

Spectral analysis and bioactive profiling of hot methanolic extracts from *Phoenix dactylifera* seeds: Antibacterial efficacy and *in vitro* cytotoxicity insights

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Abstract

Phoenix dactylifera, commonly called date palm, has great importance as a fruiting plant. The hot methanolic extract of date seeds (HMEDSE), was further fractionated into three fractions (F1, F2, and F3) through column chromatography. The three fractions were composed of various bioactive constituents which was analysed through GC-MS and FT-IR analysis. The results revealed remarkable antibacterial properties of crude HMEDSE against various pathogenic microorganisms affecting humans. The spectrum of activity of HMEDSE against various human pathogenic bacteria showed the following sequence based on its efficacy, *Escherichia coli* (17.6 ± 2.5 mm), *Klebsiella pneumoniae* (16.3 ± 2.5 mm), *Staphylococcus aureus* (16.3 ± 1.5 mm), *Streptococcus pyogenes* (15 ± 2.6 mm), *Pseudomonas aeruginosa* (15 ± 2 mm), and lastly, *Bacillus subtilis* (14.3 ± 2 mm). Furthermore, HMEDSE exhibited cytotoxicity, with an IC_{50} of 73.5 ± 0.5 μ g/mL against MCF-7 ATCC breast cancer cells, leading to gradual apoptosis.

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Keywords: anti-bacterial effect; hot methanolic extract; human pathogenic bacteria; *in vitro* cytotoxicity; MCF-7 breast cancer cells; *Phoenix dactylifera*; seeds

Introduction

In ancient times, herbs and plants used for medicinal purposes were the main sources of treatment for various diseases. Therefore, natural products contribute significantly to the maintenance of good health (Moni *et al.*, 2021). *Phoenix dactylifera*, commonly known as date palm, is a flowering botanical species in the Arecaceae family. This plant, scientifically named *Phoenix dactylifera* L., is cultivated throughout the Arabian Peninsula in arid and semi-arid regions (Al-Alawi *et al.*, 2017; Mariod *et al.*, 2017; Mohamed *et al.*, 2021; Al Arabiya, 2022). The date palm is an important fruiting plant revered in numerous regions of the world, especially in the Middle East and North Africa, with cultivation spanning thousands of years. In Saudi Arabia, the date fruit not only plays a crucial role in strengthening the local economy but is also a staple food for the inhabitants of the areas where this esteemed plant thrives. More than 300 different varieties of dates have been shipped out from the Kingdom of Saudi Arabia. In a report, the Saudi Arabian Ministry of Environment, Water and Agriculture (EWA) stated that the country's date exports amounted to \$324 million. The Food and Agriculture Organization (FAO), which has declared 2027 the International Year of Dates, claims that the ministry has succeeded in having dates included in the International Superfruit Index. Palms and the industries derived from their processing are among the most important in the world (Al Arabiya, 2022).

Dates are considered ideal for their numerous nutritional and therapeutic benefits. They have been used for centuries for medicinal purposes, and they contain various bioactive compounds that benefit human health. Dates are an excellent source of sugar, fiber, minerals, vitamins, and protein. Extracts of pulp, seeds, pollen, and ovaries of dates are used to treat and prevent various chronic diseases. Dates contain a variety of secondary metabolites such as flavonoids, carotenoids, anthocyanins, procyanidins and sterols. In addition, dates contain bioactive compounds that have a variety of therapeutic properties, such as anticancer, anti-inflammatory, antioxidant, antibacterial, and hepatoprotective properties (Rahmani *et al.*, 2014; Sassi *et al.*, 2020; Mohamed *et al.*, 2021).

Materials and Methods

Fruit sample, processing, and extraction

A bundle of dates was obtained from a nearby market in Jazan, Saudi Arabia. The research plan is visually depicted in Figure 1. The fruits were carefully packed in a sterile plastic bag and identified under expert guidance of Dr. Remesh Mochikkal, the herbarium curator of Jazan College Herbarium. After identification, the voucher specimen was officially marked with the identification code JZUH 1226. The fruit skin was removed, and the seeds were separated, washed in tap water, then washed in Millipore water, followed by two-week drying in a shade. Thereafter, they were blended to a coarse powder in a mill. Subsequently, 200 grams of the desiccated seed powder underwent a 4-hour Soxhlet extraction with methanol maintained at 60 °C. Once the extraction concluded, the derivatives were segregated using a silica gel 60 column into three distinct fractions, aptly labeled as F1, F2, and F3. Then, F1, F2 and F3 were clarified by spinning at 3000 × rpm for 15 minutes. The supernatants were transferred one by one into a new glass jar, and the lid was removed to allow the solvent to evaporate during the air-drying phase of the process. After the seed extract was thoroughly air-dried, the dried samples were removed from the beakers by scraping, and were combined, aggregated, and weighed.

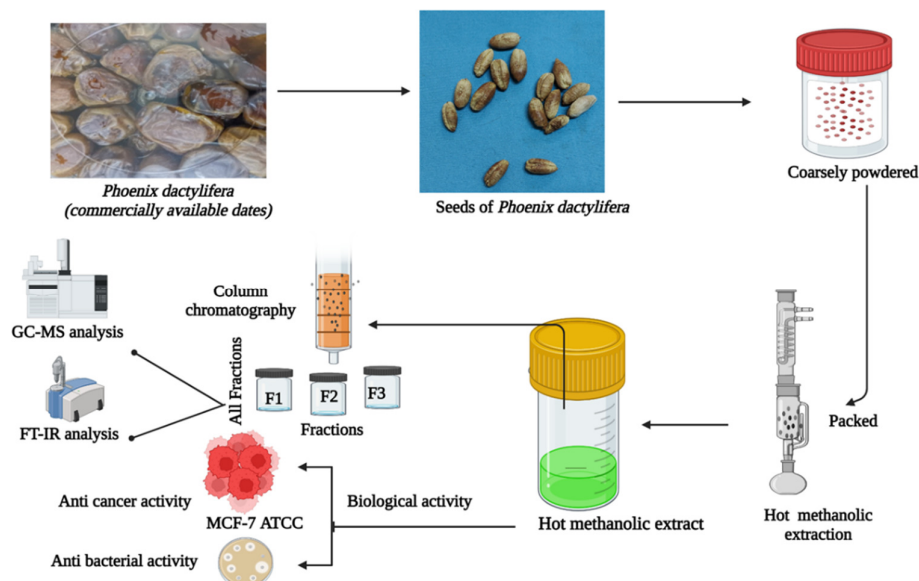


Figure 1. Schematic representation on the processing of present study

This figure was Created with BioRender.com, Bio Render, Canada

GC-MS analysis

The Thermo Scientific TR Gas Chromatography-Mass Spectrometry (GC-MS) system, a sophisticated analytical tool, was used to detect potentially useful compounds in samples F1, F2 and F3. It includes an AS 3000 autosampler for precise sample introduction, a Trace Ultra-GC for the separation of compounds via a capillary column and an ISQ mass detector for the identification and quantification of compounds based on their mass-to-charge ratio. This comprehensive setup, inspired by the methods developed by Sivakumar *et al.* (2021), ensures accurate and reliable analysis, allowing the chemical constituents of the samples to be accurately recognized and evaluated.

Fourier transform infrared spectroscopy (FT-IR) studies

The functional groups in samples F1, F2, and F3 were determined by Fourier transform infrared spectroscopy (FT-IR). The analysis was facilitated by a Nicolet iS10 FT-IR spectrophotometer, a product of Thermo Fisher Scientific Inc. in the USA. Samples were prepared using the KBr pellet technique, and their spectral analysis was performed in the resolution range of 400-4000 cm^{-1} (Sivakumar *et al.*, 2021).

Determination of antibacterial effect

The study aimed to examine the antibacterial properties against specific human pathogenic bacteria, namely *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*.

Bacterial strains

The study employed bacterial strains: *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Streptococcus pyogenes* ATCC 19615, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Klebsiella pneumoniae* ATCC 700603. All bacterial cultures utilized in this research were consistently preserved in the microbiology laboratory at the College of Pharmacy through routine subculturing at specified intervals.

Standardization of bacterial culture

A 24-hour culture of each bacterium was prepared in nutrient broth, a growth medium providing essential nutrients for bacterial growth, and subsequently diluted in nutrient broth using a series of dilutions, with concentrations ranging from 10^{-1} to 10^{-7} . The viability of the bacterial cultures was tested by determining the number of colony-forming units (CFU) in 1 mL. To determine CFU/mL, aliquots of the diluted culture are plated on agar plates and incubated. After incubation, the number of colonies is counted. The standardized bacterial cultures enable the quantification of antibacterial activity.

Determination of antibacterial susceptibility

The antibacterial susceptibility test was conducted following the protocol outlined by Moni *et al.*, 2018 and Sivakumar *et al.*, 2021. In summary, Muller Hinton agar plates were produced to conduct antibacterial investigation. Bacterial subcultures were derived from the original culture, and after incubating for 24 hours, the culture was examined for antibacterial activity. The Agar well diffusion technique was used to analyze the HMEDSE and standard ciprofloxacin disc with a 50 mcg/mL concentration. The inoculation was performed by immersing a sterile cotton swab into the standardized (CFU/mL) culture of different organisms and spreading it equally on the MH agar plate by rotating the petri dish. The plates were dried for approximately 10 minutes before the delivery of sample analytes. The agar well diffusion approach was executed by creating perforations on the inoculated MH agar plates using a conventional sterile stainless-steel borer. The plates were placed in an incubator at 37 °C for 24 hours. The antibacterial spectrum was evaluated by observing the formation of zones that block bacterial growth after 24 hours of incubation.

Cytotoxic effect and morphological analysis

MCF-7 ATCC human breast cancer cells were cultivated in RPMI-1640 media, buffered with 2.0 g/L sodium bicarbonate, and adjusted to a pH of 7.4. These cells were housed in a CO₂ incubator (Heraeus, Germany), under conditions of 37 °C, 90% relative humidity, and an atmosphere of 5% CO₂. The media was further supplemented with 10% v/v fetal bovine serum (FBS), 100 U/mL of penicillin, and 100 µg/mL of streptomycin. Studies detailing the cytotoxic characteristics of HMEDSE and associated morphological examinations have been documented in prior work (Sultan *et al.*, 2022; Suvitha *et al.*, 2022).

Statistical analysis

Each experiment was repeated three times ($n = 3$), and data were analyzed using one-way ANOVA. The Prism 9 GraphPad InStat software, USA was used to analyse statistical significance. Tukey-Kramer test as post-hoc analysis was performed to compare the values of test samples with those of standard drugs. The statistical significance was assumed at $p < 0.05$.

