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In-vitro Antioxidant and Anticancer Studies of Date Palm (Phoenix dactylifera L.) Seed Extract and Its Oil on Seven Cell Lines with Molecular Docking Study Targeting PI3K and EGFR Inhibition

Amina El-Shaibany[1], Gehad M. Elnagar[2,3], Ali E. Raslan[4], Ahmed S. Abdelkhalek[5]*, Naif A. Al-Gabri[6,7], Mahmoud M. Elaasser[8], Nesreen M. I. M. Elkomy[9]*

[1]Pharmacognosy Department, University of Sana'a, Pharmacy College, Yemen; amina.doc.pharm@gmail.com
[2]Biochemistry Department, Faculty of Pharmacy, Zagazig University, 44519, Egypt; jmabdulrahman@pharmacy.zu.edu.eg
[3]Biochemistry Department, Faculty of Pharmacy, El Saleheya El Gadida University, El Saleheya El Gadida 44813, Egypt; gehadelnagar@sgu.edu.eg
[4]Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut 71524, Egypt; aliraslan@azhar.edu.eg
[5]Medicinal Chemistry Department, Faculty of Pharmacy, Zagazig University, Zagazig, 44519, Egypt; ashilal@pharmacy.zu.edu.eg
[6]Department of Pathology, Faculty of Agriculture and Veterinary Medicine, Thamar University, Dhamar, Yemen, naifaljabry2014@gmail.com
[7]Laboratory of Pathology, Salam Veterinary Group, Buraydah, Al-Qassim, Saudi Arabia.
[8]The Regional Center for Mycology and Biotechnology, Al-Azhar University, 11787 Nasr City, Cairo, Egypt; mmelaasser@gmail.com
[9]Pharmacology and toxicology department, Faculty of Pharmacy, Zagazig University, Egypt; nmmousa@pharmacy.zu.edu.eg

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ABSTRACT

Background: Natural products and nutraceuticals are considered important sources of anticancer medicines. The study was conducted to investigate the in-vitro antioxidant and anticancer activities of date palm (Phoenix dactylifera L.) seed extract (DPSE) and its oil (Oi-Y) against seven cancer cell lines with new insights for the proposed anticancer mechanism of action via molecular docking study focusing on the major active constituents with inhibitory effect on phosphatidylinositol-3-kinase (PI3K) and antagonistic action of epidermal growth factor receptor (EGFR). **Materials and methods:** The antioxidant assay was conducted using DPPH radical scavenging activity. Besides, the anticancer activities were investigated using a variety of cell lines, including HCT-116 (colon carcinoma), HepG-2 (hepatocellular carcinoma), PC-3 (prostate cancer), A-549 (lung adenocarcinoma), HeLa (cervical cancer), HEP-2 (human larynx epithelial carcinoma), and MCF-7 (breast carcinoma). They were quantitatively determined for their in-vitro anti-neoplastic activities using a colorimetric technique. The IC50 values were computed by using optical density. Positive control was performed using doxorubicin. **Results:** DPSE and Oi-Y showed stronger antioxidant activity than ascorbic acid. The oil had stronger cytotoxic effects on the tested cell lines than DPSE. The HepG-2 cell line was the most susceptible cell, with an IC50 value of 11.18 µg/mL. Molecular docking results showed that oleuropein, luteolin-7-O-glucoside, apigenin-7-O-glucoside, and chromone-1 interestingly bind with high scores with the selected PI3K and

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*Corresponding author & copyright holder: Ahmed S. Abdelkhalek, Nesreen M. I. M. Elkomy Email address: ashilal@pharmacy.zu.edu.eg , nmmousa@pharmacy.zu.edu.eg

EGFR target sites. **Conclusions:** Our experiment demonstrated that date palm seed extract and its oil could considerably be a novel source of anticancer agents

1. Introduction

According to the World Health Organization (WHO) official statistical report and the American Cancer Society (ACS), cancer is a leading cause of mortality worldwide [1-3]. Overall, the most prevalent malignancies' cumulative 5-year survival rates were listed in the following decreasing order: prostate 70-100%, breast 80-85%, rectum 60-70%, colon and cervix 50-70%, ovary 30-50%, stomach and brain 20-40%, oesophagus 10-30%, lung 10-20%, liver 5-30%, and pancreas 5-15% [3]. According to estimates, there will be almost 10 million cancer-related deaths and 19.3 million new instances of cancer in 2020 (excluding skin cancer and nonmelanoma) (9.9 million including skin cancer and nonmelanoma) [4]. Female breast cancer has surpassed lung cancer as the most prevalent cancer diagnosed, with an anticipated 2.3 million new cases (11.7%), followed by lung (11.4%), colorectal (10.0%), prostate (7.3%), and stomach (5.6%) cancers. Additionally, with 1.8 million deaths, lung cancer continued to be the greatest cancer killer (18%), followed by colorectal (9.4%), liver (8.3%), stomach (7.7%), and breast cancer (6.9%) [3, 5]. Cancer is characterized by the transforming of normal cells into neoplastic cells by altering their genetic material, leading to the expression of oncogenes, inhibition of tumor suppressor genes, and uncontrolled growth. Cell cycle division and tumor growth are controlled by some enzymes such as tyrosine kinase [6] and growth factor as an epidermal growth factor that is involved in replication and metastasis [7]. Drugs targeting tyrosine kinase enzyme or growth factors receptors are very effective as anticancers with high selective index and fewer side effects and resistance [8, 9]. PI3K and EGFR inhibitors are widely used in different cancer types [10].

Natural products and nutraceuticals are considered important sources of anticancer medicines and have been approved by the National Cancer Institute (NCI). This significantly advances the search for novel, potent anticancer drugs derived from natural sources [11]. There are numerous potent anti-neoplastic substances originating from natural sources, such as indole alkaloids [12]. Various indole alkaloids are found in the Ervatamia heyneana woods and stem bark, including camptothecin and its derivatives [13]. Rhazya stricta and Anthocephalus cadamba also have anticancer indole alkaloids [14, 15]. Vincristine, vinblastine, and podophyllotoxin (etoposide and teniposide) are among the most widely utilized broad-spectrum anticancer Vinca alkaloids derivatives that have been used to treat various cancers [16]. Natural products offer a superior safety profile and fewer adverse effects than synthetic chemicals [17, 18]. Parallel to this, the research found that anticancer chemotherapy medications have serious adverse effects, including baldness, bone marrow suppression, and gastrointestinal side effects such as extreme nausea and vomiting [19]. This makes the idea of testing various natural extracts and oils for potential anticancer properties while minimizing adverse effects appealing.

Moreover, some plants are a good source of antioxidant constituents such as Aloe vera flower [20], black pepper [21], and okra mucilage [22]. Due to its possible health benefits, date palm (Phoenix Dactylifera L.) significant herbal product utilized in traditional treatments [23]. Phenolics, tocopherols, sterols, and saturated and unsaturated fatty acids are among the significant constituents that give the extract its desirable properties, which include antiviral, antioxidant, antimicrobial, antihyperlipidemic, hepatoprotective effect, neuroprotective effect, and hypoglycemic properties [23-29]. Additionally, it has low quantities of fat and protein and high levels of carbs (between 70 and 80 percent) [30, 31]. Furthermore, date seed oil can shield the skin against oxidative stress damage brought on by hydrogen peroxide as well as UV-A and UV-B rays, which harm cells and cause cellular damage [32].

