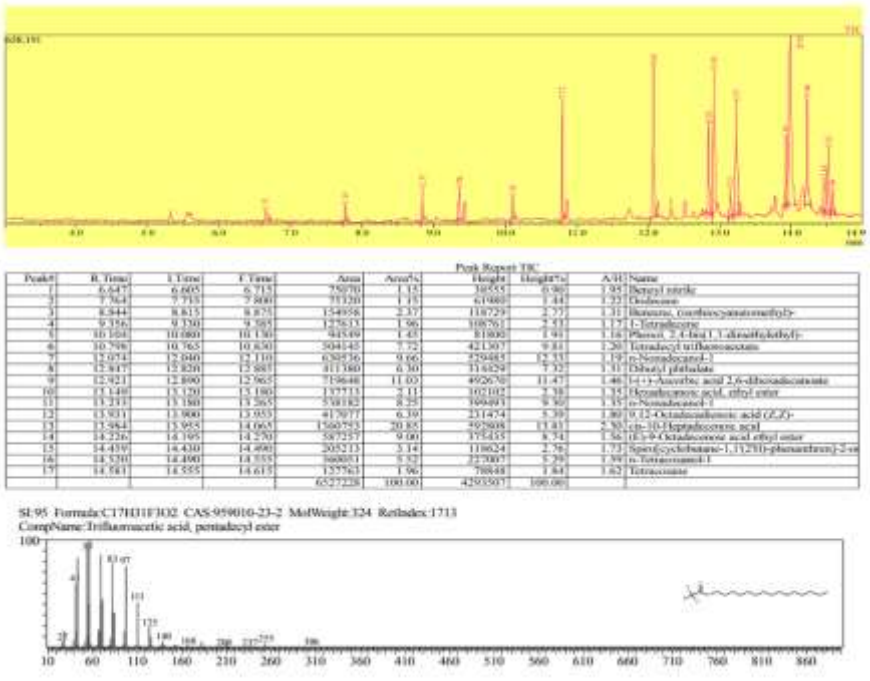

Figure 5. The GC-MS spectrum for the crude.

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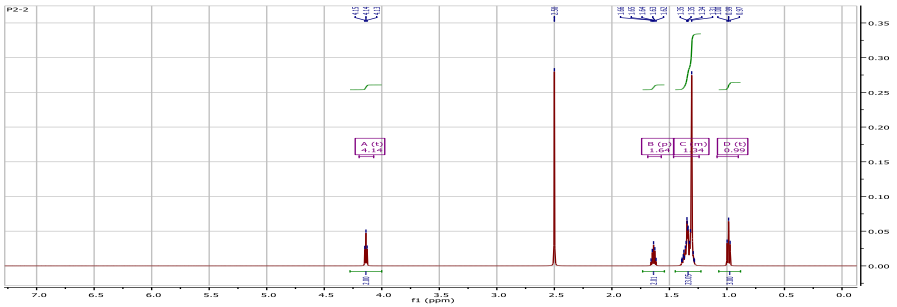

Figure 6. The $^{1H\text{-}NMR}$ spectrum for the isolated compound.
^{13}C NMR (125 MHz, DMSO-d_6): δ 159.36–113.94 (aromatic), 66.96–13.46 (aliphatic).

