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ORIGINAL ARTICLE

Anticancer Potential of Seed Extract and Pure Compound from *Phoenix dactylifera* on Human Cancer Cell Lines

Ebtesam S. Al-Sheddi

Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

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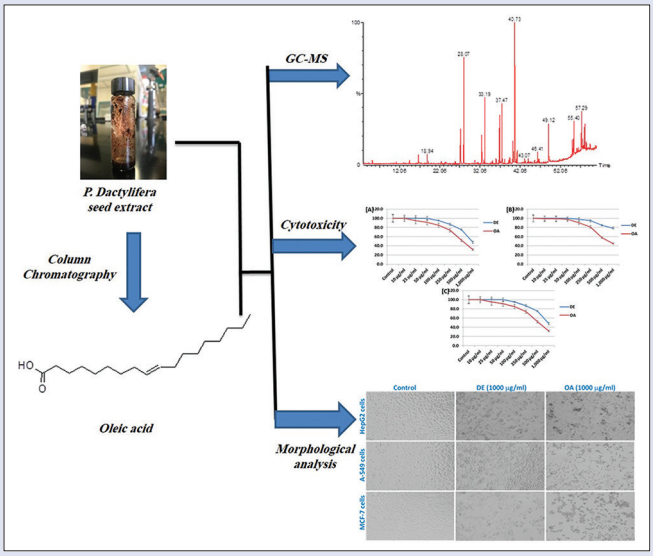
ABSTRACT

Background: *Phoenix dactylifera* (Palmacea), known as date palm is a widespread economical plant in the Middle Eastern. The dietary fiber in *P. dactylifera* seeds has important therapeutic use in medical condition such as diabetes, obesity, hypertension, colorectal, and prostate cancers. **Objectives:** The objective is to isolate, characterize the major bioactive components and evaluate the cytotoxic activity of extract and isolated pure compound of *P. dactylifera*. **Materials and Methods:** *P. dactylifera* extract (DE) was obtained by maceration. The pure compound, identified as oleic acid (OA) was isolated by column chromatography. Cytotoxicity assessment was done by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide assay and morphological alterations in HepG2, A-549, and MCF-7 cells and bioactive compounds were evaluated by gas chromatography/mass spectrometry. **Results:** The DE showed a dose-dependent cytotoxicity in all the testes cell lines. The cell viability at doses of 250, 500, and 1000 µg/ml of DE was found as 87%, 75%, and 48% in HepG2; 95%, 85%, and 78% in A-549; and 77%, 51%, and 35% in MCF-7 cells, respectively. The GCMS analysis indicated the presence of 37 compounds. The fatty acids and esters, fatty alcohols, and steroid ester were predominant in the DE. The IC_{50} value of isolated pure compound (OA) was determined at 735.2 µg/ml in HepG2, 909.1 µg/ml in A549, and 675.6 µg/ml in MCF-7 cells. **Conclusion:** These results suggest that DE has promising anticancer potential and OA could be the compound contributing to cytotoxicity.

Key words: Breast cancer, gas chromatography/mass spectrometry, liver cancer, lung cancer, oleic acid, *Phoenix dactylifera*

SUMMARY

- Major bioactive compounds in *Phoenix dactylifera* seed extract were identified by gas chromatography/mass spectrometry
- The fatty acids and esters, fatty alcohols, and steroid ester were predominant
- Pure compound namely oleic acid was isolated by column chromatography
- The cytotoxicity of extract and pure compound of *P. dactylifera* was assessed
- A concentration-dependent cytotoxic response was observed in HepG2, A549, and MCF-7 cell lines.



Abbreviations used: *P. dactylifera*: *Phoenix dactylifera*; MTT: 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide; DE: *Phoenix dactylifera* extract; OA: Oleic acid; DMSO: Dimethyl sulfoxide.