The present study aimed to assess the in-vitro antioxidant potential and anticancer activity of DPSE and its oil against different types of human cancer cells and a molecular docking study targeting PI3K and EGFR for exploring the anticancer mechanism of action of major active constituents.

2. Materials and methods

2.1. The plant material

2.1.1. Identification and preparation

Dates were collected from Hadramout city, Yemen in 2019. The Botany Department of the Faculty of Science, Sana'a University, Yemen, performed plant taxonomy and seed authentication. At Sana'a University's herbarium pharmacy department, a voucher specimen with the number (Sa/Phar/Pharm 44) was placed. Date seeds were soaked in hot water, washed, dried at 60 °C for a day then the seeds were roasted and grounded followed by a sieving process that resulted in a powder.

2.1.2. Extraction and preparation of the oily samples of the date palm seed

Phoenix dactylifera L dried powder (500 g) were extracted with n-hexane and methanol and concentrated using rotavap (Supplementary data).

2.2. Cell lines

Seven cancer cells including HepG-2, HeLa, HEP-2PC-3, HCT-116, A-549 and MCF-7 were used to investigate the activity of DPSE and Oi-Y (Supplementary data).

2.2.1. Experimental design

MTT assay using 1.56-50 µg/ mL of extract for 24 h were performed and analyzed by the tissue culture unit at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt (Supplementary data).

2.2.2. Cytotoxicity effect

Methanolic DPSE and Oi-Y were investigated for their cytotoxicity against previously mentioned cancer cell lines a using Doxorubicin as positive control.

2.2.3. Cytological examination

The 50% inhibitory concentration (IC50), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose-response curve for each concentration using GraphPad Prism software (San Diego, CA. USA) [33, 34] (supplementary data).

2.2.4. Microscopic observation of the tumor cells treated with the samples

The cellular morphology was observed using an inverted microscope (CKX41, Olympus, Japan) equipped with a digital microscopy camera to capture the images representing the morphological changes compared to the control cells [35]. The cytopathic effects (morphological alterations) were microscopically detected at 100X (supplementary data).

2.3. DPPH radical scavenging activity

A methanol solution of the test compound was prepared. A 40 µL methanol solution of the test compound was added to 3 mL of DPPH solution. Absorbance were recorded with a UV-visible spectrophotometer (Milton Roy, Spectronic 1201) at 515 nm was determined continuously. The absorbance of the DPPH radical without antioxidant (control) and ascorbic acid were also measured [36] in three replicates and averaged (supplementary data). The percentage inhibition (PI) of the DPPH radical was calculated according to the formula:

$$\% \text{ Inhibition} = (AC - AS)/AC \times 100$$

Ascorbic acid was used as the standard [17].

2.4. Molecular docking studies

From PDB, the crystal structures of EGFR (2RGP.pdb) and PI3K-δ (PDB ID:2WXG) were obtained. Protonation, energy minimization, and the addition of hydrogen were used to get the two targets ready for docking. The PubChem database provided the 2D structures of the palm oil molecules in the form of SMILES, and MOE 2019 (Molecular Operating Environment (MOE) version MOE 2019.0102 (Chemical Computing Group, Montreal, CA) was used to create the 3D structure followed by protonation and energy reduction. All parameters are kept in the default. The co-crystallized compounds of SW13 and N4-(1-(3-fluorobenzyl)-1H-indazol-5-yl)-5-((piperidin-1-ylimino)methyl)pyrimidine-4,6-diamine were docked at PI3K and EGFR,

respectively, using the identical docking methodology to validate the docking protocol (Fig. S1).

2.5. Statistical analysis

The mean ± SD was used to summarize and show the data. An Independent Student's t-test, a two way ANOVA test were used for statistical analysis. Differences were considered significant at P values of less than 0.05. Experimental results were expressed as means ± standard error of the mean (SEM). The IC50 values were calculated from linear regression analysis. Results for DPPH and anticancer activity were analyzed by Pearson correlation coefficient.

3. Results

3.1. Antitumor activity

The findings of the present study revealed that DPSE revealed higher activity against HCT-116 and HepG-2 followed by MCF-7, A-549, PC-3, HeLa, and HEP-2 with IC50 28.8, 34.0, 40.2, 44.8, 61.12 and 65.34 µg/ mL respectively. Oi-Y exhibited the strongest cytotoxic activity against HepG-2 and MCF-7 followed by HCT-116, A-549, HeLa, PC-3, and HEP-2 with IC50 11.18, 18.60, 19.10, 19.20, 22.10, 23.40 and 23.95 µg/ mL, respectively. Oi-Y showed the highest strong cytotoxic activity against all tested cell lines compared with DPSE. The highest anticancer activity of DPSE was reported against HCT-116 with IC50 at 28.81 µg/mL, while Oi-Y presented the highest anticancer activity against HepG-2 with an values of 11.18 µg/mL. Conversely, the lowest anticancer activities of DPSE and Oi-Y were observed against HEP-2 with IC50 23.95 and 57.23 µg/ml, respectively (Table 1).

Table 1 IC_{50} values (µg/mL) of DPSE and Oi-Y in different human cancer cell lines using doxorubicin as a reference drug.

Cell line	Doxorubicin	DPSE			Oi-Y		
	Mean ± SD	Mean ± SD	t-test	*p*-value	Mean ± SD	t-test	*p*-value
MCF-7	4.39 ± 0.23	40.23 ± 12.31	5.04	0.037	18.60 ± 4.22	5.82	0.028
HCT-116	4.58 ± 0.44	28.81 ± 5.26	7.95	0.015	19.10 ± 7.95	3.16	0.034
HepG-2	4.71 ± 0.37	34.04 ± 7.08	7.17	0.019	11.18 ± 4.82	2.32	0.146
PC-3	7.07 ± 0.59	47.53 ± 11.23	6.23	0.025	23.40 ± 6.03	4.67	0.043
A-549	8.47 ± 0.61	44.82 ± 10.04	6.26	0.025	19.20 ± 6.24	2.96	0.097
HeLa	12.04 ± 0.58	51.12 ± 12.05	5.61	0.030	22.10 ± 4.53	3.82	0.062
HEP-2	10.13 ± 0.79	55.3 ± 14.72	5.31	0.034	23.95 ± 5.67	4.18	0.053

IC_{50}: 50% inhibitory concentration; MCF-7: breast carcinoma cells; HCT-116: colon carcinoma cells; HepG-2: hepatocellular carcinoma, PC-3: prostate cancer, A-549: lung adenocarcinoma, HeLa: cervical cancer; HEP-2: human larynx epithelial carcinoma.

Fig. 1 depicted the inhibition of the selected cancer cell lines by DPSE and Oi-Y. It demonstrates that significant dose-dependent cytotoxic activity against the cell lines was observed at all concentrations ranging from 50-1.56 µg/mL.

3.2. Selective cytotoxic effect of DPSE and Oi-Y on a non-cancerous cell vs. cancerous cells

DPSE and Oi-Y were tested for cytotoxicity against seven cancerous cell types (HCT-116, HepG-2, PC-3, A-549, HeLa, HEP-2, and MCF-7) as well as the non-cancerous human fetal lung fibroblast cells (MRC-5). The selectivity index was calculated by dividing the CC50 of the non-cancer cell by the IC50 values of each cancer cell line as presented in Table 2. DPSE and Oi-Y protected normal non- cancer cells compared to the standard anticancer doxorubicin. These results showed partially selective cytotoxic action of the DPSE and Oi-Y against cancerous cell lines.