Results

The biochemical compounds in fractions F1, F2, and F3 from HMEDSE, as confirmed using GC-MS spectrum shown in Figure 2. As shown in Tables 1-3, several bioactive components were detected in fractions F1, F2, and F3 in the current study. The structures of the compounds are shown in Figures 3, 4, and 5. Dasycarpidan-1-methanol, acetate, an alkaloid was found in all three methanolic fractions, with retention time (RT) of 67.23, 67.29, and 60.28 min in F1, F2, and F3, respectively. 1-Monolinoleoylglycerol was determined in F1 and F2. 1-Monolinoleoylglycerol, with a retention time of 61.29 min, was found in F1 and F2. Ethyliso-allocholate have been determined significantly in F2. A steroidal molecule, ethyl isoallocholate, was found in F2 but not in F1 and F3. Ethyliso-allocholate eluted with a probability index of 25.58% and occupied 10.73% of the chromatogram, with a RT of 53.12 min (Figure 2B).

Table 1. GC-MS detection of possible bioactive compounds of fraction 1 (F1) of the hot methanolic extract from the seeds of *Phoenix dactylifera* (Dates)

Compound name	Molecular formula	Molecular weight	Retention time (Min)	Probability Index	Percent area of curve
Dasycarpidan-1-methanol, acetate (ester)	C ₂₀ H ₂₆ N ₂ O ₂	326	67.23	33.79	0.29
1-Monolinoleoylglycerol	C ₂₇ H ₅₄ O ₄ Si ₂	498	61.29	52.3	0.89
Oleic acid, eicosyl ester	C ₃₈ H ₇₄ O ₂	562	53.95	28.78	2.60
Cyclodecasiloxane, eicosamethyl-	C ₂₀ H ₆₀ O ₁₀ Si ₁₀	741.5	48.46	59.72	2.87
17-Pentatriacontene	C ₃₅ H ₇₀	490	39.06	38.66	0.33
Heptadecane, 9-hexyl-	C ₂₃ H ₄₈	324	35.77	36.80	0.29
n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	32.44	17.74	1.72
Tetratetracontane	C ₄₄ H ₉	618	43.88	11.76	1.85
1-Tricosanol	C ₂₃ H ₄₈ O	340	40.55	38.76	6.68
Octadecane, 3-ethyl-5-(2-ethylbutyl)-	C ₂₆ H ₅₄	366	37.15	14.39	1.70
Cholestan-3-one, cyclic 1,2-ethanediyl acetal, (5á)-	C ₂₉ H ₅₀ O ₂	430	31.37	31.42	2.06
2-Phenylpropenal	C ₉ H ₈ O	132	12.97	57.62	0.20

Table 2. GC-MS detection of possible bioactive compounds of fraction 2 (F2) of the hot methanolic extract from the seeds of *Phoenix dactylifera* (Dates)

Compound name	Molecular formula	Molecular weight	Retention time (Min)	Probability Index	Percent area of curve
Ethyl iso-allocholate	C ₂₆ H ₄₄ O ₅	436	53.12	25.58	10.73
Dasycarpidan-1-methanol, acetate (ester)	C ₂₀ H ₂₆ N ₂ O ₂	326	67.29	49.55	8.46
Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	31.51	71.18	7.43
3,12-Oleandione	C ₃₀ H ₄₈ O ₂	440	28.25	16.77	6.77
Stigmasterol	C ₂₉ H ₄₈ O	412	59.14	41.18	6.43
Oleic acid, eicosyl ester	C ₃₈ H ₇₄ O ₂	562	61.90	21.07	3.05
Lycoxanthin	C ₄₀ H ₅₆ O	552	68.03	34.89	1.92
1-Monolinoleoylglycerol	C ₂₇ H ₅₄ O ₄ Si ₂	498	61.29	52.3	0.96
Heptadecane, 9-hexyl-	C ₂₃ H ₄₈	324	19.77	40.06	0.29
17-Pentatriacontene	C ₃₅ H ₇₀	490	41.6	38.66	0.25
Octadecanoic acid, methyl ester	C ₁₉ H ₃₈ O ₂	298	36.47	63.96	4.46
Octadecane, 3-ethyl-5-(2-ethylbutyl)-	C ₂₆ H ₅₄	366	37.15	14.39	1.70

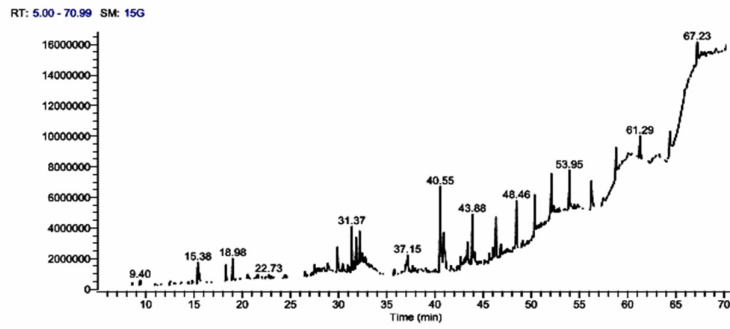
Table 3. GC-MS detection of possible bioactive compounds of fraction 3 (F3) of the hot methanolic extract from the seeds of *Phoenix dactylifera* (Dates)

Compound name	Molecular formula	Molecular weight	Retention time (Min)	Probability Index	Percent area of curve
Dasycarpidan-1-methanol, acetate (ester)	C ₂₀ H ₂₆ N ₂ O ₂	326	60.28	38.23	0.26
Oleic acid, eicosyl ester	C ₃₈ H ₇₄ O ₂	562	60.28	18.49	0.20
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C ₁₉ H ₃₈ O ₄	330	46.15	61.60	2.21
cis-13-Eicosenoic acid	C ₂₀ H ₃₈ O ₂	310	43.88	20.13	3.97
Octadecanoic acid, 4-hydroxy-, methyl ester	C ₁₉ H ₃₈ O ₃	314	40.53	25.58	7.78
Heptacosane	C ₂₇ H ₅₆	380	37.06	15.8	0.97
n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	32.12	18.96	3.27
Oleic Acid	C ₁₈ H ₃₄ O ₂	282	32.10	17.49	3.27
17-Pentatriacontene	C ₃₅ H ₇₀	490	31.6	18.66	0.27
9-Octadecenamide	C ₁₈ H ₃₅ NO	281	50.34	18.93	1.37
α -N-Normethadol	C ₂₀ H ₂₇ NO	297	20.32	15.35	0.12
Benzoic acid, methyl ester	C ₈ H ₈ O ₂	136	10.91	70.28	1.18

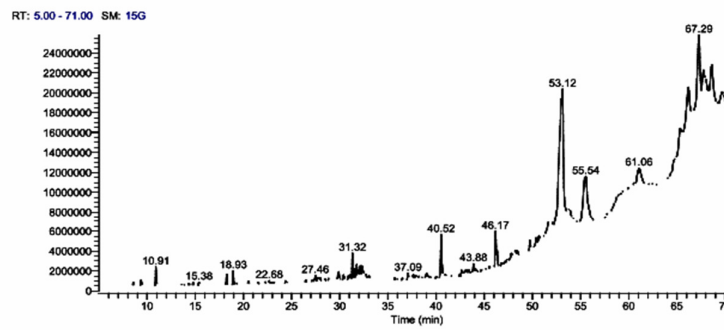
Oleic acid eicosyl ester, was present in all three fractions, with F1 being the component with a retention time of 61.90 min, occupying a chromatogram area of 3.05% (Figure 2B), followed by fraction 3 at 60.28 min, occupying only an area of 0.20% (Figure 2A). At retention time of 53.95 min, oleic acid, eicosyl ester was identified in F1 and occupied 2.60% of the peak area (Figure 2A). As a result, oleic acid eicosyl ester was the most abundant compound in F2 and F1. In the chromatogram of GC-MS, oleic acid was found only in fraction 3 at 32.10 min, with a probability index of 17.49% and a concentration of 3.27%.

Cyclodecasiloxane eicosamethyl- is, otherwise called eicosamethyl-cyclodecasiloxane was detected only in F1 at 48.46 min, with a probability index of 59.72% and a concentration of 2.87% in the GC-MS chromatogram. 1-Cholestan-3-one cyclic 1,2-ethanedial acetal (5 α)- is a steroidal compound detected in F1 at RT of 31.37 min, with a probability index of 31.42%, and it occupied 2.06% of the area of the GC-MS chromatogram curve. Tetratetracontane, a long-chain alkane, was detected only Tricosanol, a plant metabolite, and a long-chain primary fatty alcohol, was detected in F1 at 40.55 min and maximum peak occupancy of 6.68% in GC-MS chromatogram. in F1 at RT of 43.88 min, and it accounted for 1.87 percent of the GC-MS chromatogram curve. The alkane derivative octadecane, 3-ethyl-5-(2-ethylbutyl), was also found in all three HMEDSE fractions. Compared to the other fractions, F3 had a maximum RT of 40.53 min and occupied 7.78% of the area in the GS-MS chromatogram.

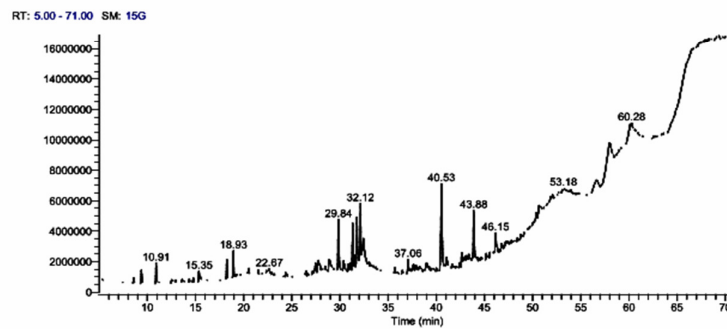
In samples F1 and F3, the presence of n-Hexadecanoic acid was conclusively established. This compound exhibited a retention time (RT) of 32.44 minutes in F1, accompanied by a probability index of 17.74%. Within the GC-MS chromatogram, n-Hexadecanoic acid constituted 1.72% of the total area. Similarly, n-Hexadecanoic acid was also detected in F3 at an RT of 32.12 minutes, with a probability index of 18.96%. In this case, the compound occupied a greater portion, specifically 3.27%, of the GC-MS chromatogram. Additionally, a steroidal compound known as Cholestan-3-one cyclic 1,2-ethanedial acetal, (5 α)-, emerged in F1 at an RT of 31.37 minutes. This compound's presence was supported by a probability index of 31.42%, and it encompassed 2.06% of the entire GC-MS chromatogram curve. Furthermore, the analysis revealed the presence of Tetratetracontane in F1, observed at an RT of 43.88 minutes. The probability index associated with this identification was 11.76%. Tetratetracontane contributed to 1.85% of the area within the GC-MS chromatogram.