Correspondence:

Dr. Ebtesam S. Al-Sheddi,
Department of Pharmacognosy,
College of Pharmacy, King Saud University,
Riyadh - 11451, Saudi Arabia.
E-mail: ealsheddi@ksu.edu.sa

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INTRODUCTION

Cancer is the major cause of deaths worldwide. Despite enormous efforts, a large number of cancer deaths are reported.[1] According to the American Cancer Society and International Union against cancer 17 million deaths are expected by 2030 due to various cancers.[2] The treatment methods of cancer include surgery, radiotherapy, and chemotherapy.[3] Chemotherapy (the use of chemical compounds to fight neoplastic diseases), in addition to surgery, has been effective in different types of cancers including breast, colorectal, ovarian, and lung cancer.[4] However, chemotherapeutic agents face the challenge of low selectivity and toxic effects on other nontarget tissue.[5] Consequently, the use of alternative and complementary therapies such as herbal medication are used increasingly. The bioactive compounds from plants are used to treat various diseases like cancer for thousands of years.[6] Hence, the screening of plants for anticancer effects has been actively pursued on an international scale.[7]

Dates from *Phoenix dactylifera* (Family Palmocea) are popular among the population of middle eastern countries and provide a staple food for people in arid and semi-arid regions of the world. Date seeds, also known as stones or pits form an integral part of date fruit and contribute between 10% and 15% of date fruits weight.[8] It has been reported that seeds of *P. dactylifera* encompass important bioactive

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compounds, therefore, the utilization of these is extremely needed.[9] The functional compounds such as fiber, fat, protein, moisture, vitamins, and high amount of phenolics are also reported in date seeds.[10] The dietary fiber in date seed has important therapeutic uses for certain medical conditions such as diabetes, obesity, hypertension, coronary heart disease, intestinal disorders, hyperlipidemia, colorectal, and prostate cancers.[11-13] In addition, seed extracts have also been found beneficial on carbon tetrachloride-induced live cell death.[14] However, the anticancer potential of *P. dactylifera* seed extract from Yemen has not been extensively studied. Hence, this study was designed to assess the anticancer effects of *P. dactylifera* seed extract and characterize its chemical composition by gas chromatography/mass spectrometry (GC-MS).

MATERIALS AND METHODS

Cell culture

Human cancer cell lines, HepG2, A-549, and MCF-7, were grown in DMEM with fetal bovine serum (FBS) (10%), 0.2% $NaHCO_3$, and 1% antibiotics-antimycotic solution. All the cell lines were cultured in a CO_2 incubator at 37°C.

Reagents

Chemicals, solvents, cell culture medium, and dyes used in this study were purchased from Sigma and FBS, antibiotics-atimycotic solution, and trypsin from Invitrogen. Plastic wares and other consumables were obtained from Nunc.

Experimental design

Extract of *P. dactylifera* was prepared in n-hexane by maceration. The identification of bioactive compounds in dried extract was done by GC-MS. Isolation and identification of pure compound (oleic acid [OA]) were analyzed by column chromatography and ^{1}H-NMR and ^{13}C-NMR. The *P. dactylifera* extract (DE) and pure OA were then analyzed for their anticancer potential. HepG2, A-549, and MCF-7 cells were treated with different (10–1000 µg/ml) concentrations of DE for 24 h. Following the exposure, cytotoxic responses were assessed using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay, and cellular morphology.

Preparation of *Phoenix dactylifera* seed extract

The seeds of *P. dactylifera* were manually isolated from *P. dactylifera* fruits obtained from native shop, Sana'a, Yemen. The sun-dried seeds were grounded and extracted at room temperature using n-hexane. To obtain a thick gummy mass, the collective n-hexane extract was evaporated under reduced pressure. To prepare the different concentrations for cytotoxicity and other assays, the extract was dissolved in dimethyl sulfoxide (DMSO).