3.3. Microscopic examination of Oi-Y

The A-549 cell line with an attached continuous sheet of the carcinoma cells was characterized by large vascular cells with abundant chromatin with very forms of mitotic indexes in the non-treated control group. Appeared a few empty areas besides numerous small cells with condensed chromatin in the Oi-Y 10 µg/mL group.

Oi-Y 100 µg/mL group showed severely destructed neoplastic cells with still cellular debris in large empty areas in Oi-Y 500 µg/mL group (Fig. 2). The

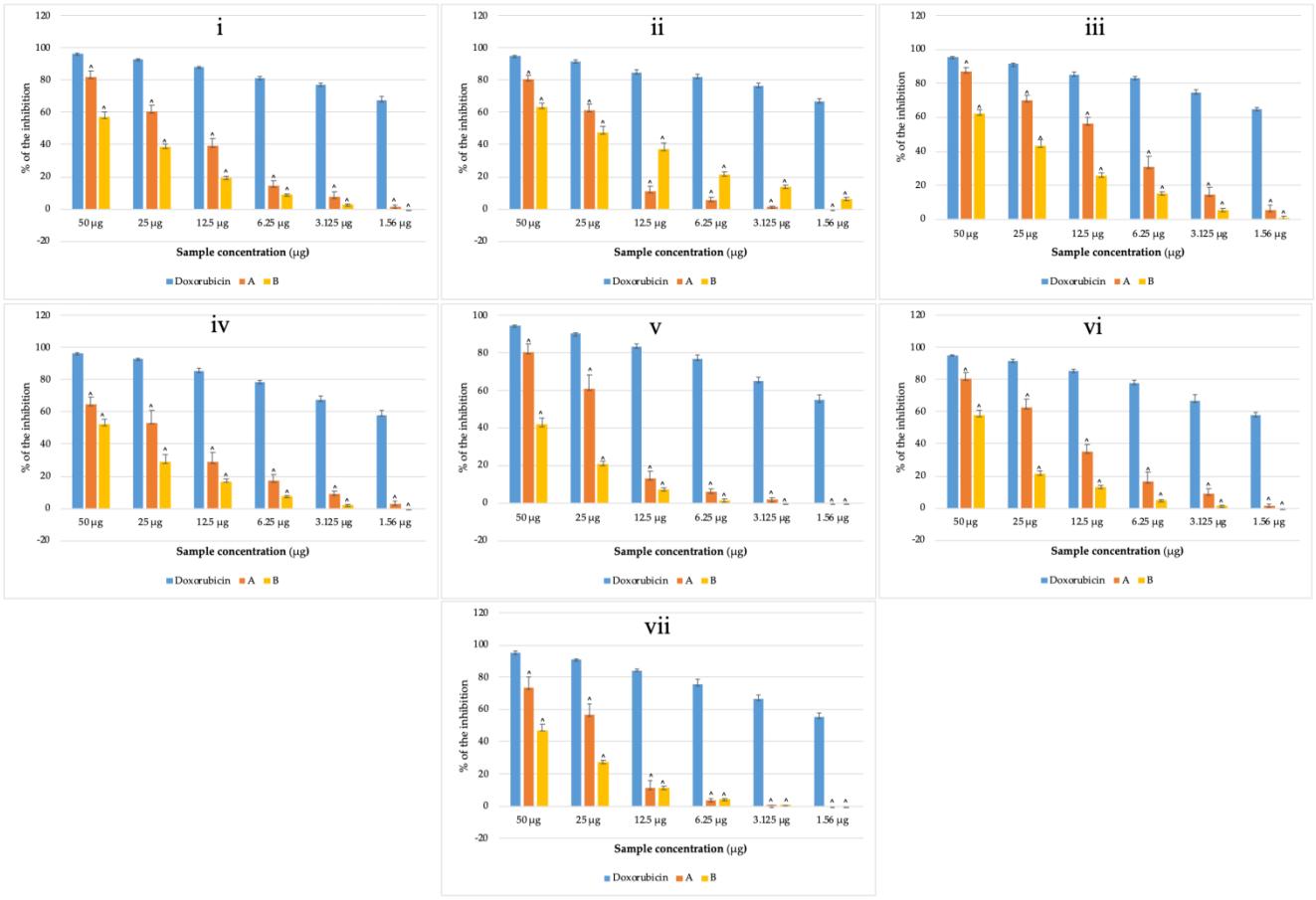


Fig. 1. % of inhibition of date palm seed extract and yellow oil against; i) MCF-7, ii) HCT-116, iii) HepG-2, iv) PC-3, v) HeLa, vi) A-549, vii) HEP-2 cell lines; A: Oi-Y; B: DPSE. ^*p* value <0.001 compared to reference drug.

Table 2. The selectivity index of DPSE, Oi-Y and reference drug on cancerous cell lines vs. a non-cancerous cell line.

Extract	(MRC-5) CC_{50}	SI = CC_{50}/IC_{50}						
		HCT-116	HeLa	HepG-2	HEP-2	PC-3	MCF-7	A-549
DPSE	14.28	0.496	0.245	0.42	0.27	0.3	0.355	0.32
Oi-Y	14.28	0.748	0.646	1.298	0.621	0.61	0.468	0.75
Doxorubicin	9.67	21.02	7.79	20.574	8.558	13.62	21.977	12.08

CC_{50}: 50% cytotoxic concentration; IC_{50}: concentration required to inhibit the cell growth by 50% compared to non-cancerous cell line; MCF-7: breast carcinoma cells; HCT-116: colon carcinoma cells; HepG-2: hepatocellular carcinoma, PC-3: prostate cancer, A-549: lung adenocarcinoma, HeLa: cervical cancer; HEP-2: human larynx epithelial carcinoma.

HeLa cell line with an attached continuous sheet of the carcinoma cells was characterized by large vascular cells and bazars cell (arrow) with abundant chromatin in the non-treated control group. Moreover, little effects except for condensed chromatin in a few cells in all Oi-Y 10, 100, and 500 µg/mL groups (Fig. 2). The HepG cell line with attached continuous cancer cell sheet appeared as a large vascular cell with abundant chromatin in the non-treated control group. Marked condensed chromatin of the major sheet cells which moderately dissociated due to loss of adhesion materials with a few vacuolar cytoplasms in the 10 µg/mL group. Prominent spaces between cells condense chromatin and destructed its cytoplasmic organelles in Oi-Y 100 µg/mL groups. Lysis of all carcinoma sheets with still remnant debris in Oi-Y 500 µg/mL group (Fig. 2). The HCT-116 cell line with the attached continuous cancer cell sheet appeared as a large vascular cell with abundant chromatin especially in bear cells in the non-treated control group. The carcinoma sheet is still attached with a few apoptotic cells in Oi-Y 10 µg/mL groups. Individual spaces between clustered cells and condense chromatin and