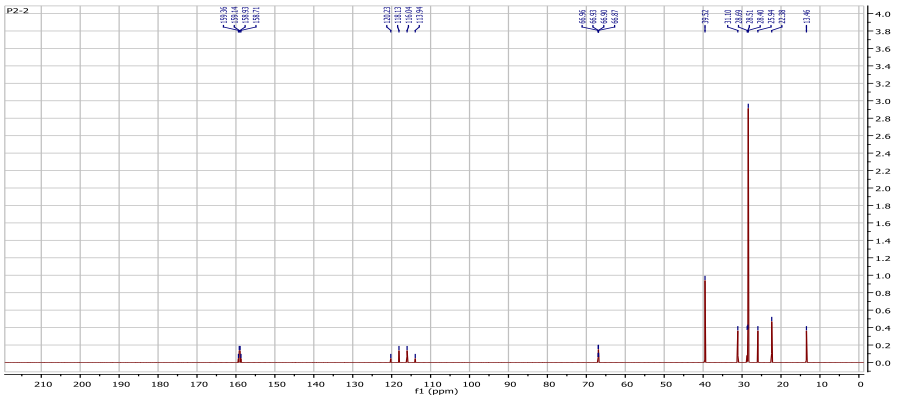

Figure 7. The $^{1H\text{-}NMR}$ spectrum for the isolated compound.
FTIR: C-H (2922, 2850 cm^{-1}), C=O (1740 cm^{-1}), and C-O (1150–1250 cm^{-1}) stretches confirmed an ester (Figure 4). The isolated compound was identified as Trifluoroacetic acid.

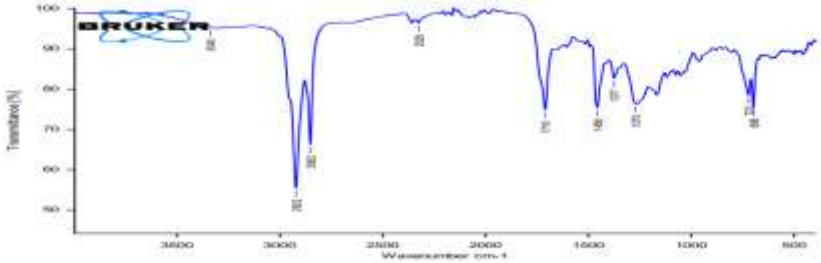

Figure 8. The IR spectrum for the isolated compound.

Ethanol crude

First component

GC-MS (Figure 9) showed tetracosane (16.93%, highest), benzylamine (15.56%), and diethyl phthalate 3.38%

^{1}H NMR (500 MHz, $CDCl_3$): δ 1.39–1.29 (m, 18H), 0.99 (t, J = 6.4 Hz, 6H).

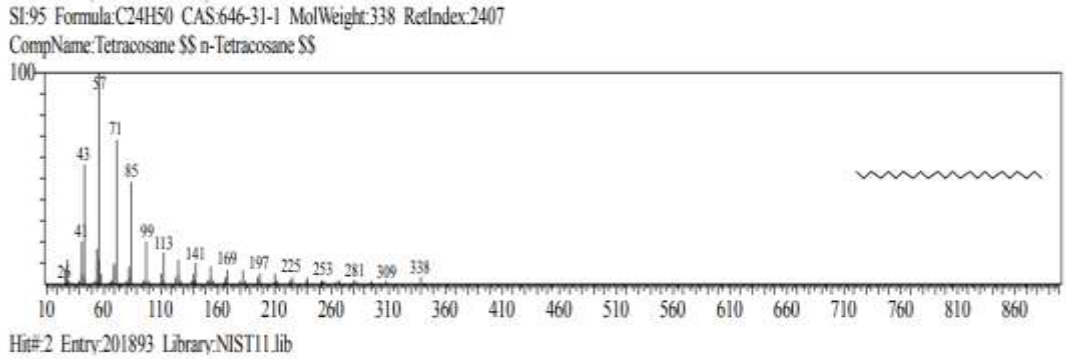

Figure 9. The GC-MS spectrum for the crude.

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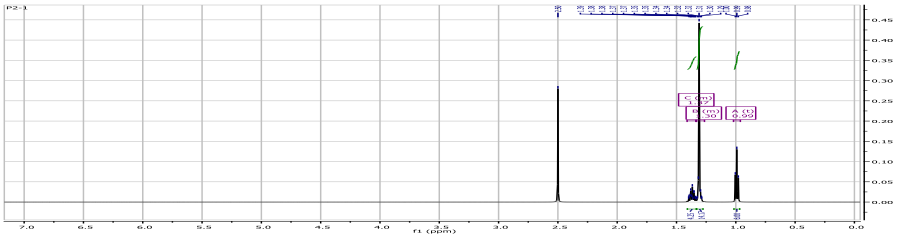

Figure 10. The $^{1H\text{-}NMR}$ spectrum for the isolated compound.
^{13}C NMR (125 MHz, $CDCl_3$): δ 31.64, 29.06, 28.95, 22.93, 14.01.