Phytochemical screening of *Phoenix dactylifera*

To analyze the presence of various phytoconstituents, the DE was subjected to phytochemical examinations.[15]

Gas chromatography/mass spectrometry analysis

Chromatographic analysis was performed in Perkin Elmer Clarus 600T series GC–MS equipped with an Elite 5MS capillary column (30 m × 0.25 µm × 25 mm). The initial temperature of 40°C was held for 2 min and then raised to 150°C at a rate of 5°C/min. Again, increases to 300°C at rate of 5°C/min for 5 min. The total run time was 61 min. All samples were injected in split mode. The injection temperature was at 280°C, inlet line temperature was 240°C, and source temperature of filament was 220°C. The mass spectrometer was operated in EI mode (positive ion, 70 eV). Mass spectra were acquired in full scan mode with repetitive scanning from 40 m/z to 600 m/z in 1 s. Total run time was 61 min. The compounds were identified by comparing with the NIST and WILEY library.

Isolation of oleic acid

Dried extract (5 g) of *P. dactylifera* was subjected to column chromatography (10 cm × 3 cm, Si gel 60–120 mesh, 250 g). Gradient elution was carried out using various proportions of n-hexane and ethyl acetate. A small sample of each eluent was evaluated by thin layer chromatography and the fractions with similar composition were combined and concentrated under vacuum to yield a total of 15 fractions (F1-F15). The fraction F4 (n-hexane ethyl acetate, 80:20 [v\v]) was the only fraction with pure compound and was hence subjected to spectral analysis (^{1}H-NMR and ^{13}C-NMR).

Cytotoxicity assessment

MTT assay was performed to assess the cytotoxicity of DE and compound following the protocol.[16] In brief, 1×10^4 cells were seeded in 96-well plates and grown overnight in CO_2 incubator. After respective treatment, 10 µl MTT solution (5 mg/ml stock) was added in each well and plates were incubated for 4 h further. The supernatant was then aspirated, and DMSO (200 µl) was added in each well and mixed. The plate was then read at 550 nm wavelengths using microplate reader.

Morphological assessment

Alterations in the morphology of HepG2, A-549, and MCF-7 cell lines were assessed under the microscope. HepG2, A-549, and MCF-7 cell lines were treated with 10–1000 µg/ml of extract and compound for 24 h. After the treatment, the images were grabbed at ×20 under phase contrast inverted microscope.

Statistical analysis

The differences between control and exposed group were analyzed using one-way analysis of variance. $P < 0.05$ was measured as statistically significant.

RESULTS

Phytochemical investigation of *Phoenix dactylifera*

The phytochemical investigation of n-hexane extract of *P. dactylifera* seeds exhibited the presence of amino acids, steroids, terpenoids, and fatty acids [Table 1].

Table 1: Phytochemical screening of *Phoenix dactylifera* extract

Phytoconstituent	*P. dactylifera* extract
Fatty acids	+
Amino acids	+
Terpenoids	+
Steroids	+
Flavanoids	-
Free sugars	-
Phenolics	-
Alkaloids	-

+ indicates Present ; - indicates Absent

Gas chromatography/mass spectrometry analysis

The gas chromatogram of *P. dactylifera* [Figure 1] revealed a large number of compounds, most of them were recognized by the comparison of their mass spectra with those in NIST and Wiley library. In all 37 compounds were identified (1–37) and listed in Table 2. The fatty acids (9-octadecenoic acid, heptadecanoic acid, dodecanoic acid, and decanoic acid), esters (ethyl ester of pentadecanoic acid, ethyl ester of tetradecanoic acid), paraffin alcohol (1-dodecanol), and steroid ester (22,23-dibromostigmasterol acetate) are predominant in this fraction.

Identification of oleic acid

The pure compound was obtained by subjecting the n-hexane extract of *P. dactylifera* seeds to column chromatography. The identification of the structure of the compound was achieved by ^{1}H-NMR and ^{13}C-NMR spectroscopy [Figures 2 and 3]. The ^{1}H-NMR exhibited signals at δ_H 0.86 (t, J = 7.3 Hz, 3H, CH_3) for methyl, δ_H 1.25–1.30 (m, 20H, 10 × CH_2) for long aliphatic chain, δ_H 1.61 (m, 2H, CH_2), δ_H 2.00 (m, 4H, CH_2), and δ_H 2.32 (t, J = 7.8 Hz, 2H, CH_2). The double-bonded protons were also observed at δ_H 5.32 (m, 2H, CH = CH).