mild vacuolated cytoplasm in Oi-Y 100 µg/mL groups. Lysis of the all-carcinoma cell sheet with still remnant destructed clusters in Oi-Y 500 µg/mL groups (Fig. 2). The PC-3 cell line with attached continuous cancer cell sheet characterized by large vascular cells with abundant chromatin in the non-treated control group. Clear mild spaces between cells due to the absence of attached character with early apoptotic features in Oi-Y 10 µg/mL groups. Increase empty spaces with destructed cells in Oi-Y 100 µg/mL groups. Increase spaces between cells with condensed chromatin with still remnant destructed clusters in Oi-Y 500 µg/mL groups (Fig. 2). The HEP-2 cell line with the attached continuous cancer cell sheet appeared as a large vascular cell with abundant chromatin in each non-treated control, Oi-Y 10 and Oi-Y 100 µg/mL groups. Nearly half of the cells were lost and the remaining have moderate apoptotic features besides empty spaces and loss of cellular adhesions in the seam degree in Oi-Y 500 µg/mL groups (Fig. 2). The MCF-7 cell line with an attached continuous cancer cell sheet appeared as a large vascular cell with abundant chromatin in the non-treated control group. The majority of cells were without any apoptotic or destructed effects except a few condensed chromatins with still intercellular adhesion features in Oi-Y 10 µg/mL group (Fig. 2). Still strong intercellular adhesions with prominent condensed chromatin gradually appear in small empty spaces in Oi-Y 100 and 500 µg/mL groups. All groups were semi-quantitative grades summarized in Table S1.

3.4. DPPH radical scavenging findings

The IC50 of the Oi-Y extract was found to be lower than the IC50 of the standard (50.82 vs. 13.9 µg/mL), DPSE exhibited lower IC50 (66.42 µg/mL) compared with Oi-Y and standard (Table 3).

3.5. Pearson correlations coefficient analysis

Pearson correlation analysis for DPSE presented a strong significant correlation between antioxidant activity (DPPH) against the anticancer activity for both A-546 ($r = 0.983$, $p < 0.01$) and HCT-116 ($r = 0.960$, $p < 0.05$) cells and good correlation was detected for HEP-2 ($r = 0.765$), HeLa ($r = 0.743$) and MCF-7 ($r = 0.661$) cells. While, strong negative correlation was detected for HEPG-2 ($r = –0.965$, $p < 0.01$) and PC-3 ($r = –0.920$, $p < 0.01$) cells. Oi-Y showed a strong significant correlation for both MCF-7 ($r = 0.998$, $p < 0.01$) and HCT-116 ($r = 0.952$, $p < 0.05$), and a weak correlation was observed for both HEP-2 ($r = 0.362$) and PC-3 ($r = 0.355$). Whereas, a strong negative non-significant correlation was detected for HeLa ($r = –0994$), A-546 ($r = –0.937$), and HEPG-2 ($r = –0.845$) (Table 4).

3.6. Molecular Docking

Oleuropein, apigenin-7-O-glucoside, luteolin-4`-O-glucoside, luteolin-7-O-glucoside, hyperoside, cirsilineol, acacetin, tyrosol, hydroxytyrosol, homoprotocatechuic acid, and homovanillic acid are the phenolics present in Phoenix dactylifera L. seed oil (Fig. S2) [23, 32, 37]. The date palm seed extract and its oil revealed caffeic acid, catechin, cinnamic acid, ferulic acid, gallic acid, p-hydroxybenzoic acid, syringic acid, vanillin, vanillic acid, luteolin, protocatechuic acid, chlorogenic acid, cryptochlorogenic acid, o-coumaric acid, p-coumaric acid, naringenin, and rutin (Fig. S3) [23, 32, 37-44], along with quercetin, epicatechin, isorhamnetin, kaempferol, dactylifric acid (caffeoylshikimic acid), m-coumaric acid, coumarin, resorcinol, salicylic acid, benzoic acid, and sinapic acid [38-43], chromone-1 and chromone -2 [45] reported in the total seed extract (Fig. S4). The fatty acids reported in the date palm seed oil are caprylic, pelargonic, capric, lauric, tridecyclic, myristic, pentadedecylic, palmitic, palmitoleic, hexadecenoic, margaric, heptadecenoic, stearic, oleic, linoleic, linolenic, arachidic, gondoic, behenic, tricosylic, lignoceric, cerotic and 9,10-epoxystearic acid (Fig. S5) [23, 32, 37, 44, 46, 47]. The seed oil of the date palm contained seven vitamin E isomers, that is, α-, β-, γ-, and δ-tocopherol and α-, γ-, and δ-tocotrienol (Fig. S6) [37, 44, 47, 48]. The oil also showed the presence of sterols like cholesterol, β-sitosterol, stigmasterol, campesterol, and Δ5-avenasterol (Fig. S7) [44, 48]. The date palm active constituents can be used for the treatment of various diseases including cancer. In this article, the date palm extract was evaluated against seven cancer cell lines and showed anticancer/antioxidant activities. Docking studies were used as a tool to deduce a possible mechanism of action of this extract. Cancer growth, proliferation, and apoptosis are controlled by various mechanisms among these mechanisms are PI3K [49] and EGFR [50]. Docking revealed an interesting high binding score at PI3K (2WXG.pdb) and EGFR (2RGP.pdb) for oleuropein, luteolin-7-O-glucoside, apigenin-7-

O-glucoside, chromone-1 and dactylifric acid as well as other constituents including fatty acids (lignoceric acid, arachidic acid) and tocopherols (γ- and δ-tocopherol and α-tocotrienol) (Table 5).