A



B



C

Figure 2. GC-MS chromatogram of fractions of hot methanolic extract from the seeds of *Phoenix dactylifera* (Dates)
(A). Chromatogram of Fraction 1 (F1) (B) Chromatogram of Fraction 2 (F2) (C) Chromatogram of Fraction 3 (F3)

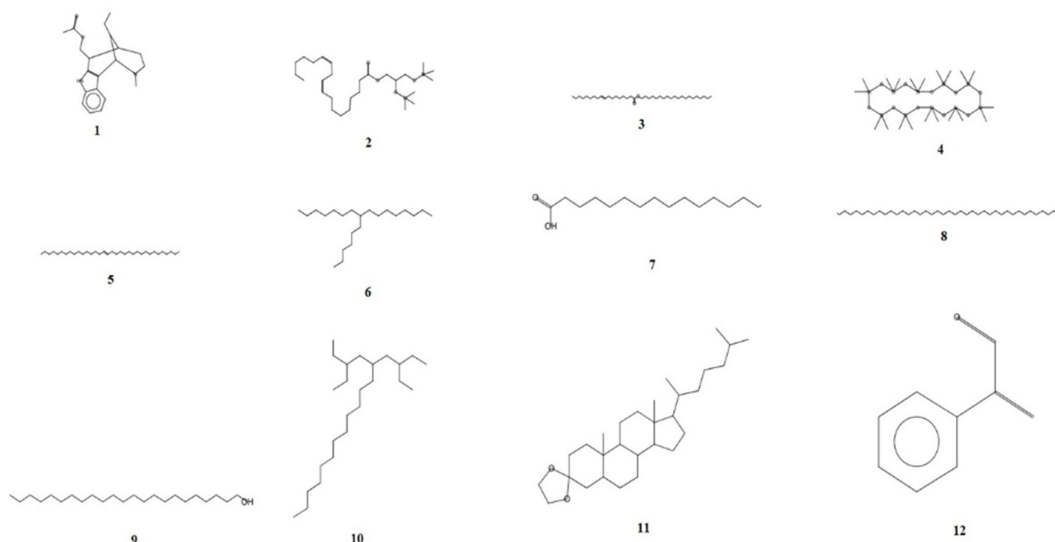


Figure 3. The chemical structure of bioactive compounds of fraction 1 (F1) of hot methanolic extract from the seeds of *Phoenix dactylifera* (Dates)

(1) Dasycarpidan-1- methanol, acetate (ester) (2) 1-Monolinoleoylglycerol (3) Oleic acid, eicosyl ester (4) Cyclodecasiloxane, eicosamethyl- (5) 17-Pentatriacontene (6) Heptadecane, 9-hexyl- (7) n-Hexadecanoic acid (8) Tetratetracontane (9) 1-Tricosanol (10) Octadecane, 3-ethyl-5-(2-ethylbutyl)- (11) Cholestan-3-one, cyclic 1,2-ethanediyl acetal, (5 α)- (12) 2-Phenylpropenal

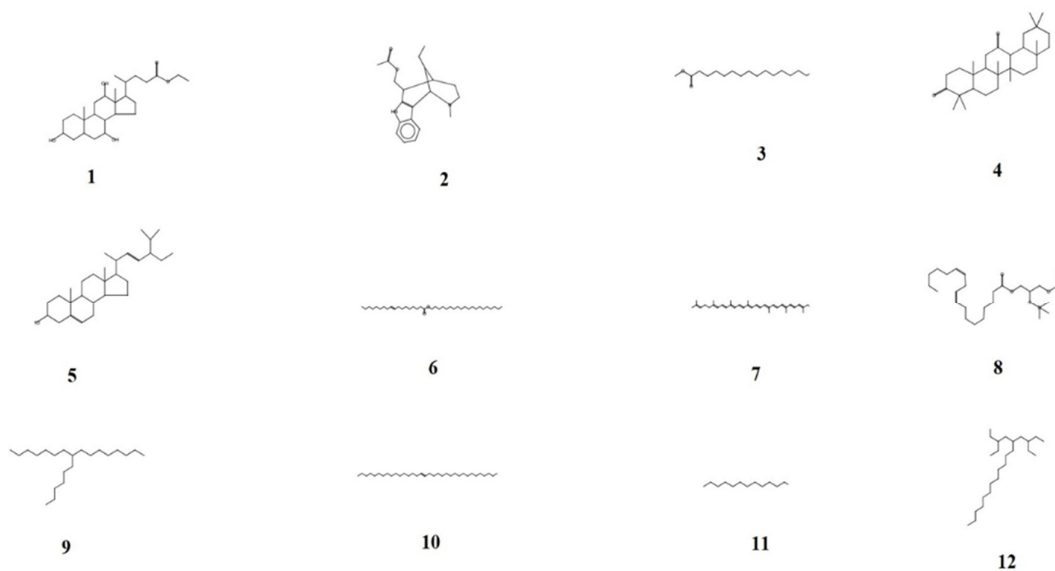


Figure 4. The chemical structure of bioactive compounds of fraction 2 (F2) of hot methanolic extract from the seeds of *Phoenix dactylifera* (Dates)

(1) Ethyl iso-allocholate (2) Dasycarpidan-1-methanol, acetate (ester) (3) Hexadecanoic acid, methyl ester (4) 3,12-Oleandione (5) Stigmasterol (6) Oleic acid, eicosyl ester (7) Lycoxanthin (8) 1-Monolinoleoylglycerol (9) Heptadecane, 9-hexyl- (10) 17-Pentatriacontene (11) Octadecanoic acid, methyl ester (12) Octadecane, 3-ethyl-5-(2-ethylbutyl)-

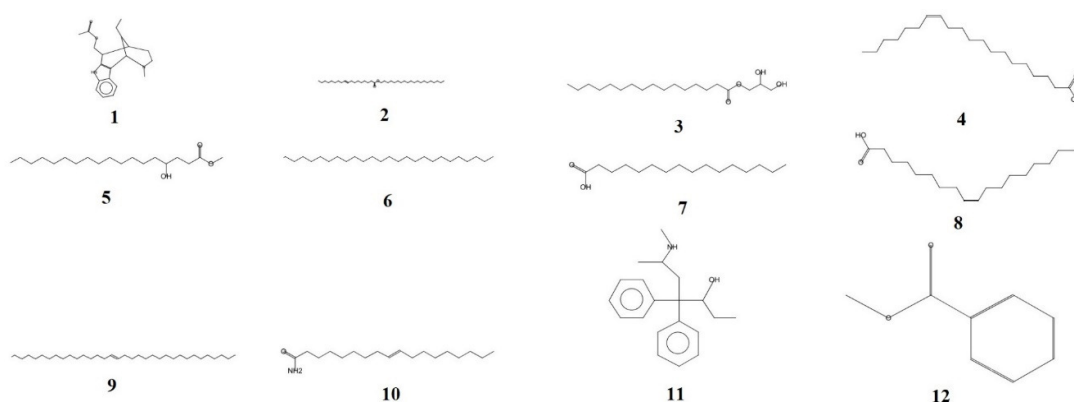


Figure 5. The chemical structure of bioactive compounds of fraction 3 (F3) of hot methanolic extract from the seeds of *Phoenix dactylifera* (Dates)

(1) Dasycarpidan-1-methanol, acetate (ester) (2) Oleic acid, eicosyl ester (3) Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (4) cis-13-Eicosenoic acid (5) Octadecanoic acid, 4-hydroxy-, methyl ester (6) Heptacosane (7) n-Hexadecanoic acid (8) Oleic Acid (9) 17-Pentatriacontene (10) 9-Octadecenamamide (11) α -N-Normethadol (12) Benzoic acid, methyl ester

An unsaturated phytosterol called stigmasterol was found only in the F2, at RT of 59.14 min. Heptadecane, was detected in F1 and F2. The presence of 17-pentatriacontene was detected in all three fractions F1, F2, and F3. 17-Pentatriacontene is a hydrocarbon molecule that combines with electrophiles. Hexadecanoic acid, methyl ester, is also known as hexadecanoic acid methyl ester and palmitic acid methyl ester. It belongs to the class of organic compounds called fatty acid methyl esters. Hexadecanoic acid, methyl ester was determined only in F2 at 31.51 min RT, with a probability index of 71.18% and a chromatogram of 7.43% GC-MS. Likewise, lycoxanthin is a metabolite of fucoxanthin, a carotenoid found mainly in brown algae was detected in F2 at 68.03 min RT, with 34.89% of probability index and occupied an area of 1.92% in the GC-MS chromatogram. The present study detected lycoxanthin only in F2 but not in the other fractions.

9-Octadecenamamide, also known as oleamide, is a fatty acid amide naturally produced within the human body, but it is also found in various plants and animals. It was detected only in fraction 3 of HMEDSE at a retention time of 50.34 min. Benzoic acid methyl ester, a chemical compound known as methyl benzoate, was detected at a retention time of 10.91 min. At various locations in the fingerprint, FT-IR spectroscopy revealed multiple peaks of HMEDSE for F1, F2, and F3 (Figures 6, 7 and 8). Spectra analysis of HMEDSE, which showed distinct peaks, indicated the existence of reactive moieties and the corresponding chemical entities (Tables 4, 5 and 6). The FT-IR spectrum analysis found bell-shaped curves for HMEDSE F1, F2, and F3 at 3454, 3375, and 3346 cm^{-1} , respectively. As indicated in Table 4, the frequency peak showed the presence of several polyphenol peaks corresponding to the stretching vibrations of hydroxyl groups and related substances.

The FTIR spectrum peaked at about 3340-3330 cm^{-1} , corresponding to the stretching vibration of hydroxyl groups in tannins (Hayat *et al.*, 2020). Flavonoids are another class of polyphenols that are abundant in dates. These compounds exert several pharmacological effects, e.g., anti-cancer, anti-inflammatory, and antioxidant properties. The frequency peaks at 2876, 1646, 1595, 1380, and 1159 cm^{-1} of F1; 1689, 1620, 1215, 1132, 735, and 661 cm^{-1} of F2; and the frequency peaks at 1639, 1558, 1506, and 660 cm^{-1} of F3 showed unique compounds, which are shown in Table 5. As found in alkanes or alkyl groups, the 2876 cm^{-1} frequency peak is associated with C-H stretching vibrations of aliphatic (straight or branched chain) hydrocarbons (Nakamoto *et al.*, 2008). Frequency peaks at 1689, 1646, 1639, 1620 cm^{-1} represent the presence of C=O and C=C stretching vibrations (Pavia *et al.*, 2014). Other peaks at 1595, 1558 and 1506 cm^{-1} are often attributed to the stretching vibrations of the C=C double bond in the aromatic ring. The 1380 cm^{-1} peak is probably due to symmetric stretching of carboxylate anion which is commonly found in organic acids and their derivatives. The peak at 1215 cm^{-1} may correspond to the C-O stretching vibration of an ester group, a frequently occurring

functional group in carboxylic acid derivatives such as esters and lactones. The peak at 1159 cm^{-1} is likely due to the C-N stretching of an amine ($-\text{NH}_2$), a functional group commonly found in amino acids and their derivatives.