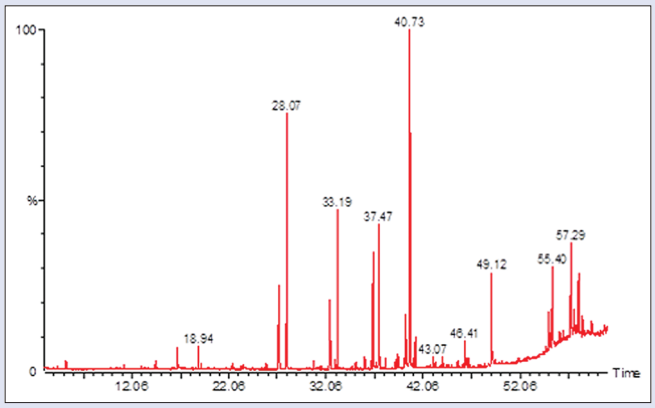

Figure 1: Gas chromatography/mass spectrometry chromatogram of *Phoenix dactylifera* extract

The ^{13}C-NMR showed signals at δ_C 180.36 for carboxylate functional group (-COOH), δ_C 130.04 and δ_C 129.74 for double bond (−CH = CH−), δ_C 14.14 for methyl group (−CH_3), and δ_C 34.10 for a long aliphatic chain. The assessment of the spectral data and its comparison with available literature led to the identification of isolated compound as OA ($C_{18}H_{34}O_2$).

3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide assay

The results of the cytotoxicity assessment of DE and isolated compound OA in HepG2, A-549, and MCF-7 are provided in Figure 4a-c, respectively. MTT assay showed that after 24 h and exposure of DE and OA decreased the cell viability of HepG2, A-549, and MCF-7 cells in a concentration-dependent manner. The percent cell viability at doses of 250, 500, and 1000 µg/ml of DE was found as 87%, 75%, and 48% in HepG2, 95%, 85%, and 78% in A-549 and 77%, 51%, and 35% in MCF-7 cells, respectively. Following treatment with 250, 500, and 1,000 µg/ml doses of OA, percentage cell viability was found as 74%, 52%, and 32% in HepG2; 81%, 58%, and 45% in A-549; and 66%, 48%, and 26% in MCF-7 cells, respectively [Figure 4a-c]. The IC_{50} values for DE and OA were found at 961.5 and 735.2 µg/ml in HepG2 cells, >1000 and 909.1 µg/ml in A-549 cells, and 769.2 and 675.6 µg/ml in MCF-7, respectively [Table 3].

Morphological analysis

P. dactylifera seed extract and OA-induced alterations in the morphology of HepG2, A-549, and MCF-7 cells are presented in Figure 5. The alterations in the morphology of cells were observed after 24 h and exposure. After the exposure of *P. dactylifera* seed extract and OA, a concentration-dependent prominent effect was observed in the morphology of all cell lines. As shown in Figure 5, as compare to control, all the cells lose their typical shape and morphology, become rounded and loss their adherence capacity after the exposure of 1000 µg/ml of DE and OA.