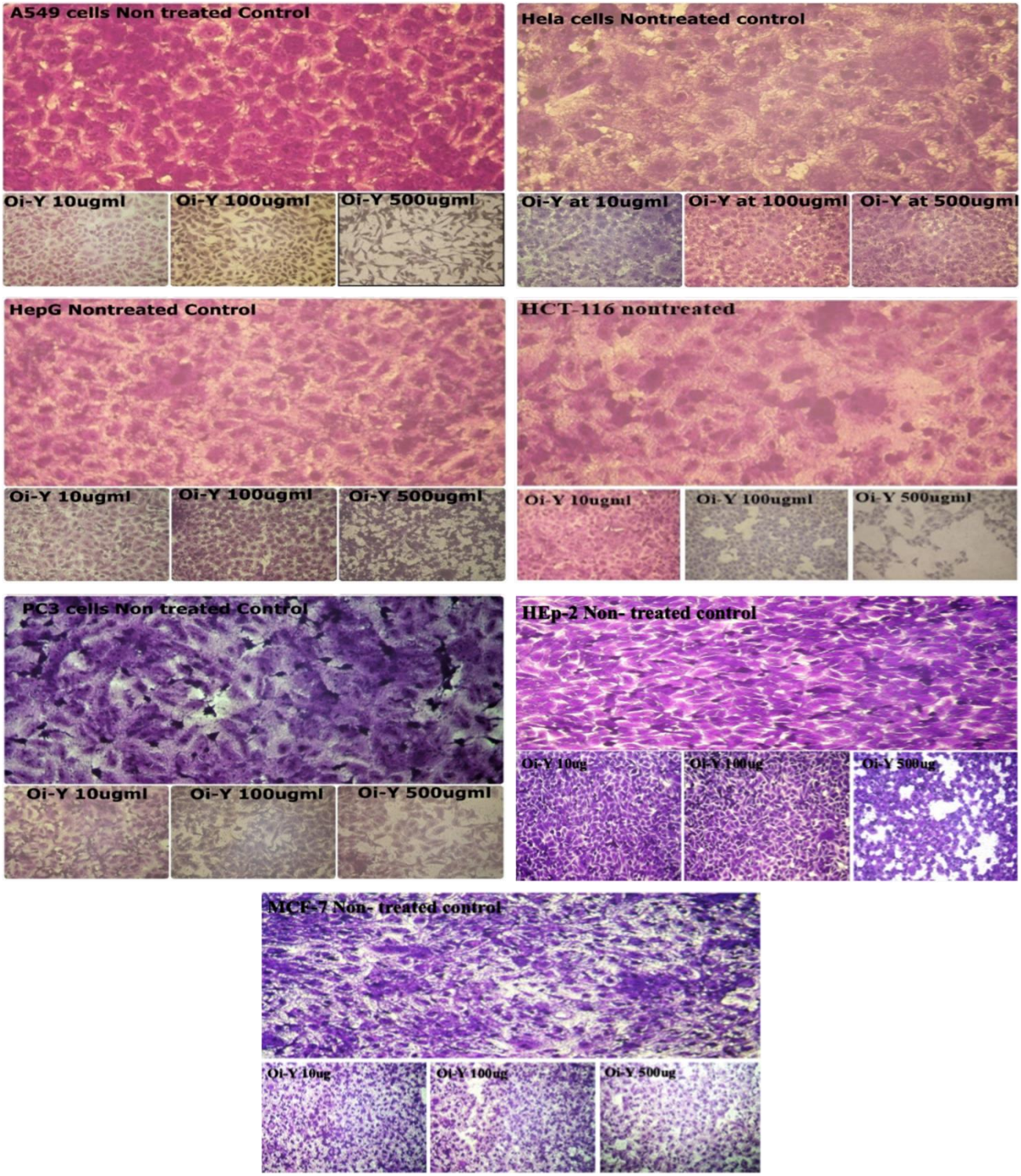


Fig. 2. Representative photomicrograph of the (400X magnification) HeLa cell line including large vascular non-treated cervical cancer cells group, little effects except condense chromatin in a few cells in all Oi-Y 10, 100 and 500 µg/mL groups, HepG-2 cell line including hepatocellular carcinoma without destruction sings in each nontreated control group, gradual loss of adhesions materials with a few vacuolar cytoplasm in both Oi-Y 10 and 100 µg/mL group and marked cell lysis in Oi-Y 500 µg/mL group, HCT-116 cell line including colon carcinoma cells without improvement changes in each non-treated, no change in Oi-Y 10 µg/mL group, gradual increase spaces and vacuolation in both Oi-Y 100 and 500 µg/mL groups, PC-3 prostate cancer cell line showing no changes in non-treated. Gradually increasing empty spaces with destructed cells in each Oi-Y 10, 100 and 500 µg/mL groups, HEP-2 human larynx epithelial carcinoma cell line showed without changes in all groups except half of cells losses and the remaining have moderate apoptotic features beside empty spaces and loses of cellular adhesions in the seam degree in Oi-Y 500 µg/mL groups, and MCF-7 breast carcinoma cells line

showing a large vascular cell with abundant chromatin in the control group, gradual increase cellular destruction in each Oi-Y 10, 100 and 500 µg/mL groups.

Table 3. Antioxidant activity of DPSE and Oi-Y using DPPH scavenging assay.

Test Sample	IC_{50} (µg/mL)		
	Mean ± SD	*t*-test	*p*-value
DPSE	66.42 ± 1.24	72.34	<0.001
Oi-Y	50.82 ± 4.38	14.59	<0.001
Ascorbic acid	13.89 ± 0.21	-	-

**P*-value is significant at <0.05 compared to reference standard (ascorbic acid), DPSE; date palm seed extract, Oi-Y; yellow oil group.

Table 4. Correlation coefficients between anticancer results (IC_{50}) against the antioxidant activity of DPSE and Oi-Y

Extract	Anticancer activity (IC_{50}%)						
DPPH	**MCF-7**	**HCT-116**	**HEPG-2**	**PC-3**	**A-546**	**HeLa**	**HEP-2**
DPSE	.6610	.960*0	.9650–	.9200–	0.983**	.7430	.7650
Oi-Y	.998**0	.952*0	.8450–	.3550	.9370–	.9940–	.3620

*&**: means correlation is significant at the 0.05 and 0.01 levels (2-tailed), respectively.

Table 5. Docking results for date palm active constituents at the ATP binding site of PI3K-d (2WXG.pdb) enzyme compared to reference ligand SW13 and at the EGFR (2RGP.pdb) compared to its co-crystallized hydrazone inhibitor

No.	Active constituent	PI3K (2WXG.pdb)		EGFR (2RGP.pdb)	
		S	**rmsd**	**S**	**rmsd**
1	Oleuropein	–8.17	1.75	–10.54	1.82
2	Apigenin-7-*O*-glucoside	–7.83	1.40	–8.87	1.95
3	Luteolin-7-*O*-glucoside	–7.98	1.22	–8.61	1.52
4	Luteolin-4`-*O*-glucoside	–7.21	1.46	–7.81	2.28
5	Chromone 1	–8.78	1.39	–10.26	1.90
6	Chromone 2	–7.50	2.05	–9.15	2.03
7	Hyperoside	–7.36	1.51	–7.78	1.27
8	Cirsilineol	– 6.76	1.10	–6.90	1.28
9	Acacetin	–6.06	1.70	–6.07	0.98
10	Isorhamnetin	–6.35	1.55	–7.24	1.69
11	Naringenin	–5.18	1.20	–6.05	1.16
12	Kaempferol	–5.89	1.68	–5.97	0.85
13	Rutin	–7.01	1.83	–8.75	2.13
14	Quercetin	–5.72	0.70	–6.16	0.83
15	Luteolin	–6.53	1.06	–6.84	1.78
16	Catechin	–5.61	1.04	–6.00	1.32
17	Homovanillic acid	–5.49	0.97	–5.60	1.84
18	Homoprotocatechuic acid	–5.11	1.85	–5.17	0.78
19	Cryptochlorogenic acid	–6.37	1.39	–5.96	1.26
20	Caffeoylshikimic acid	–6.59	1.54	–7.16	1.54
21	Vanillic acid	–5.28	1.16	–5.70	0.63
22	Sinapic acid	–5.76	0.72	–5.71	1.41
23	Gallic acid	–5.27	0.41	–5.08	1.29
24	Syringic acid	–5.58	0.75	–5.29	1.51
25	*p*-coumaric acid	–5.12	1.63	–5.33	0.84
26	Ferulic acid	–5.49	0.96	–5.56	1.02
27	Chlorogenic acid	–7.14	0.86	–7.22	1.59
28	Caffeic acid	–5.20	1.34	–5.11	1.90
29	*α*-Tocopherol	–7.40	1.73	–8.79	2.21
30	*β*-Tocopherol	–7.63	1.63	–9.27	1.55