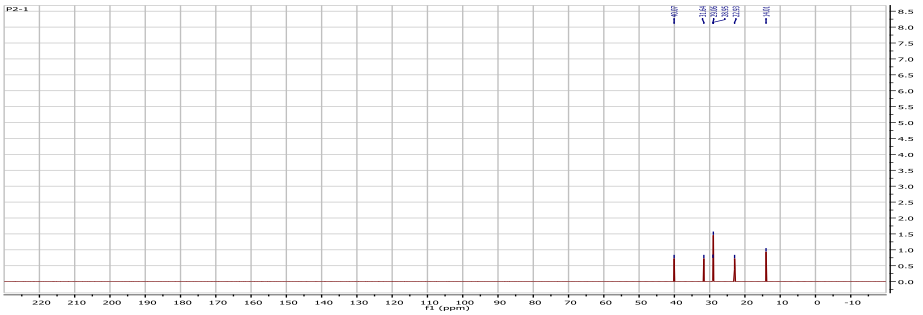

Figure 11. The $^{13C\text{-}NMR}$ spectrum for the isolated compound.
FTIR: C-H stretches (2923–2853 cm^{-1}) and bending (1456 cm^{-1}) (Figure 12).

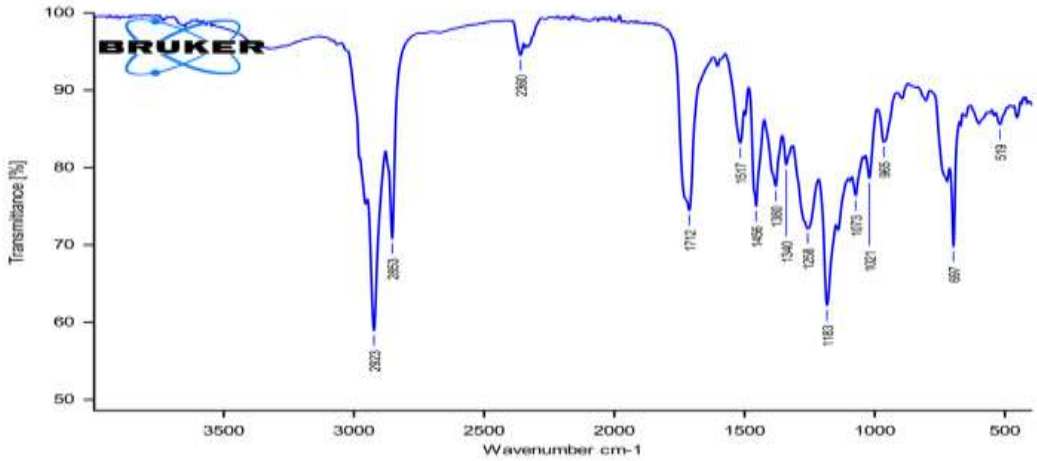

Figure 13. The IR spectrum for the isolated compound.
Second component (Cis-vaccenic acid)

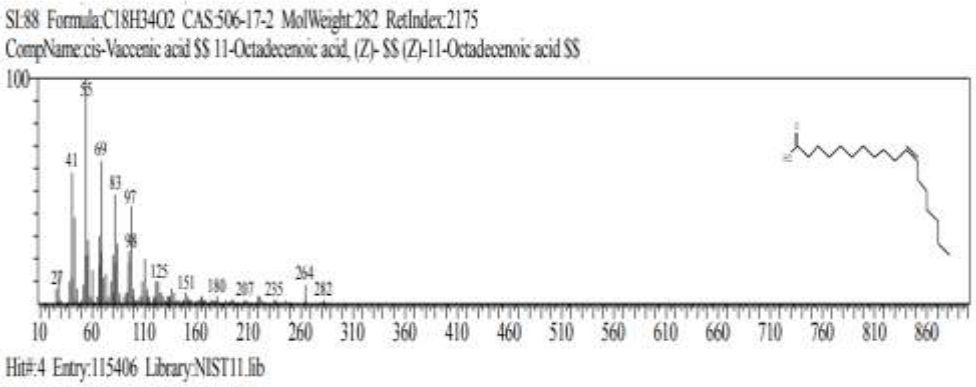

Figure 14. The GC-MS spectrum for the crude.
^{1}H NMR (500 MHz, Chloroform-d) δ 9.47 (s, 1H), 5.50 – 5.44 (m, 2H), 2.29 (t, *J* = 8.1 Hz, 2H), 2.11 – 2.03 (m, 4H), 1.64 (p, *J* = 8.0 Hz, 2H), 1.36 – 1.31 (m, 23H), 0.99 (t, *J* = 6.4 Hz, 3H).