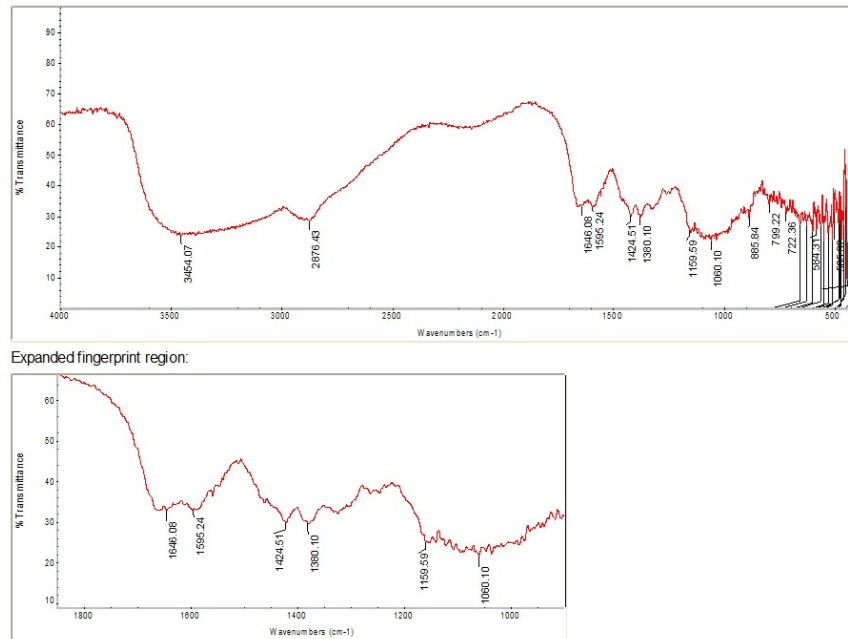


Figure 6. FT-IR analysis of fraction 1 (F1) of hot methanolic extract from the seeds of *Phoenix dactylifera* (Dates)

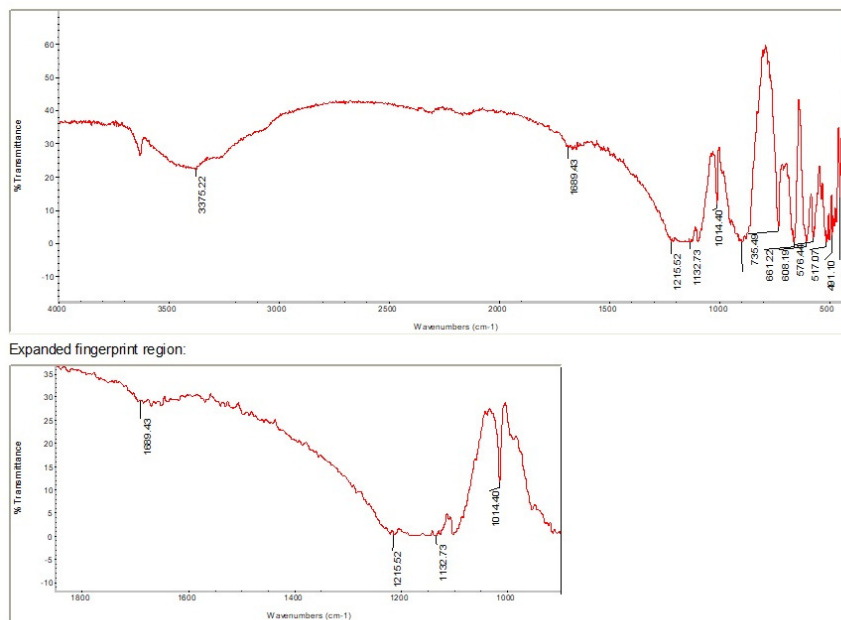


Figure 7. FT-IR analysis of fraction 2 (F2) of hot methanolic extract from the seeds of *Phoenix dactylifera* (Dates)

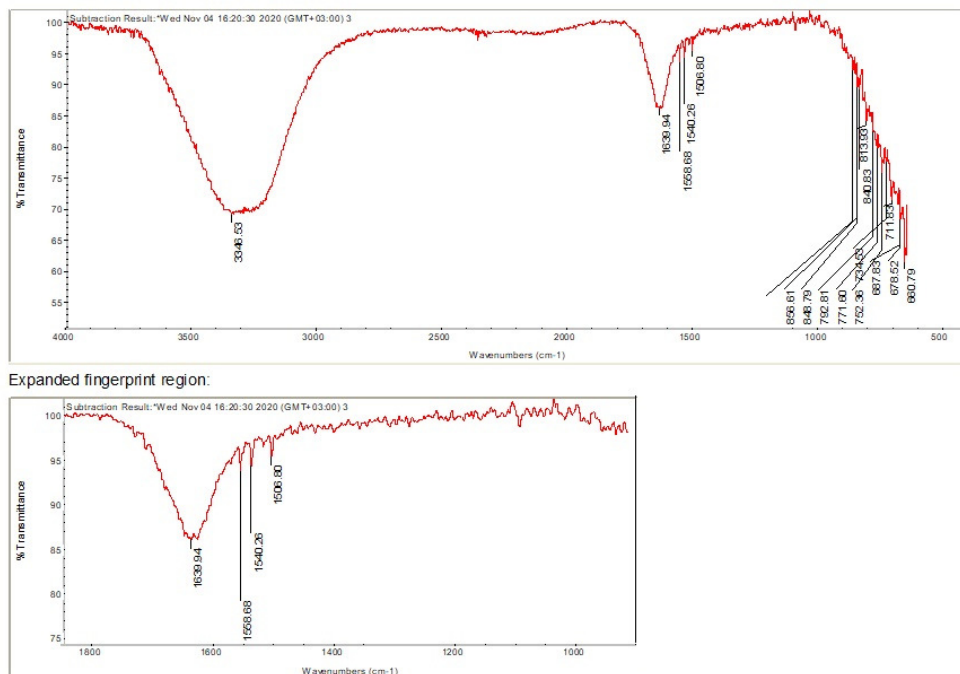


Figure 8. FT-IR analysis of fraction 3 (F3) of hot methanolic extract from the seeds of *Phoenix dactylifera* (Dates)

Table 4. FT-IR detection of possible bioactive compounds of fraction 1 (F1) of the hot methanolic extract from the seeds of *Phoenix dactylifera* (Dates)

Wave number (cm ⁻¹)	Intensity Estimation	Functional group	Type of vibration	Possible Compounds
3454	Weak	O-H	Stretching	1-Tricosanol, Oleic acid, eicosyl ester, 1-Monolinoleoylglycerol
2876	Strong	CH ₂ . str. (Symmetrical)	Stretching	1-Monolinoleoylglycerol, Oleic acid, eicosyl ester, Cholestan-3-one, Octadecane
1646	Strong	C=O, N-H	C=O Stretching NH bending	Dasycarpidan-1-methanol, acetate (ester), 1-Monolinoleoylglycerol, Oleic acid, eicosyl ester, n-Hexadecanoic acid, cyclic 1,2-ethanediyl aetal, 2-Phenylpropenal
1595	Strong	C=C	Stretching	Alkenes, Dasycarpidan-1-methanol, acetate (ester), 1-Monolinoleoylglycerol, Oleic acid, eicosyl ester, 17-Pentatriacontene,
1380	Strong	C-H	bending	Aliphatic compounds, Octadecane, 1-Tricosanol, Tetratetracontane, Heptadecane, 17-Pentatriacontene
1159	Weak	C-O	Stretching	Esters, Dasycarpidan-1- methanol, acetate (ester), 1-Monolinoleoylglycerol

Table 5. FT-IR detection of possible bioactive compounds of fraction 2 (F2) of the hot methanolic extract from the seeds of *Phoenix dactylifera* (Dates)

Wave number (cm ⁻¹)	Intensity Estimation	Functional group	Type of vibration	Possible Compounds
3375	Weak	O-H	Stretching	Ethyl iso-allocholate, Hexadecanoic acid, methyl ester, Stigmasterol, Oleic acid, eicosyl ester, Lycoxanthin, Octadecanoic acid, methyl ester
1689	Strong	C=O, N-H	C=O Stretching NH bending	Ethyl iso-allocholate, Dasycarpidan-1-methanol, acetate (ester), Hexadecanoic acid, methyl ester, 3,12-Oleandione, 1-Monolinoleoylglycerol, Octadecanoic acid, methyl ester
1620	Strong	C=C	Stretching	Alkenes, Dasycarpidan-1-methanol, acetate (ester), Stigmasterol, Oleic acid, eicosyl ester, Lycoxanthin, 1-Monolinoleoylglycerol, 17-Pentatriacontene, Octadecanoic acid, methyl ester
1215	Strong	C-N	Stretching	Aromatic amine, Dasycarpidan-1-methanol, acetate (ester)
1132	Strong	C-O	Stretching	Esters, Ethyl iso-allocholate, Dasycarpidan-1-methanol, acetate, Hexadecanoic acid, methyl ester, Stigmasterol, Oleic acid, eicosyl ester, Lycoxanthin, 1-Monolinoleoylglycerol, Octadecanoic acid, methyl ester
735	Strong	C-H (Ar)	bending	Dasycarpidan-1-methanol
661	Strong	C-H (Alkene)	bending	Stigmasterol, Oleic acid, eicosyl ester, Lycoxanthin, 1-Monolinoleoylglycerol, 17-Pentatriacontene, Octadecanoic acid, methyl ester

Table 6. FT-IR detection of possible bioactive compounds of fraction 3 (F3) of the hot methanolic extract from the seeds of *Phoenix dactylifera* (Dates)

Wave number (cm ⁻¹)	Intensity Estimation	Functional group	Type of vibration	Possible Compounds
3346	Strong	O-H	Stretching	Oleic acid, eicosyl ester, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, cis-13-Eicosenoic acid, Octadecanoic acid, N-Normethadol
1639	Strong	C=O, N-H	C=O Stretching NH bending	Dasycarpidan-1-methanol, acetate (ester), Oleic acid, eicosyl ester, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, cis-13-Eicosenoic acid, Octadecanoic acid, 9-Octadecenamide, N-Normethadol, Benzoic acid
1558	Medium	C=C	Stretching	Alkenes, Dasycarpidan-1-methanol, Oleic acid, eicosyl ester, cis-13-Eicosenoic acid, 17-Pentatriacontene, N-Normethadol, Benzoic acid
1506	Medium	C-H	bending	Aliphatic compounds, Dasycarpidan-1-methanol, cis-13-Eicosenoic acid
660	Medium	C-H (Alkene)	bending	Oleic acid, eicosyl ester, cis-13-Eicosenoic acid, 17-Pentatriacontene

Table 7 shows the antibacterial spectrum of HMEDSE. According to the results, the antibacterial activity of HMEDSE was comparable to that seen in other human pathogenic microorganisms studied. It was not statistically significant at $p < 0.05$ (Figure 9). Moreover, the HDMSE spectrum was significantly smaller than that of the reference drug ciprofloxacin ($p < 0.05$; Figure 10). Figure 11 shows the IC₅₀ of HMEDSE with respect to growth of MCF -7 ATCC cells. The IC₅₀ value was $73.5 \pm 0.5 \mu\text{g/mL}$, indicating that it inhibited growth. The untreated cells displayed characteristic normal and complete appearance, suggesting confluent and healthy status, with each well-differentiated cell having a distinct nucleus. The morphological changes in MCF-7 reflected the cytotoxic action of HMEDSE. The extracts resulted in unattached, lifeless, and shrivelled cells showing shattered nuclei due to apoptosis.