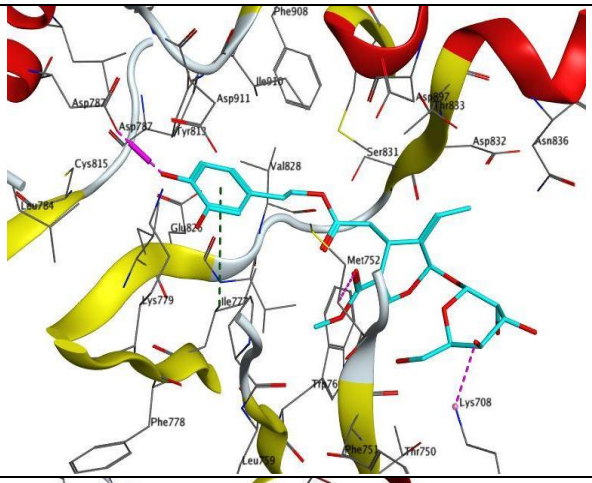
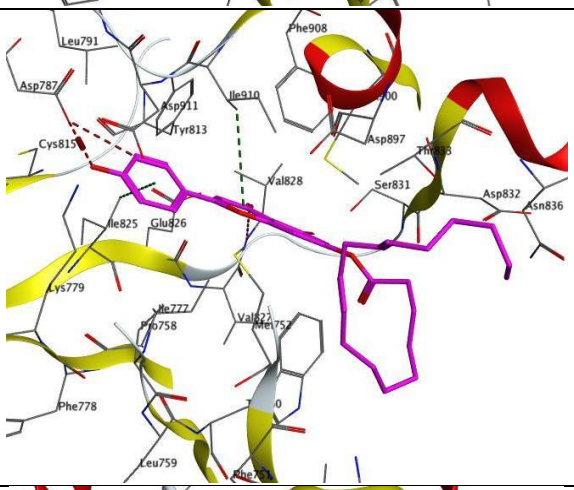
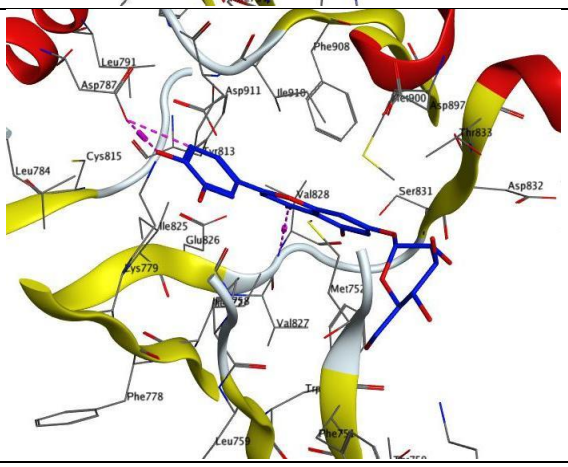
31	γ-Tocopherol	–8.36	1.31	–9.33	1.66
32	δ-Tocopherol	–7.61	1.86	–10.03	1.61
33	α-Tocotrienol	–8.53	1.18	–9.87	1.61
34	γ-Tocotrienol	–7.57	1.50	–8.26	2.37
35	δ-Tocotrienol	–7.87	2.34	–8.98	1.58
36	Caprylic acid	–5.48	0.71	–5.54	0.93
37	Pelargonic acid	–5.80	1.29	–5.91	1.33
38	Capric acid	–6.01	0.57	–6.11	1.68
39	Lauric acid	–5.83	1.82	–6.78	1.76
40	Tridecylic acid	–6.07	1.10	–7.40	1.76
41	Myristic acid	–6.85	0.84	–7.48	1.45
42	Pentadecylic acid	–6.72	0.87	–7.67	1.20
43	Palmitoleic acid	–6.71	1.15	–8.33	1.71
44	Palmitic acid	–7.09	1.43	–7.74	1.28
45	Heptadecenoic acid	–6.56	1.74	–7.83	1.59
46	Margaric acid	–6.75	1.23	–8.28	1.50
47	Linolenic acid	–6.45	1.33	–8.14	1.79
48	Linoleic acid	–6.78	1.83	–8.04	1.43
49	Oleic acid	–6.74	2.34	–8.14	1.56
50	Vaccenic acid	–6.90	1.80	–7.81	1.73
51	Stearic acid	–7.20	1.74	–8.00	1.13
52	Gondoic acid	–6.60	1.62	–8.95	1.96
53	Arachidic acid	–7.17	1.83	–8.78	1.80
54	Lignoceric acid	–6.30	1.94	–9.40	1.36
55	9,10-Epoxystearic acid	–6.62	1.61	–8.32	1.41
56	Stigmasterol	–5.51	1.56	–6.86	1.60
57	Campesterol	–6.31	1.68	–7.48	1.55
58	SW13 (co-crystalized ligand)	–9.56	1.91	-	-
59	Co-crystalized hydrazone ligand	-	-	–11.36	1.24

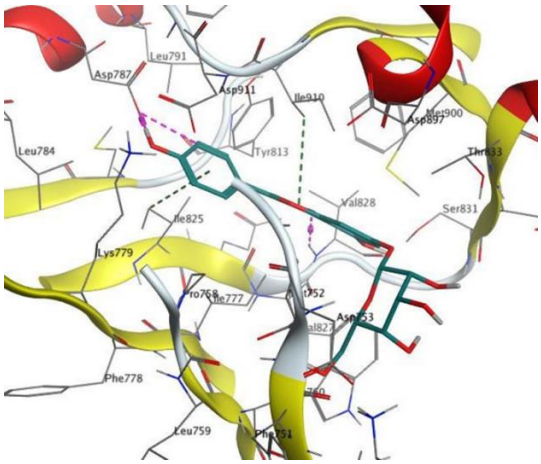
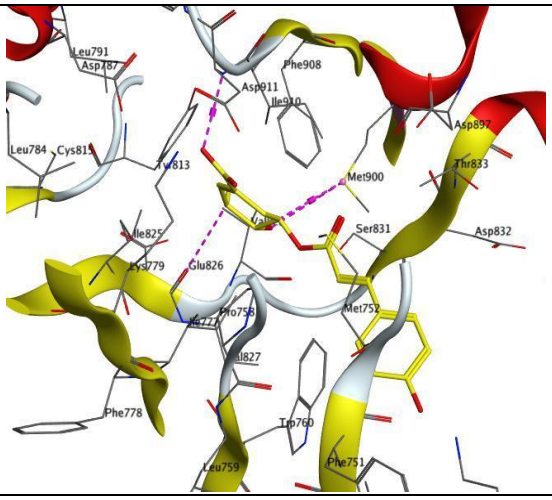
S, Binding scores; rmsd, root mean square deviation.