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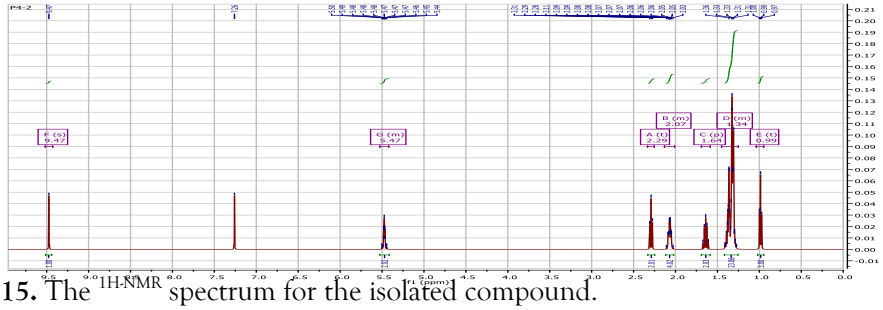

Figure 15. The ^{1}H-NMR spectrum for the isolated compound.

^{13}C NMR (125 MHz, Chloroform-d) δ 177.13, 130.72, 76.97, 34.64, 31.64, 29.24, 29.06, 28.95, 28.93, 28.72, 28.27, 24.80, 22.93, 14.01.

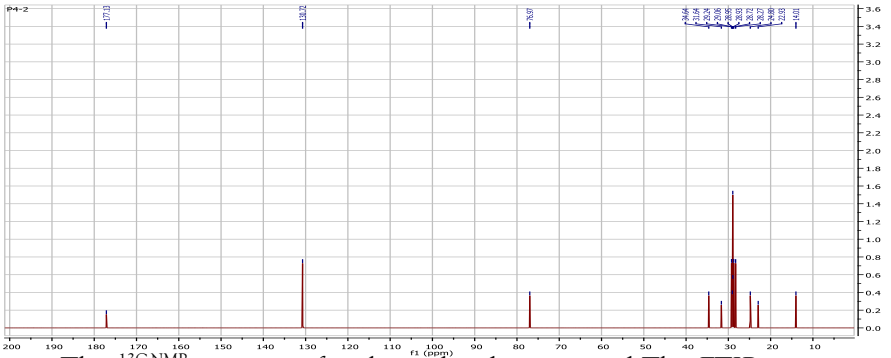

Figure 16. The ^{13}C-NMR spectrum for the isolated compound.The FTIR spectrum indicated significant absorption peaks at O-H Stretch (Carboxylic Acid):3346 cm^{-1}, C=O 1716cm^{-1}, and Peaks 2923-2853cm^{-1} for C-H stretching in alkyl chains. (Haj et al., 2020; Haj et al., 2023) (Figure 17)

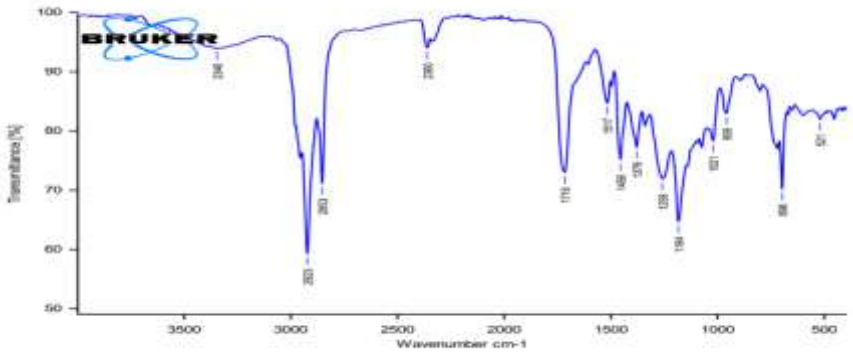

Figure 117. The IR spectrum for the isolated compound.