Table 7. Anti-bacterial study of hot methanolic extract from the seeds of *Phoenix dactylifera* (Dates)

Organisms	Concentration CFU [#] /mL	Zone of inhibition (mm)	
		HMEDSE	Ciprofloxacin (50 $\mu\text{g/mL}$)
<i>Bacillus subtilis</i> ATCC 6633	2×10^5	14.3 ± 2	29.3 ± 1.5
<i>Staphylococcus aureus</i> ATCC 25923	4×10^5	16.3 ± 1.5	27.3 ± 2
<i>Streptococcus pyogenes</i> ATCC 19615	4×10^3	15 ± 2.6	27.6 ± 1.1
<i>Escherichia coli</i> ATCC 25922	3×10^5	17.6 ± 2.5	33.3 ± 2.3
<i>Pseudomonas aeruginosa</i> ATCC 27853	2×10^3	15 ± 2	29.3 ± 3.7
<i>Klebsiella pneumoniae</i> ATCC 700603	2×10^4	16.3 ± 2.5	32.6 ± 3

Each value is the mean of 3 batches (n=3) with standard deviation. All the values are compared to standard ciprofloxacin disc by performing Tukey Kramer test (post hoc test). The standard values were more significant than HMEDSE.

HMEDSE: Hot methanolic extract of dates seed

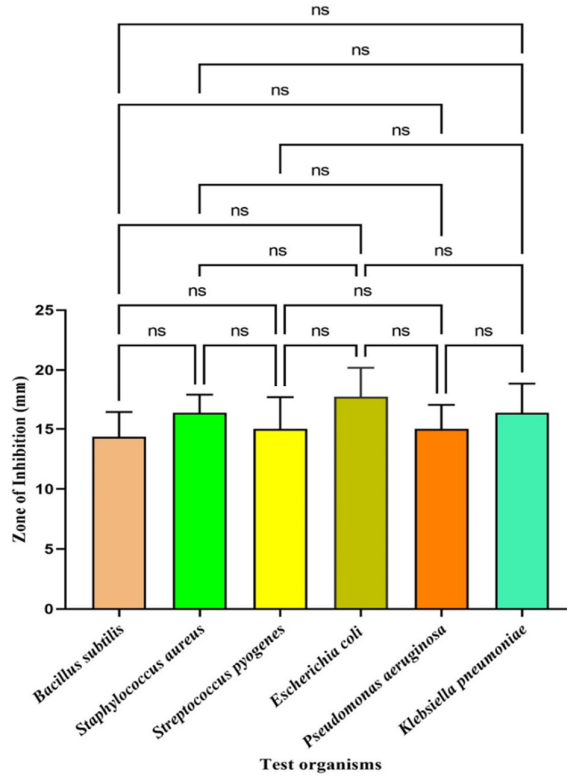


Figure 9. Spectrum of antibacterial activity of hot methanolic extract from the seeds of *Phoenix dactylifera* (Dates)
 ns: Nonsignificant at $p > 0.05$

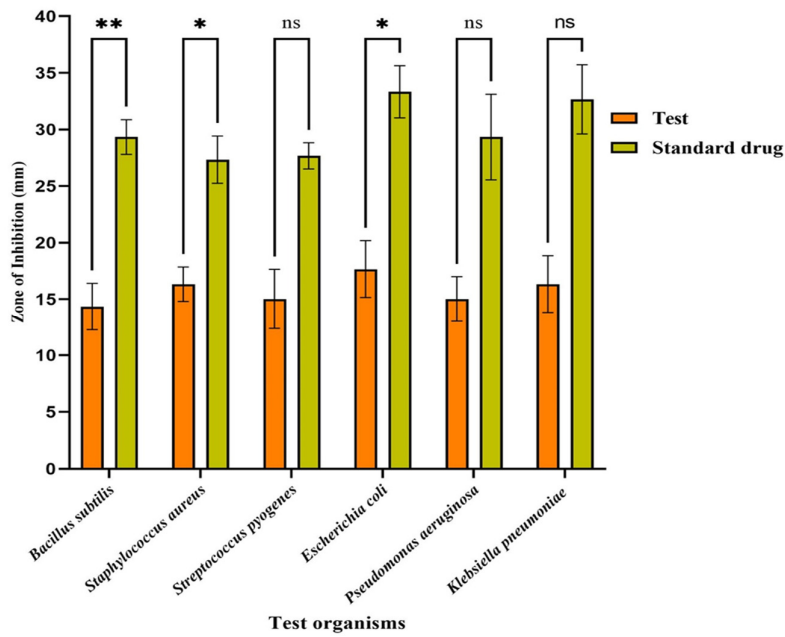


Figure 10. Comparative anti-bacterial study Test vs Standard
 ** Extremely significant at $p < 0.05$; * Significant at $p < 0.05$; ns: Nonsignificant at $p > 0.05$

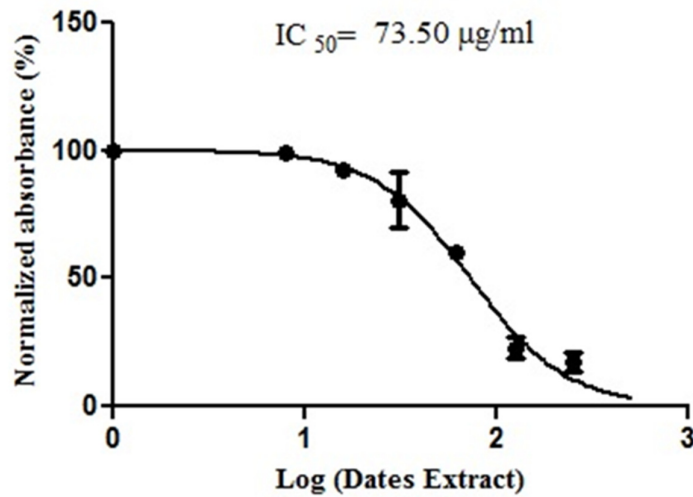


Figure 11. The dose-response curve on the effect of hot methanolic extract from the seeds of *Phoenix dactylifera* (Dates) against MCF-7 cells

In this current study, the extract showed a remarkable cytotoxic effect on cancer cells, this influence being primarily due to the induction of apoptosis. A fluorescence microscope was used to evaluate the cell state to quantify parameters such as viability, early apoptosis, late apoptosis, and necrosis, as shown in Figure 12. A total of 200 cells were differentially and randomly counted, along with untreated negative control. Early apoptosis was detected through staining of the DNA fragments with acridine orange. Moderate apoptosis reflected in blebbing, and condensation of nuclear chromatin, were observed upon treatment with the extract for 24 h. Moreover, in the late stages of apoptosis, after 48 and 72 h of treatment, changes were observed, e.g., a reddish-orange colour due to the binding of AO to denatured DNA. Thus, HMEDSE time-dependently induced morphological features related to apoptosis. There was a significant difference in cell number when treated cells (200 cells) were differentially evaluated ($p < 0.05$).

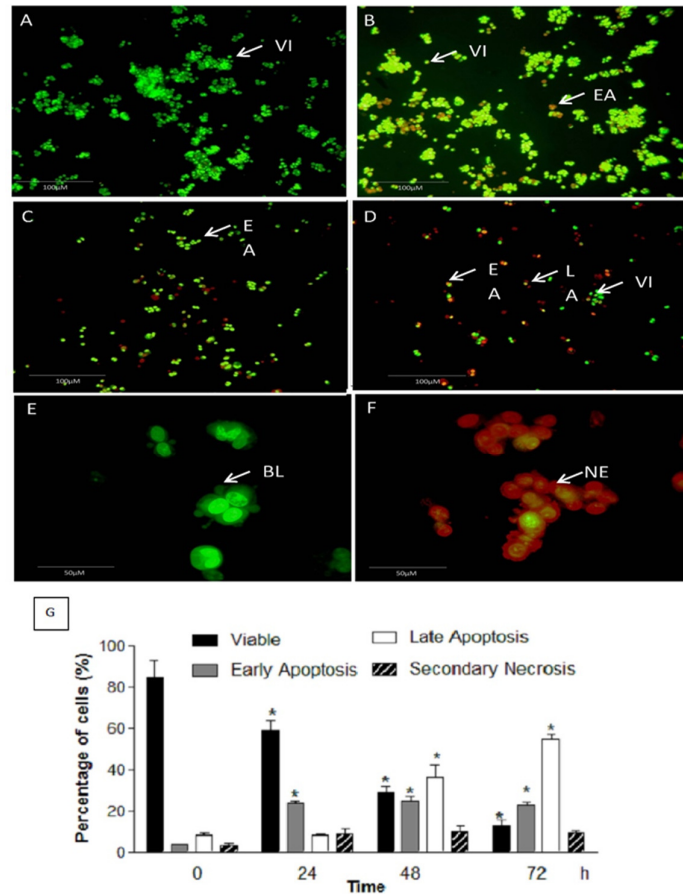


Figure 12. Apoptosis study. Fluorescent photomicrograph of AO/PI double-stained MCF-7 cells. The cells were treated with hot methanolic extract from the seeds of *Phoenix dactylifera* (Dates) at 73 µg/ml for 24, 48 and 72 h

(A) Control (untreated) cells showed a normal structure with no remarkable features of apoptosis and necrosis. (B) At 24 h, AO was interconnected with fragmented DNA (bright green) (C) An orange colour, representing late apoptosis, was noticed at 48 h. (D) At 72 h, the cells were orange with a blebbing membrane that indicated late apoptosis. At 72 h, secondary necrotic cells (bright red colour) were noticed. (E) the blebbing cells as an indicator of early apoptosis start to be seen at higher magnification. (F) secondary necrotic cells (bright red colour) seen at 72 h at higher magnification. (G) The percentages of viable early and late apoptosis and secondary necrotic cells were increased after MCF-7 cells were treated with extract in a time-dependent manner. Apoptosis increased significantly ($*p < 0.05$) in a time-dependent manner. VI: viable cells, EA: early apoptosis, LA: late apoptosis, NE: necrosis.