3.7. Docking studies at PI3K (2WXG.pdb)

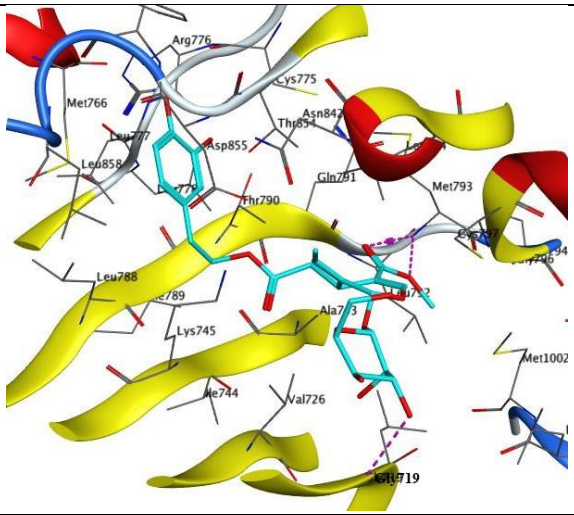
PI3K is related to the lipid kinase enzyme that regulates the cell cycle (i.e., growth, proliferation, and apoptosis) through the PI3K/AKT/mTOR pathway [49]. PI3K is considered one of the promising targets for the treatment of anticancer. It has four isomeric forms including PI3K-δ, PI3K-γ, PI3K-α, and PI3K-β [51]. It was revealed that amino acids Asp787, Glu826, and Val828 have a valuable contribution towards the stabilization of co-crystalized PI3K inhibitor (2WXG.pdb) through the formation of three HB interactions and H-arene interactions with Met752. Docking studies showed a promising binding affinity for oleuropein which formed three hydrogen bonds (HB) interactions with Lys708 (2.57 Å), Asp787 (1.88 Å), Met752 (2.34 Å), H-π interaction with Ile777 and π-π interaction with Trp760 [52] (Table 6). Also, chromone-1 revealed three hydrogen bonds; two with Asp787 (2.32, 1.96 Å), one HB with Val828 (2.64 Å) [53] and two H-π interactions with Ile825 and Ile 910 (Table 6). Interestingly, luteolin-7-O-glucoside formed two hydrogen bonds with Asp787 (1.81 Å for HO---H and 2.27 Å for OH- π HB) [54] and one HB with Val828 (2.43 Å). In addition to hydrophobic interactions with Tyr813 and Trp760. Moreover, apigenin-7-O-glucoside showed HB interactions with Asp787 (1.89 A) and Val828 (2.55 A). Also, H-π interactions with Ile910 and Ile825 in addition to Hydrophobic interaction with Trp760 (Table 6). Dactylifric acid has formed four hydrogen bonds; two of them with Met900 (2.75 Å, 3.70 Å), one HB with Asp911 (2.25 Å) [55] and one HB with Glu826 (2.47 Å) as well as hydrophobic interaction with Trp760 (Table 6).

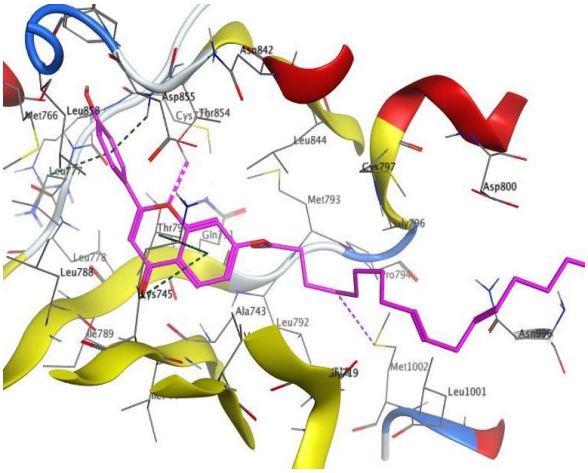
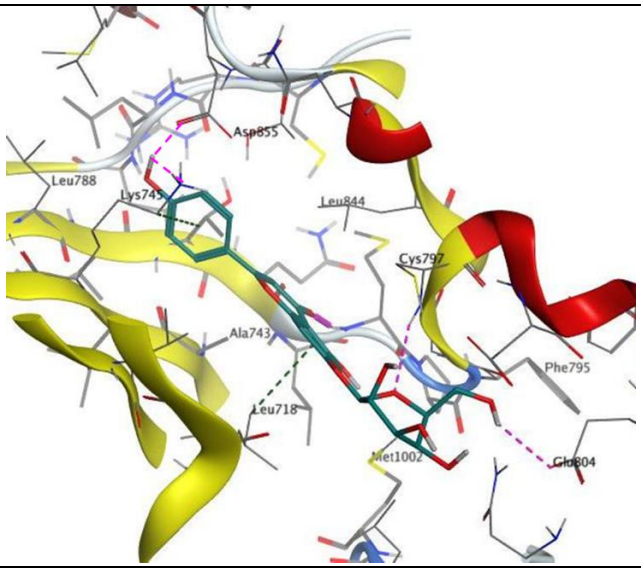
Table 6. Binding scores (S), root mean square deviation (rmsd), 3D binding interactions of selected palm extract compounds inside the binding pocket of the PI3K (2WXG.PDB) and EGFR (2RGP.pdb).

Target PI3K(2WXG)				
Molecule	**Binding Pose (3D structure)**	**S**	**rmsd**	**Contacts with amino acids**
Oleuropein		–8.17	1.75	Trp760, Thr750, Val827, Asp911, **Lys708, Asp787, Met752, Ile777**, Ile910, Lys779, Pro758
Chromone 1		–8.78	1.39	Trp760, Thr750, **Val827**, Asp911, Lys708, **Asp787**, Met752, Ile777, **Ile825**, **Ile910**, Lys779, Pro758
Luteolin-7-*O*-glucoside		–7.98	1.22	**Trp760**, **Tyr813**, Thr750, **Val827**, Asp911, Lys708, **Asp787**, Met752, Ile777, Ile825, Ile910, Lys779, Pro758

Apigenin-7-*O*-glucoside		–7.83	1.40	**Trp760**, Tyr813, Thr750, Val827, **Val828,** Asp911, Lys708, **Asp787**, Met752, Ile777, **Ile825**, **Ile910**, Lys779, Pro758
Caffeoylshikimic acid		–6.59	1.54	Trp760, Tyr813, Thr750, Val827, **Asp911**, Lys708, Asp787, **Glu826**, **Met900**, Ile777, Ile825, Ile910, Lys779, Pro758

EGFR (2RGP.pdb)

Molecule	**Binding Pose (3D structure)**	**S**	**rmsd**	**Contacts with amino acids**
Oleuropein		–10.54	1.82	**Gly719**, Val726, Lys745, Leu788, **Leu777**, Asp855, Thr790, **Met793**, Thr754, Gln791, Cys797.

Chromone 1		–10.26	1.90	Leu718, Val726, **Lys745**, Leu780, **Leu777**, Met766, **Thr854**, **Asp855**, Ala743, **Met1002**, Met793
Apigenin-7-*O*-glucoside		–8.87	1.95	**Leu718**, Val726, **Lys745**, Leu780, **Leu777**, Met766, **Glu804**, Thr854, **Asp855**, Ala743, Met793, **Cys797**

S, Binding scores; rmsd, root mean square deviation.

3.8. Docking studies at EGFR (2RGP.pdb)

EGFR or ErbB1/HER1 is a tyrosine kinase that is included in cell proliferation, progression (cyclin D-CDK4 activation) and apoptosis in various types of cancers [50]. It was revealed that both amino acids Met793 and Leu844 have a valuable contribution towards the stabilization of co-crystalized EGFR inhibitor (2RGP.pdb) through the formation of two HB interactions and H-π interaction, respectively [56]. The docked compounds showed a docking score range of –5.11 to –10.54 kcal/mol compared to the co-crystallized HYZ inhibitor (–11.36 kcal/mol) (Table 5). Herein, oleuropein (S= –10.54 kcal/mol) formed three hydrogen bonds: two hydrogen bonds with Met793 at 2.30 Å and 2.58 Å (O---H---N) and one hydrogen bond with Gly719 at 2.42 Å (H---OH) besides H-π interactions with Ile777 (Table 7). Furthermore, chromone 1 (S = –10.26 kcal/mol) formed two HB interactions with Thr854 (2.10 Å) and Met1002 (3.49 Å) besides H-π interactions with Lys745, Leu777, and Asp855 (Table 7). Apigenin-7-O-glucoside (S = –7.83 kcal/mol) showed four HB interactions Asp855 (2.74 Å), Cys797 (2.59 Å), Lys745 (3.29 Å), and Glu804 (2.53 Å) plus H-π interactions with Leu718 and Lys745 (Table 6).