Third component (9-octadecenoic ethyl ester)

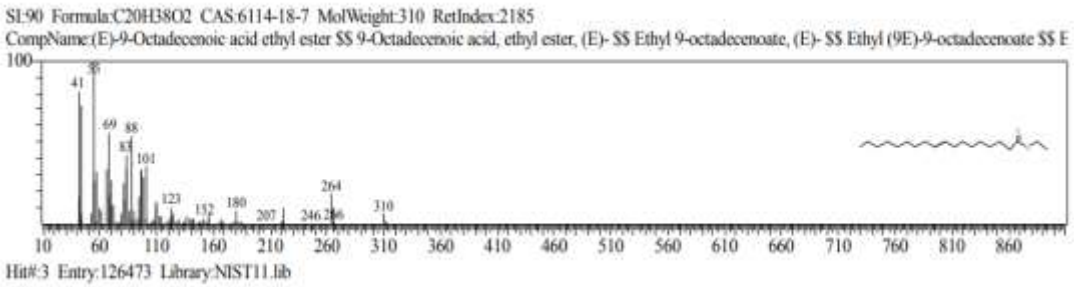

Figure 18. The GC-MS spectrum for the crude.

^{1}H NMR (500 MHz, DMSO-6d) δ 5.51 (ddd, *J* = 5.2, 3.4, 1.9 Hz, 2H), 4.18 (q, *J* = 6.0 Hz, 2H), 2.34 (t, *J* = 8.0 Hz, 2H), 2.08 – 1.93 (m, 4H), 1.68 (p, *J* = 7.9 Hz, 2H), 1.46 – 1.23 (m, 22H), 0.99 (t, *J* = 6.4 Hz, 3H).
^{13}C NMR (125 MHz, DMSO-6d) δ 173.56, 129.22, 60.62, 33.47, 31.10, 30.80, 28.69, 28.51, 28.40, 28.38, 28.17, 24.78, 22.38, 14.14, 13.46.
The FTIR spectrum figure 20 indicated significant absorption peaks at C-O (Ester C-O stretch)1258 cm^{-1}, [1]:Absorption Bands C=O peaks 1714 cm^{-1}. (Haj et al., 2020; Haj et al., 2023) (Figure 19)

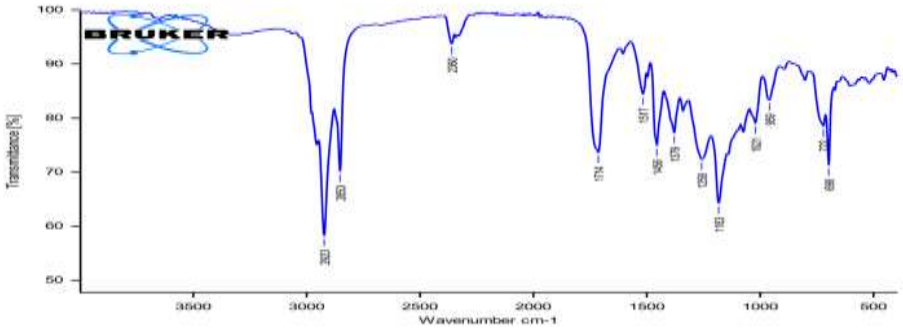

Figure 19. The IR spectrum for the isolated compound.

Antimicrobial Testing
The extracts were evaluated against E. coli and S. aureus using nutrient agar (37°C, pH 7). DMSO served as the control.
Method: Two eukaryotic and two bacterial strains were employed to assess the antibacterial activity of the newly unique descendants. The tested microorganisms included two species: E. coli and S. aureus. These microbes were gathered from Kirkuk's health labs. Nutrient agar was used as a culture medium. The strains were revived by inoculating Luria-Bertani (LB) culture medium, 1% peptone, 0.5% yeast extract, and 1% NaCl, and then maintained at 37°C and pH 7 ±0.2 for 18 hours. A 48-well plate was filled with samples, and 250 µL of the animal delay (100 CFU/mL) was kept at 37°C alongside the LB culture. The final product was incubated at 37 °C for 1 day. Dimethyl sulfoxide (DMSO) was used as a solvent and control sample [7].