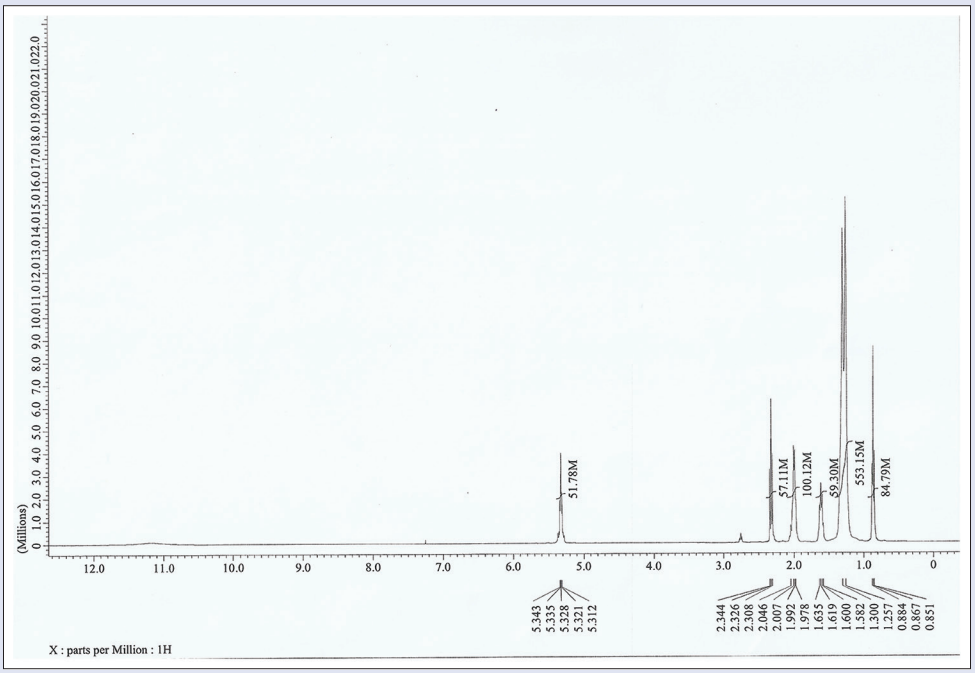

Figure 2: ^{1}H-NMR spectra of pure compound of *Phoenix dactylifera*

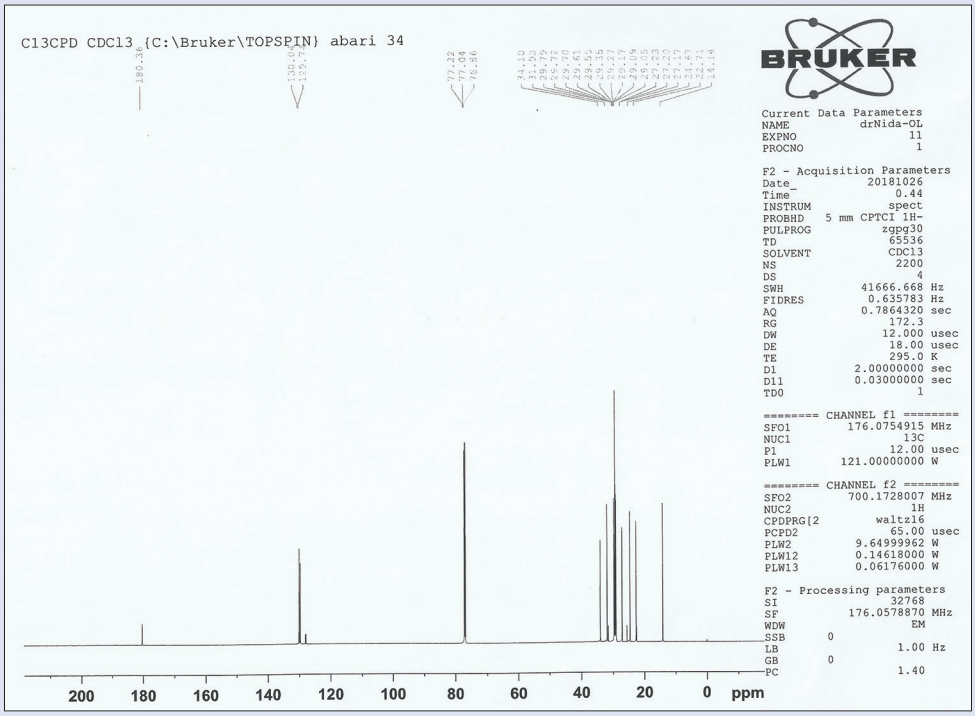

Figure 3: ^{13}C-NMR spectra of pure compound of *Phoenix dactylifera*

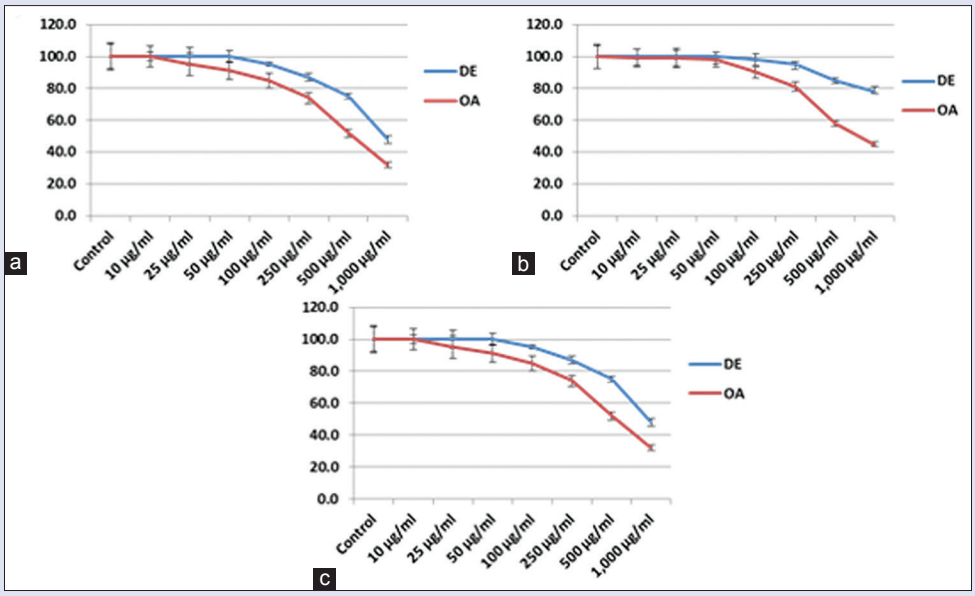

Figure 4: Cytotoxicity assessments by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide assays in (a) HepG2 cells, (b) A549 cells, and (c) MCF-7 cells. All the cells were exposed to various concentrations (10–1000 µg/ml) of *Phoenix dactylifera* extract and oleic acid for 24 h. Values are mean ± standard error of three independent experiments

DISCUSSION

Natural products, such as plants have been demonstrated beneficial against various diseases including cancer.[1,2] Consequently, to search for new anticancer agents at low cost are in continuous demand.[3,4] The natural products have received accumulative devotion since over three decades for their use in the search of a new therapeutic agent for preventing the cancer diseases.