Discussion

Phoenix dactylifera, the date palm, is a plant grown primarily for its delicious and nutritious fruits. While the fruits of *Phoenix dactylifera* are often highlighted, few researchers worldwide have discovered that the seeds have a variety of therapeutic properties. The seeds have shown promising medicinal properties in the present study. Many bioactive substances have been identified in the fraction of HMEDESE. Dasyarpidan-1-methanol, acetate (ester), ethyliso-allocholate, 1-Monolinoleoylglycerol trimethylsilyl ether, Oleic acid icosyl ester, N-Hexadecanoic acid, cyclononasiloxane-octadecamethyl, octadecane, 3-ethyl-5-(2-ethylbutyl), Cholestan-3-one, cyclic 1,2-ethanediy-etal, Stigmasterol, hepadecane, etc.

Dasycarpidan-1-methanol has been previously shown to have antibacterial activity (Alqahtani *et al.*, 2022). A previous study showed that ethyliso-allocholate may be a potent antibacterial agent (Malathi *et al.*, 2016). 1-Monolinoleoylglycerol trimethylsilyl ether has been shown to possess antibacterial, anti-inflammatory, and antioxidant properties (Parthipan *et al.*, 2015; Şimşek Sezer and Uysal, 2021). Oleic acid icosyl ester (OIE), also known as icosyl oleate or 9-octadecenoic acid (Z)- eicosyl ester have significant medicinal property. Gurunathan *et al.* (2016) discovered that OIE is an effective larvicidal agent against mosquito larvae. In another study, OIE was found to have antifungal effects, especially against *Candida* (Yang *et al.*, 2022). According to Schwingshackl and Hoffmann (2014), oleic acid (OE) is a monounsaturated fatty acid (MUFA) found in a variety of animal and vegetable fats and oils. It has been reported that OE provides several health benefits, including a lower risk of cardiovascular disease and improvement of insulin sensitivity (Viguiliouk *et al.*, 2015). In addition, PUFAs are better than saturated fats, as they decrease the levels of LDL cholesterol and minimize the risk of heart disease. According to a previous study, oleic acid is one of the most important fatty acids in date seeds, accounting for about 20-30% of the total fatty acid content (Boukouada *et al.*, 2014).

In another study conducted in Iran, the fatty acid profiles of different parts of the date palm were investigated, including the fruit, seed, and leaves. The researchers found that oleic acid was present in all parts of the date palm, but its concentration was highest in the seed oil (Nehdi *et al.*, 2018). Oleic acid is the most abundant fatty acid in olive oil. It is known for its health-promoting properties, including reducing inflammation, improving blood sugar control, and reducing the risk of certain cancers (Jimenez-Lopez *et al.*, 2020). A previous report suggested that cyclononasiloxane-octadecamethyl, a related compound, possesses potent antifungal properties (Ni, 2016).

Cholestan-3-one, cyclic 1,2-ethanediyl-etal, (5 α)- has been described as an antibacterial agent (National Center for Biotechnology Information, 2023). According to Ertas *et al.* (2015), the essential oils of *Euphorbia macroclada* contain a significant amount of tetratetracontane as a major constituent. N-Hexadecanoic acid, also known as palmitic acid (PA), has demonstrated anti-inflammatory properties. In addition, it showed significant antibacterial activity against biofilm-forming microbes (Aparna *et al.*, 2012; Bakar *et al.*, 2017). Recent studies have shown that palmitic acid diminishes the proliferation of prostate cancer cells both in lab cultures and animal models (Zhu *et al.*, 2021). Additionally, palmitic acid has been documented to restrict the growth of a range of pathogens such as bacteria and fungi (Casillas-Vargas *et al.*, 2021). Nguyen *et al.* (2018) also reported on the antimicrobial properties of 3-ethyl-5-(2-ethylbutyl).

The potential of stigmaterol as a medical agent for treating various diseases, including cancer, has been explored. It was observed that stigmaterol exerted anti-cancer effects by enhancing apoptosis and reducing tumor growth (Bakrim *et al.*, 2022). Breast cancer cells underwent stigmaterol-induced apoptosis in a dose-dependent manner (Zhang *et al.*, 2022). In a previous study, stigmaterol was shown to exert anti-cancer effects in ovary carcinoma cells. According to the study, stigmaterol may be a new treatment strategy for ovarian cancer. It has been reported that stigmaterol plays a crucial role in altering lipid metabolism due to its cholesterol-lowering solid effect (Feng *et al.*, 2018). Stigmaterol has demonstrated notable anti-osteoarthritic effects by effectively inhibiting pro-inflammatory mediators (Feng *et al.*, 2018). Stigmaterol has been described as an immunomodulatory compound, as it suppressed the release of tumor necrosis factor- α , nitric oxide, and proinflammatory cytokines, and inhibited cyclooxygenase-2 (Bakrim *et al.*, 2022).

Earlier research revealed the occurrence of stigmaterol in the pollen of Iraqi date palms (Taiseer, 2021). Another study examining the antibacterial capabilities of stigmaterol found it to be notably effective in inhibiting both Gram-positive and Gram-negative bacteria, such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Bae *et al.*, 2020). The compound is versatile, finding use across multiple industries. Its solubility qualifies it as an effective solvent, and its emollient and conditioning features make it apt for personal care products. Studies have indicated that 9-hexylheptadecane possesses antifungal capabilities

(Maghdu and Palaniyappan, 2015). Its potential role as a surfactant in the realm of nanotechnology also holds promise for future innovations. Similarly, heptadecane offers exciting possibilities in nanotechnological applications. Serving as a surfactant, heptadecane can aid in the creation of nanoparticles from a variety of materials including metals, metal oxides, and polymers. Its surfactant qualities assist in stabilizing these nanoparticles, preventing them from clustering together. Numerous studies have illustrated heptadecane's effectiveness as a surfactant, particularly in crafting nanoparticles with specific dimensions and forms (Can *et al.*, 2022). Furthermore, it has been attributed with anti-inflammatory, anti-cancer, antibacterial, and anti-arthritic properties (Dinesh *et al.*, 2018). Methyl palmitate is an ester formed from methanol and palmitic acid. The compound is known for its antioxidant and anti-inflammatory effects, as well as its potential to lower blood cholesterol levels (Asghar *et al.*, 2011; Amir *et al.*, 2020). It is formed in the digestive system of mammals by bacterial degradation of the fucoxanthin molecule (Kotake *et al.*, 2001). Research suggests that lycoxanthin may have even stronger antioxidant capabilities than fucoxanthin (Kotake *et al.*, 2001). In a study by Ponder *et al.* (2021), trace amounts of lycoxanthin were found in extracts of peppers, tomatoes, and carrots; these amounts were significantly lower than other carotenoids such as lutein and beta-carotene. Methyl palmitate exhibits a wide range of biological effects and shows promise for various therapeutic applications. In another study, lipid analysis of date palm fruit showed that both the fruit and its pulp contain oleamide (Alkhoori *et al.*, 2022)

The detection of oleamide in date palm raises the possibility that this compound contributes to the plant's biological functions and potential health advantages. Earlier research has indicated that oleamide serves as a regulator of the endocannabinoid system, which plays a pivotal role in a range of physiological activities such as pain management, appetite control, and immune response (Basu, 1999). Separate research has demonstrated that oleamide can act as a sedative, enhancing both the duration and quality of sleep in rodents (Reyes Prieto *et al.*, 2012).

Additionally, oleamide has been investigated for its potential anti-inflammatory and anticancer benefits. One study found it to hinder the proliferation of human breast cancer cells (Zibara *et al.*, 2015), while other research noted its ability to decrease inflammation in the brain (Nam *et al.*, 2017; Kita *et al.*, 2019). Methyl benzoate is widely used as a flavoring agent and fragrance in various products such as perfumes, soaps, and cosmetics (National Center for Biotechnology Information, 2023). Benzoic acid methyl ester has been identified as one of the major volatile compounds in the fruits of *Phoenix dactylifera*, contributing to its characteristic aroma (Al-Shahib and Marshall, 2003). The presence of this compound in the fruit could also have association with its use as a natural preservative due to its antimicrobial properties.

Dates are a significant source of polyphenols which have anti-inflammatory and antioxidant activities. In one study, the polyphenol content of three different date varieties was investigated, and the spectrum of FT-IR showed a large peak between 3400 and 3200 cm^{-1} , corresponding to the existence of OH groups in these polyphenols (Wongsa *et al.*, 2022). Tannins are a class of polyphenols found in many plant foods, including dates. These compounds have various pharmacological properties such as antioxidant, anticancer, and antimicrobial effects. The FT-IR analysis of a methanolic extract of date showed a peak at about 3400-3200 cm^{-1} , which was consistent with OH groups in flavonoids (Al-Shahib and Marshall, 2003).

The peak at 1132 cm^{-1} could correspond to the C-N stretching vibration of a secondary amine (-NH-), a functional group commonly found in amides and amines (McNaught and Wilkinson, 1999). The peaks at 735, 661, and 660 cm^{-1} may be due to bending vibration of a substituted benzene ring, a functional group found in many organic compounds (Coates, 2006). According to studies by Al-Farsi and Lee (2008), extracts from the fruits, seeds, and leaves of date palm exerted antibacterial effect on a variety of bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. In a study by Samad *et al.* (2016), ethanolic extract of date palm fruit showed potent antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. In addition, in previous studies, *Bacillus subtilis*, *Escherichia coli*,

Staphylococcus aureus, and *Streptococcus pyogenes* were significantly inhibited by the methanolic extract of date palm kernels (Alsohaimi and Fouda, 2019; Bhaskaracharya and Bhaskaracharya, 2022).