Material concentration and Inhibition zone Diameter
Inhibition zone Diameter for (cyclohexane amine N, N-dimethyl 5%)
Escherichia. coli: 24 mm, Staphylococcus aureus: 13 mm.
Inhibition zone Diameter for (trifluoroacetic acid, pentadecyl ester 5%)
Escherichia. coli: 22 mm, Staphylococcus aureus: 18 mm.
Inhibition zone Diameter for (Tetracosane 3%)
Escherichia. coli: 17 mm, Staphylococcus aureus 14 mm.
Inhibition zone Diameter for (Cis vaccenic acid 5%)
Escherichia. coli: 22 mm, Staphylococcus aureus.: 25 mm.
Inhibition zone Diameter for (9-octadecenoic ethyl ester, 7%)
Escherichia. coli: 15 mm, Staphylococcus aureus: 28 mm.

CONCLUSION
This study highlights the promising potential of Salvadora persica L. as a natural resource for promoting oral hygiene and serving various biological applications. The plant includes bioactive chemicals with antibacterial, anti-inflammatory, and antioxidant effects. Trifluoroacetic acid is crucial in pharmaceutical synthesis and antimicrobial treatments, highlighting the synergy between natural compounds and advanced chemical methodologies. This study reaffirms Salvadora persica's traditional uses and positions it as a viable candidate for developing innovative medical and pharmaceutical applications. Future

research could explore scalability and integration into modern therapeutic frameworks. We extracted Salvadora persica using solvents of different polarities with a Soxhlet apparatus and obtained five compounds, which are cyclohexane amine N, N-dimethyl, trifluoroacetic acid, pentadecyl ester, tetracosane, and 9-octadecenoic ethyl ester. They all affect E. coli and S. aureus bacteria.

REFERENCES

1. Ameen, S. M. (2024). Natural dentin remineralizing agents. In Natural conservative dentistry: An alternative approach to solve restorative problems (p. 59).
2. Aljarbou, F., Almobarak, A., Binrayes, A., & Alamri, H. M. (2022). Salvadora persica's biological properties and applications in different dental specialties: A narrative review. Evidence-Based Complementary and Alternative Medicine, 2022(1), 8667687.
3. Azizan, N. F., Mohd, N., Nik Azis, N. M., & Baharin, B. (2023). Effectiveness of Salvadora persica toothbrush and Salvadora persica chewing stick in plaque and gingivitis control: A randomised control trial. BMC Complementary Medicine and Therapies, 23(1), 456. https://doi.org/10.1186/s12906-023-04295-z
4. Haj, N. Q., Mohammed, M. O., & Mohammood, L. E. (2020). Synthesis and biological evaluation of three new chitosan Schiff base derivatives. ACS Omega, 5(26), 15885–15894.
5. Haj, N. Q., Mohammed, M. O., & Mohammood, L. E. (2023). Synthesis, identification, and anti-cancer evaluation of some heterocyclic chitosan-thiosemicarbazones compounds and their nickel (II)
6. Hunaydi, Z. F. O., Shafiai, N. A. A., Noor, S. N. F. M., & Rahman, N. R. A. (2023). Clinical effects, uses, and applications of Miswak Salvadora persica on oral health over the last three decades: A scoping literature review. Journal of Health and Translational Medicine, 26(3), 310–324.
7. Jassim, S. A., Hammond, K. K., & Alsheekhly, B. (2021). Valuable dental materials from Salvadora persica plants. Medico-Legal Update, 21(1), 1–5.
8. Khojasteh-Taheri, R., Ghasemi, A., Meshkat, Z., Sabouri, Z., Mohtashami, M., & Darroudi, M. (2023). Green synthesis of silver nanoparticles using Salvadora persica and Caccinia macranthera extracts: Cytotoxicity analysis and antimicrobial activity against antibiotic-resistant bacteria. Applied Biochemistry and Biotechnology, 191(8), 5120–5135.