[5,6] *P. dactylifera* (Palmocea) plant is popular among the Middle Eastern population. The dietary fiber in *P. dactylifera* seeds has important therapeutic use in medical conditions such as diabetes, obesity, hypertension, colorectal, and prostate cancers.[11-13] The pharmacological potential of *P. dactylifera* seeds have led to design present investigation to explore the anticancer activity of extract and isolated pure compound from *P. dactylifera*. The n-hexane extract was found as a viscous liquid at room temperature and appeared to be semi-solid at lower temperatures. The semi-solid nature of the extract indicated the existence of saturated and unsaturated fatty

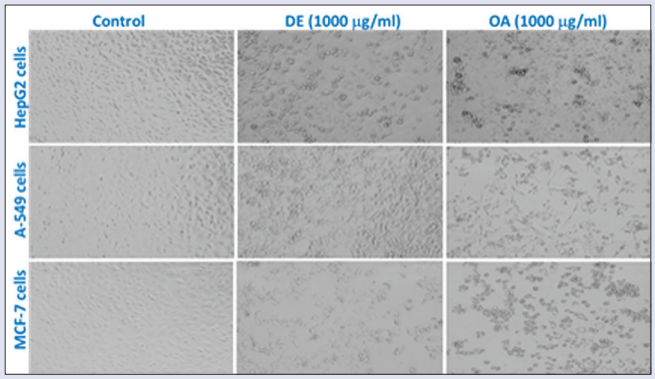

Figure 5: Morphological changes in HepG2, A-549, and MCF-7 cells following the exposure of *Phoenix dactylifera* extract and oleic acid at 1000 µg/ml for 24 h. Images were taken under the phase-contrast inverted microscope at ×20