The date palm, *Phoenix dactylifera*, is cytotoxic against numerous cancer cell lines. The potential of date palm extracts and their bioactive components to induce apoptosis, suppress cell proliferation, and modify cell cycle progression indicates their cytotoxic effect (Ghazzawy, 2022). A previous study revealed that *Phoenix dactylifera* leaf extracts produced cytotoxic effect by decreasing the proliferation of human breast cancer cells via apoptosis induction (Khan *et al.*, 2022). Another study on date palm extract's cytotoxic and antioxidant properties discovered that it exerted considerable cytotoxicity against multiple cancer cell lines (Shahbaz *et al.*, 2022). Al-Sayyed *et al.* (2021) studied the cytotoxic effect of date palm seed extract on MCF-7 breast cancer cells. The study results showed that the extract produced a dose-dependent cytotoxic effect on cancer cells (Al-Sayyed *et al.*, 2021). Alkhoori *et al.* (2022) studied the cytotoxic effect of date palm seed extract on human HepG2 and healthy hepatocytes (WRL-68). The study's results revealed that the extract had a strong cytotoxic effect on cancer cells but had little effect on normal cells. An earlier study looked at the cytotoxicity of a date seed methanolic extract on human colon cancer cells (HT29). It was discovered that the extract had substantial cytotoxic effect on cancer cells through a mechanism involving induction of apoptosis (Rezaei, 2015). Furthermore, Khan *et al.* (2022) reported the anti-cancer effect of *Phoenix dactylifera* L. seed extract on human mammary carcinoma. Hence, it might be employed as an essential option for developing anti-cancer medications.

Conclusions

This investigation has demonstrated the anti-cancer and antibacterial potential of *Phoenix dactylifera* seeds. In addition, numerous useful bioactive phytochemicals of medical significance were identified in the seed extract. The hot methanolic extract of *Phoenix dactylifera* seeds showed promising biological activity. Further research is needed to fully understand the potential benefits and mechanisms of action of these seeds. However, it is important to note that *Phoenix dactylifera* seeds should be consumed in moderation to combat numerous health problems.

Authors' Contributions

SMS: Principal investigator, conceptualization, experimentation, processing of results, writing and editing; SM, ZUR, MSA, VKB, MEE, AJ: Performed experiments; FYS, GG: Funding sources; AMHA, AMOA, DAH, MAM, HFA: Project management, Resources; SS: Project consultation.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

- Abubacker MN, Devi PK (2014). In vitro antifungal potentials of bioactive compound oleic acid, 3-(octadecyloxy) propyl ester isolated from *Lepidagathis cristata* Willd. (Acanthaceae) inflorescence. Asian Pacific Journal of Tropical Medicine 7:S190-S193. [https://doi.org/10.1016/s1995-7645\(14\)60230-3](https://doi.org/10.1016/s1995-7645(14)60230-3)
- Al Arabiya (2022). Saudi Arabia tops in dates exports, with more than 300 species fetching \$324mln. <https://english.alarabiya.net/business/economy/2022/08/28/Saudi-Arabia-tops-in-dates-exports-with-more-than-300-species-fetching-324-mln#>
- Al-Alawi RA, Al-Mashiqri JH, Al-Nadabi JSM, Al-Shihi BI, Baqi Y (2017). Date palm tree (*Phoenix dactylifera* L.): Natural products and therapeutic options. Frontiers in Plant Science 8:845. <https://doi.org/10.3389/fpls.2017.00845>
- Al-Farsi MA, Lee CY (2008). Nutritional and functional properties of dates: a review. Critical Reviews in Food Science and Nutrition 48(10):877-887. <https://doi.org/10.1080/10408390701724264>
- Alkhoodri MA, Kong ASY, Aljaafari MN, Abushelaibi A, Erin Lim SH, Cheng WH, Lai KS, (2022). Biochemical composition and biological activities of date palm (*Phoenix dactylifera* L.) seeds: A review. Biomolecules 12(11). <https://doi.org/10.3390/biom12111626>
- Alqahtani SS, Moni SS, Sultan MH, Ali Bakkari M, Madkhali OA, Alshahrani SZ, ... Mohamed Z (2022). Potential bioactive secondary metabolites of Actinomycetes sp. isolated from rocky soils of the heritage village Rijal Alma, Saudi Arabia. Arabian Journal of Chemistry 15(5):103793. <https://10.1016/j.arabjc.2022.103793>
- ALrajhi M, AL-Rasheedi M, Elnaeem, MES, Alhazmi Y, Mustafa MM, ALreshidi MA (2019). Antibacterial activity of date palm cake extracts (*Phoenix dactylifera*). Cogent Food & Agriculture 5(1). <https://doi.org/10.1080/23311932.2019.1625479>
- Al-Sayyed HF, Abu-Qatouseh LF, Malkawy M, Al-Wawi S, Al Kafaween M (2021). Extracts of Jordanian date palm fruit (*Phoenix dactylifera* L.) inhibit human mammary adenocarcinoma (MCF-7) cells *in vitro* by inducing cell viability. Current Research in Nutrition and Food Science Journal 9(2):423-430. <https://doi.org/10.12944/crnfsj.9.2.06>
- Al-Shahib W, Marshall RJ (2003). The fruit of the date palm: its possible use as the best food for the future? International Journal of Food Sciences and Nutrition 54(4):247-259. <https://doi.org/10.1080/09637480120091982>
- Alsohaimi A, Fouda AM (2019). Assessment of the effect of extract formulation of date palm kernel on facial skin wrinkles: Biophysical measurements and digital profilometry. Journal of Cosmetic Science 70(6):277-290.
- Amir SM, El-Naggar HA, El-Damhougy KA, Mansour AEB, Mohamed A, Hamdy AHA (2020). GC-MS analysis of bioactive components in six different crude extracts from the soft coral (*Sinularia maxim*) collected from Ras Mohamed, Aqaba Gulf, Red Sea, Egypt. Egyptian Journal of Aquatic Biology and Fisheries 24(6):425-434. <https://doi.org/10.21608/ejabf.2020.114293>
- Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C, Haridas M (2012). Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. Chemical Biology & Drug Design 80(3):434-439. <https://doi.org/10.1111/j.1747-0285.2012.01418.x>
- Asghar SF, Habibur R, Choudahry MI, Rahman A (2011). Gas chromatography-mass spectrometry (GC-MS) analysis of petroleum ether extract (oil) and bio-assays of crude extract of *Iris germanica*. International Journal of Genetics and Molecular Biology 3:95-100.
- Bae H, Song G, Lim W (2020). Stigmasterol causes ovarian cancer cell apoptosis by inducing endoplasmic reticulum and mitochondrial dysfunction. Pharmaceutics 12(6):488. <https://doi.org/10.3390/pharmaceutics12060488>

- Bakar K, Mohamad H, Latip J, Tan HS, Heng GM (2017). Fatty acids compositions of *Sargassum granuliferum* and *Dictyota dichotoma* and their anti-fouling activities. The Journal of Sustainability Science and Management 12:8-16.
- Bakrim S, Benkhaira N, Bourais I, Benali T, Lee LH, El Omari N, Sheikh RA, Goh KW, Ming LC, Bouyahya A (2022). Health Benefits and Pharmacological Properties of Stigmasterol. Antioxidants 11(10):1912. <https://doi.org/10.3390/antiox11101912>
- Basu S (1999). Oxidative injury induced cyclooxygenase activation in experimental. hepatotoxicity. Biochemical and Biophysical Research Communications 254(3):764-767. <https://doi.org/10.1006/bbrc.1998.99>
- Bhaskaracharya RK, Bhaskaracharya A, Stathopoulos C (2022). A systematic review of antibacterial activity of polyphenolic extract from date palm (*Phoenix dactylifera* L.) kernel. Frontiers in Pharmacology 13:1043548. <https://doi.org/10.3389/fphar.2022.1043548>
- Boukouada M, Ghiaba Z, Gourine N, Bombarda I, Saidi M, Yousfi M.(2014). Chemical composition and antioxidant activity of seed oil of two Algerian date palm cultivars (*Phoenix dactylifera*). Natural Product Communications 9(12):1777-1780. <https://doi.org/10.1177/1934578x1400901230>
- Can G, Mert HH, Mert MS (2022). Shape-stabilized n-heptadecane/polymeric foams with modified iron oxide nanoparticles for thermal energy storage. Thermochemica Acta 714(179266):179266. <https://doi.org/10.1016/j.tca.2022.179266>
- Casillas-Vargas G, Ocasio-Malavé C, Medina S, Morales-Guzmán C, Del Valle RG, Carballeira NM, Sanabria-Ríos DJ (2021). Antibacterial fatty acids: An update of possible mechanisms of action and implications in the development of the next-generation of antibacterial agents. Progress in Lipid Research 82(101093):101093. <https://doi.org/10.1016/j.plipres.2021.101093>
- Chiraz BS, Wafa T, Tesnime G, Sana Ben A, Sami F (2020). Date palm - Chapter 42. Amit KJ (Ed). Nutritional Composition and Antioxidant Properties of Fruits and Vegetables. Academic Press, pp 681-694. <https://doi.org/10.1016/B978-0-12-812780-3.00042-8>
- Clinical and Laboratory Standards Institute (2013). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. CLSI Document M100-S22; Clinical and Laboratory Standards Institute: New York, NY, USA.
- Coates J (2006). Interpretation of infrared spectra. A practical approach. In: Encyclopaedia of Analytical Chemistry. <https://doi.org/10.1002/9780470027318.a5606>
- Dinesh Kumar G, Karthik M, Rajakumar R (2018). GC-MS analysis of bioactive compounds from ethanolic leaves extract of *Eichhornia crassipes* (Mart) Solms. and their pharmacological activities. Pharma Innovation 7(8):459-462.
- Ertas A, Yilmaz MA, Firat M (2015). Chemical profile by LC-MS/MS, GC/MS and antioxidant activities of the essential oils and crude extracts of two *Euphorbia* species. Natural Product Research 29(6):529-534. <https://doi.org/10.1080/14786419.2014.954113>
- Feng S, Dai Z, Liu AB, Huang J, Narsipur N, Guo G, ... Yang CS (2018). Intake of stigmasterol and β -sitosterol alters lipid metabolism and alleviates NAFLD in mice fed a high-fat western-style diet. Molecular and Cell Biology of Lipids 1863(10):1274-1284. <https://doi.org/10.1016/j.bbaliip.2018.08.004>
- Ghazzawy HS, Gouda MM, Awad NS, Al-Harbi NA, Alqahtani MM, Abdel-Salam, MM, ... Hikal DM (2022). Potential bioactivity of *Phoenix dactylifera* fruits, leaves, and seeds against prostate and pancreatic cancer cells. Frontiers in Nutrition 9:998929. <https://doi.org/10.3389/fnut.2022.998929>
- Gurunathan A, Senguttuvan J, Paulsamy S (2016). Evaluation of mosquito repellent activity of isolated oleic acid, eicosyl ester from *Thalictrum javanicum*. Indian Journal of Pharmaceutical Sciences 78(1):103-110. <https://doi.org/10.4103/0250-474x.180259>
- Hayat J, Akodad M, Moumen A, Baghour M, Skalli A, Ezrari S, Belmalha S (2020). Phytochemical screening, polyphenols, flavonoids and tannin content, antioxidant activities and FTIR characterization of *Marrubium vulgare* L. from 2 different localities of Northeast of Morocco. Heliyon 6(11):e05609. <https://doi.org/10.1016/j.heliyon.2020.e05609>
- Heba IM, Hossam SE, Mohan Jain S, Al-Khayri JM (2021). Date palm - Chapter 18. In: Rouf AB, Khalid RH, Moonisa AD (Eds). *Phoenix dactylifera* L. secondary metabolites: Bioactivity and pharmaceutical potential. Phytomedicine, Academic Press, pp 483-531. <https://doi.org/10.1016/B978-0-12-824109-7.00018-2>