acids.[17] The GC-MS examination of n-hexane extract of *P. dactylifera* exhibited the presence of 37 chemically important compounds [Table 3]. The compounds such as unsaturated and saturated fatty acids and esters, steroid ester, and high molecular weight hydrocarbon were predominant. Due to the presence of fatty acids, the oily fraction can be used in the cosmetics or other pharmaceutical products.[18] The lipophilic nonsteroidal anti-inflammatory drugs (NSAIDs) have been commonly used in the management of chronic rheumatic disorders. The myristic and OA s present in DE can be a source of percutaneous absorption enhancer by increasing the diffusion of NSAIDs. The literature survey revealed the possible bioactivities of identified compounds (1–37), it was suspected that OA (40.73, 15.85%) that could contribute to the cytotoxic response of *P. dactylifera* seed extract. OA (OA) as one of the major components have also been reported in seed oil of *P. dactylifera* growing in Saudi Arabia[19] and fruit flour of date palm bought from a market of Nigeria.[20] The anticancer effect of OA present in olive oil has already been reported.[21] Therefore, in this study, the anticancer potential of DE and OA on three different human cancer cell lines, i.e., liver cancer (HepG2), lung cancer (A-549), and breast cancer (MCF-7) were performed. The column chromatography of DE using various gradients of n-hexane and ethyl acetate resulted in the isolation of pure compound alleged as OA. The identity of the isolated compound was confirmed by spectral analysis, ^{1}H-NMR, and ^{13}C-NMR [Figures 2 and 3]. The triplet at δ_H 0.86 indicated the presence of the methyl group, a multiplet at δ_H 1.25–1.30 corresponded to long aliphatic methylene chain and a multiplet at δ_H 5.32 was observed for the double-bonded protons. The ^{13}C-NMR showed signals at δ_C 180.36 for carboxylate functional group (-COOH), at δ_C 130.04 and δ_C 129.74 for double bond (–CH = CH–), δ_C 14.14 for methyl group ($-CH_3$), and δ_C 34.10 for long aliphatic chain. The assessment of the spectral data and its comparison with available literature led to the identification of isolated compound as OA ($C_{18}H_{34}O_2$).

The MTT assay results revealed a concentration-dependent cytotoxicity in all three cell lines after the exposure of DE and OA. The MTT data and cellular morphological results showed higher cytotoxic response of OA as compared to *P. dactylifera* against HepG2, A-549, and MCF-7 cancer cells. MTT assay is frequently used parameter to assess the cytotoxic response because its measure cellular function, thus it is useful to assess the cytotoxic potential of extracts/compounds. This assay shows the function of mitochondria in viable cells on the basis of enzymatic reduction of tetrazolium salt through mitochondrial dehydrogenase enzyme.[22] In this study, more decrease in the viability of MCF-7 and HepG2 cells was observed as compared to A-549 cells.

Table 2: Phytoconstituents identified in *Phoenix dactylifera* extract

#	Name	RT	Area	Area %
1	Isohexyl Pentyl Ester	5.34	28388	0.810
2	Octanal	11.30	3191	0.090
3	Hexanal	14.38	6048	0.170
4	Maltol	14.52	25709	0.730
5	2-(2-butoxyethoxy)-Ethanol	16.78	67095	1.910
6	1-dodecanol	18.94	43352	1.230
7	Nonanoic Acid	19.14	13478	0.380
8	Bis-(3,5,5-trimethylhexyl) Ether	22.45	13785	0.390
9	Methyl Ester Of Heptadecanoic Acid	25.93	16601	0.470
10	Dodecanoic Acid	27.23	190805	5.430
11	2,2,4-trimethyl-pentanoic Acid,	27.88	18076	0.510
12	Ethyl Ester Of Pentadecanoic Acid	28.07	298948	8.510
13	Acrylic Acid Decyl Ester	30.75	19753	0.560
14	Tetradecanoic Acid	32.47	108801	3.100
15	Ethyl Ester Of Tetradecanoic Acid	33.19	313331	8.920
16	Dl-3,4-dimethyl-3,4-hexanediol	34.70	5348	0.150
17	1,2-benzenedicarboxylic Acid	36.64	50335	1.430
18	Decanoic Acid	36.87	176525	5.020
19	Butyl Myristate	37.31	14502	0.410
20	Heptadecanoic Acid	37.47	272124	7.740
21	Cycloeicosane	39.24	4926	0.140
22	2-nitrocyclododecanone	39.38	8887	0.250
23	D6-dodecene-1-ol	40.10	13681	0.390
24	22-tricosenoic Acid	40.25	100465	2.860
25	Eicos-2-yne	40.60	50404	1.430
26	9-octadecenoic Acid	40.73	556804	15.850
27	1,1-dimethyl Ester Of Hexadecanoic Acid	41.07	19389	0.550
28	Ethyl Ester Of Hexadecanoic Acid	41.22	54463	1.550
29	1-tridecanol	43.07	17176	0.490
30	Octadecanoic Acid	46.41	24155	0.690
31	3-acetoxydodecane	48.50	7659	0.220
32	Oleoyl Chloride	49.12	129761	3.690
33	1-tridecene	49.49	5704	0.160
34	Eicosyl Acetate	51.80	6666	0.190
35	Dieicosyl Thiodipropionate	55.05	46759	1.330
36	Octadecane	55.40	94822	2.700
37	22,23-dibromostigmasterol Acetate	57.29	95738	2.720

Table 3: The half-maximal inhibitory concentration values of the extract and pure compound of *Phoenix dactylifera*

Compound	HepG2 cells (µg/ml)	A-549 cells (µg/ml)	MCF-7 cells (mg/ml)
P.dactylifera Extract	961.5	>1000	769.2
Oleic Acid	735.2	909.1	675.6

A dose-dependent cytotoxic response induced by DE has previously been reported against MCF-7 cells after 48 h and exposure.[23] The different cytotoxic response in each cell line could be due to the specificity of the extract/compound towards cancer cell lines.[24] In this study, the cytotoxic effects of *P. dactylifera* may be due to the active components present in the extract[25] The IC_{50} value of isolated pure compound (OA) was determined at 735.2 µg/ml in HepG2 cells, 909.1 µg/ml in A549 cells, and 675.6 µg/ml in MCF-7 cells. The anticancer properties of OA have previously been reported against different cancer cell lines[26] including human breast,[27] T-leukemia/lymphoma cell lines Jurket,[28] tongue squamous cell carcinomas,[29] and human colon adenocarcinoma cells.[30]

CONCLUSION

This study demonstrated the extraction, isolation of pure compound and screening the anticancer potential of *P. dactylifera* seeds. The n-hexane extract was prepared and subjected to column chromatography for the isolation of pure compound. The isolated compound was identified as OA by ^{1}H-NMR and ^{13}C-NMR. The results from this study demonstrated that DE showed promising anti-cancer activity against HepG2, A-549, and MCF-7 cells. A concentration-dependent cytotoxic response of DE and OA was observed. This study also revealed that OA play a

role in the cytotoxicity of HepG2, A-549, and MCF-7 cells, therefore, it can be concluded that *P. dactylifera* has the ability to induce cell death/antiproliferation against cancer cells and OA could be the component responsible for such a response. The specific mechanism(s) involved in the extract or OA-mediated cytotoxicity/cell death will be focused in future studies.

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Conflicts of interest

There are no conflicts of interest.

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