- Jimenez-Lopez C, Carpena M, Lourenço-Lopes C, Gallardo-Gomez M, Lorenzo JM, Barba FJ, Prieto MA, Simal-Gandara J (2020). Bioactive compounds and quality of extra virgin olive oil. *Foods* 9(8):1014. <https://doi.org/10.3390/foods9081014>
- Khan MA, Singh R, Siddiqui S, Ahmad I, Ahmad R, Upadhyay S, ... Mishra DP (2022). Anticancer potential of *Phoenix dactylifera* L. seed extract in human cancer cells and pro-apoptotic effects mediated through caspase-3 dependent pathway in human breast cancer MDA-MB-231 cells: an in vitro and in silico investigation. *BMC Complementary Medicine and Therapies* 22(1):68. <https://doi.org/10.1186/s12906-022-03533-0>
- Kita M, Ano Y, Inoue A, Aoki J (2019). Identification of P2Y receptors involved in oleamide-suppressing inflammatory responses in murine microglia and human dendritic cells. *Scientific Reports* 9(1):3135. <https://doi.org/10.1038/s41598-019-40008-8>
- Kotake-Nara E, Miyashita K, Nagao A, Kushiro M, Zhang H, Sugawara T (2001). Carotenoids affect proliferation of human prostate cancer cells. *The Journal of Nutrition* 131(12):3303-3306. <https://doi.org/10.1093/jn/131.12.3303>
- Malathi K, Anbarasu A, Ramaiah S (2016). Ethyl Iso-allocholate from a medicinal rice karungkavuni inhibits dihydropteroate synthase in *Escherichia coli*: A molecular docking and dynamics study. *Indian Journal of Pharmaceutical Sciences* 78(6). <https://doi.org/10.4172/pharmaceutical-sciences.1000184>
- Mariod AA, Saeed Mirghani ME, Hussein I (2017). *Phoenix dactylifera* date palm kernel oil. In: Unconventional oilseeds and oil Sources. Academic Press, pp 181-185. <https://doi.org/10.1016/B978-0-12-809435-8.00029-9>
- McNaught AD, Wilkinson A (1997). IUPAC Compendium of Chemical Terminology. 2nd. Edition, Blackwell Science Publications, Oxford.
- Metoui M, Essid A, Bouzoumita A, Ferchichi A (2018). Chemical composition, antioxidant and antibacterial activity of Tunisian date palm seed. *Polish Journal of Environmental Studies*, 28(1):267-274. <https://doi.org/10.15244/pjoes/84918>
- Moni SS, Hadi Sultan M, Makeen HA, Jabeen A, Sanobar S, Rahimullah S, ... Remesh M (2021). Phytochemical and spectral analysis of the methanolic extracts of leaves of *Murraya koenigii* of Jazan, Saudi Arabia. *Natural Product Research* 35(15):2569-2573. <https://doi.org/10.1080/14786419.2019.1679137>
- Nakamoto K (2008). Infrared and Raman spectra of inorganic and coordination compounds. John Wiley & Sons, Inc. <https://doi.org/10.1002/9780470405840>
- Nam HY, Na EJ, Lee E, Kwon Y, Kim HJ (2017). Antiepileptic and neuroprotective effects of oleamide in rat striatum on kainate-induced behavioral seizure and excitotoxic damage via calpain inhibition. *Frontiers in Pharmacology* 8. <https://doi.org/10.3389/fphar.2017.00817>
- National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 7150, Methyl benzoate. Retrieved 2023 April 28 from: <https://pubchem.ncbi.nlm.nih.gov/compound/Methyl-benzoate>
- National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 567452. Retrieved 2023 April 29 from: <https://pubchem.ncbi.nlm.nih.gov/compound/567452>
- Nehdi IA, Sbihi HM, Tan CP, Rashid U, Al-Resayes SI (2018). Chemical composition of date palm (*Phoenix dactylifera* L.) seed oil from six Saudi Arabian cultivars. *Journal of Food Science* 83(3):624-630. <https://doi.org/10.1111/1750-3841.14033>
- Nguyen TB, Phan Van HL, Cao N (2018). Bioactive compounds from marine fungus *Penicillium citrinum* strain ND7c by gas chromatography-mass spectrometry. *Pharmaceutical Chemistry Journal* 5(1):211-224.
- Ni Luh S (2016). Identification of the substance bioactive leaf extract *Piper caninum* potential as botanical pesticides. *International Journal of Pure & Applied Bioscience* 4(4):26-32.
- Parthipan B, Suky M, Mohan V (2015). GC-MS analysis of phytocomponents in *Pleiospermium alatum* (Wall. ex Wight & Arn.) Swingle (Rutaceae). *Journal of Pharmacognosy and Phytochemistry* 4:216-222.
- Pavia DL, Lampman GM, Kriz GS, Vyvyan JA (2014). Introduction to Spectroscopy. 5th Edition. Cengage Learning, pp 305.
- Ponder A, Kulik K, Hallmann E (2021). Occurrence and determination of carotenoids and polyphenols in different paprika powders from organic and conventional production. *Molecules* 26(10):2980. <https://doi.org/10.3390/molecules26102980>

- Rahmani AH, Aly SM, Ali H, Babiker AY, Srikar S, Khan AA (2014). Therapeutic effects of date fruits (*Phoenix dactylifera*) in the prevention of diseases via modulation of anti-inflammatory, anti-oxidant and anti-tumour activity. *International Journal of Clinical and Experimental Medicine* 7(3):483-491.
- Samad MA, Hashim SH, Simarani K, Yaacob JS (2016). Antibacterial properties and effects of fruit chilling and extract storage on antioxidant activity, total phenolic and anthocyanin content of four date palm (*Phoenix dactylifera*) cultivars. *Molecules* (Basel, Switzerland) 21(4):419. <https://doi.org/10.3390/molecules21040419>
- Schwingshackl L, Hoffmann G (2014). Monounsaturated fatty acids, olive oil and health status: a systematic review and meta-analysis of cohort studies. *Lipids in Health and Disease* 13:154. <https://doi.org/10.1186/1476-511X-13-154>
- Shahbaz K, Asif JA, Liszen T, Nurul AA, Alam MK (2022). Cytotoxic and antioxidant effects of *Phoenix dactylifera* L. (Ajwa date extract) on oral squamous cell carcinoma cell line. *BioMed Research International* 5792830. <https://doi.org/10.1155/2022/5792830>
- Şimşek Sezer EN, Uysal T (2021). Phytochemical analysis, antioxidant and anticancer potential of *Sideritis niveotomentosa*: Endemic wild species of turkey. *Molecules* (Basel, Switzerland) 26(9):2420. <https://doi.org/10.3390/molecules26092420>
- Sivakumar SM, Muhammad HS, Hafiz AM, Osama AM, Mohammed AB, Saad SA, ... Ahmed MS (2021). Bioactive principles in exudate gel from the leaf of *Aloe fleurentiniorum*, traditionally used as folkloric medicine by local people of Aridah and Fayfa mountains, Saudi Arabia. *Arabian Journal of Chemistry* 14(11):103400. <https://doi.org/10.1016/j.arabjc.2021.103400>
- Sultan MH, Moni SS, Saad SA, Hafiz AM, Osama AM, Mohammed AB, ... Mohamed EE (2022). Formulation, characterization, and biological evaluation of injectable nanocrystals from stem exudate gel of *Caralluma retropiciens* (Ehrenb) – Part C. *Arabian Journal of Chemistry* 15(2):103579. <https://doi.org/10.1016/j.arabjc.2021.103579>
- Suvitha S, Siddig IA, Al-Mamary MA, Syam M (2012). Synthesis of chalcones with anticancer activities. *Molecules* 17(6):6179-6195. <https://doi.org/10.3390/molecules17066179>
- Taiseer Abdul-kader, S (2021). Isolation and identification of campesterol, stigmasterol and beta-sitosterol in Iraqi date palm pollen. *Journal of Education and Scientific Studies Chemistry* 5(17):135-140.
- Vigüiliouk E, Stewart SE, Jayalath VH, Ng AP, Mirrahimi A, de Souza RJ, ... Sievenpiper JL (2015). Effect of replacing animal protein with plant protein on glycemic control in diabetes: A systematic review and meta-analysis of randomized controlled trials. *Nutrients* 7(12):9804-9824. <https://doi.org/10.3390/nu7125509>
- Wongsa P, Phatikulrungsun P, Prathumthong S (2022). FT-IR characteristics, phenolic profiles and inhibitory potential against digestive enzymes of 25 herbal infusions. *Scientific Reports* 12(1):6631. <https://doi.org/10.1038/s41598-022-10669-z>
- Yang TL, Hsieh CM, Meng LJ, Tsai T, Chen CT (2022). Oleic acid-based self-micro-emulsifying delivery system for enhancing antifungal activities of clotrimazole. *Pharmaceutics* 202214(3):478. <https://doi.org/10.3390/pharmaceutics14030478>
- Zhang X, Ali Esmail G, Fahad Alzeer A, Valan Arasu M, Vijayaraghavan P, Choon Choi K, Abdullah Al-Dhabi N (2020). Probiotic characteristics of *Lactobacillus* strains isolated from cheese and their antibacterial properties against gastrointestinal tract pathogens. *Saudi Journal of Biological Sciences* 27(12):3505-3513. <https://doi.org/10.1016/j.sjbs.2020.10.022>
- Zhang X, Wang J, Zhu L, Wang X, Meng F, Xia L, Zhang H (2022). Advances in stigmasterol on its anti-tumor effect and mechanism of action. *Frontiers in Oncology* 12:1101289. <https://doi.org/10.3389/fonc.2022.1101289>
- Zhu S, Jiao W, Xu Y, Hou L, Li, H., Shao J, Xiaoliang Z, Ran W, Kong D (2021). Palmitic acid inhibits prostate cancer cell proliferation and metastasis by suppressing the PI3K/Akt pathway. *Life Sciences* 286(120046):120046. <https://doi.org/10.1016/j.lfs.2021.120046>
- Zibara K, Awada Z, Dib L, El-Saghir J, Al-Ghadban S, Ibrik A, El-Zein N, El-Sabban M (2015). Anti-angiogenesis therapy and gap junction inhibition reduce MDA-MB-231 breast cancer cell invasion and metastasis in vitro and in vivo. *Scientific Reports* 5(1):12598. <https://doi.org/10.1038/srep12598>



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