4. Discussion

Uncontrolled cell growth and proliferation are characteristics of the cancer [35]. 9.6 million individuals worldwide and 18.1 million Americans lost their lives to cancer-related causes in 2018. Statistics show that if the current cancer rate does not improve, the population's demographics will alter by 2030 when the incidence of the disease reaches 21.4

million cases [57, 58]. Because chemotherapy is expensive and has several side effects, researchers are still hunting for efficient anticancer medicines derived from natural sources [59-61].

Date seed oil has a higher phenol content compared to most edible oils except olive oil, which is considered a rich source of phenolic compounds in the Mediterranean diet [32]. Date seed oil could be considered a potential source of natural phenolic compounds and serve as a good source of natural antioxidants and could potentially be considered as a functional food or food ingredient [32, 40]. In addition to their contribution to the resistance of oil to oxidative rancidity and their participation in conferring specific flavor to the oil, it is worthy to mention that some authors report that phenols could have a positive effect in the prevention of coronary heart disease and cancer [32].

Several studies evaluating the phenolic and flavonoid content of the extract and oil have confirmed the high content of these compounds [31, 62-66]. The content and level of polyphenols play a crucial role in activities reflected in the DPSE [31]. Furthermore, previous research on the anticancer activities of Phoenix dactylifera L. used digested date extract (DDE) and date polyphenol extract (DPE) to assess their anticancer activities against human colon cancer (Caco-2) cells [67]. Another study displayed that Phoenix dactylifera L. leaves hydroethanolic extract was found to be effective in inhibiting human melanoma-derived cell lines (IGR-39) at doses of 35 and 75 µg/ml [68]. Khan and his colleagues explored the underlying mechanism by which methanolic extract of Ajwa date inhibited the growth of human breast adenocarcinoma (MCF-7) at doses of 15 and 20 µg/ml [69]. A previous study reported the effect of date palm pollen (Phoenix dactylifera L.) methanolic extract against three types of human cancer cell lines, Cervical carcinoma cell line (HeLa-1), breast carcinoma cell line (MCF-7) and intestinal carcinoma cell line (CACO). Very strong activities were recorded against the three human cell lines with IC50 3.68 µg/ml (HeLa-1), 4.88 µg/ml (CACO), and 5.00 µg/ml (MCF-7) [70].

The objective of this study is to evaluate the anticancer and antioxidant properties of DPSE and Oi-Y, which are grown in Hadramout, Yemen. The suggested anticancer mechanism of action was explored by a molecular docking study targeting PI3K and EGFR. As a result, in the current study, we examined innovative, natural, risk-free, affordable, and generally accessible extracts and oils from date palm seeds for their antioxidant and anticancer effects as DPSE and its oil (Oi-Y) using the standard antineoplastic medication with wide anticancer activity (doxorubicin) against seven cell lines were chosen. In exploring the results of antioxidant activities reflected by DPSE and Oi-Y, our results showed the strong in-vitro antioxidant activity of Oi-Y and DPSE with IC50 50.82 and 66.42 µg/ml, respectively, compared to ascorbic acid.

The results of the in-vitro anticancer study showed that, in a concentration-dependent manner, DPSE and Oi-Y greatly slowed the proliferation of several cancer cell lines, including HepG-2, HT-116, MCF-7, A-549, PC-3, HEp-2, and HeLa. The results of the current investigation showed that Oi-Y had the strongest anticancer activity, with the potent anticancer activity recorded against HepG-2 (IC50 = 11.18 µg/ml) and MCF-7 (IC50 = 18.60 µg/ml). Oi-Y showed significant anticancer activity against HepG-2, MCF-7, HCT-116, and A-549, while HeLa, PC-3, and HEP-2 showed modest anticancer activity. Oi-Y displays approximately 42.30%, 42.82%, 44.11%, and 54.48% activity against HEP-2, HepG-2, A-549, and HeLa, respectively, when compared to the reference medication (doxorubicin).

The present study results showed that the anticancer effects of Oi-Y were more potent than DPSE. DPSE had anticancer effects that varied from modest to moderate, except for HeLa and HEP-2, which showed weak anticancer activity with IC50 values of 51.12 and 55.34 µg/ml, respectively, DPSE showed moderate anticancer activity in other cell lines with IC50 values ranging from 28.81 to 47.53 µg/ml. According to the extracts' IC50 values, the strength of cytotoxic action was divided into four categories: extremely strong (1-10 µg/ml), strong (11-20 µg/ml), moderate (21-50 µg/ml), weak (51-100 µg/ml), and greater than 100 µg/ml (non-cytotoxic) [33, 71]. Oi-Y was found to have more intriguing cytotoxic effects on seven cell lines than DPSE. Of all the cell lines investigated, the HepG-2 cell line was the most susceptible one.

To explore the anticancer mechanism of action, a molecular docking study was performed targeting two important pathways for cancer progression such as PI3K and EGFR which are involved in the pathogenesis of many cancer types. According to molecular docking study results, we found important

active compounds with structure-activity relationship to active target sites as luteolin-7-O-glucoside, apigenin-7-O-glucoside, oleuropein, chromone-1 and dactylifric acid. Our results demonstrated that luteolin-7-O-glucoside, apigenin-7-O-glucoside, and dactylifric acid had an inhibitory effect on the PI3K target site while oleuropein, chromone-1, and apigenin-7-O-glucoside showed an inhibitory effect of EGFR target site. This is in harmony with previous studies' results of the luteolin-7-O-glucoside [72], apigenin-7-O-glucoside [73], oleuropein [74, 75], chlorogenic acid [76], and dactylifric acid [55].

5. Conclusion

The current study presents the in-vitro antioxidant and anti-proliferative activity of Yemeni date palm (Phoenix dactylifera L.) seed extract and its oil. This research has demonstrated a promising anticancer activity of DPSE and Oi-Y against the HepG-2, HCT-116, A-549, PC-3 (prostate cancer), HEP-2, HeLa, and MCF-7 cell lines (Graphical abstract). The obtained docking results clarify that date palm seed extract and its oil can act on cancer cell lines via multiple mechanisms of action, providing a clue that date palm seed can be used as a promising anticancer agent besides its antioxidant activity. This study indicated promising in-vitro antioxidant and anticancer activities of DPSE and Oi-Y With a stronger effect of Oi-Y against seven cell lines. Date palm seed extract and its oil are promising source of anticancer active constituents such as luteolin-7-O-glucoside, apigenin-7-O-glucoside, oleuropein, chromone 1, and dactylifric acid. This reflected that date palm seed had the potential as a novel source of anticancer agents.

Future perspective

In the future, in vivo studies and pharmacokinetics will be carried in addition to formulation of this extract and its oil.

Data Availability

The data supporting this study are available on a request from the corresponding author upon request.

Conflict of Interest Statement

This work has not been published previously and also is not under consideration for publication elsewhere. All the authors agree to the content of the paper.

Credit Authorship Contribution Statement

A.E.: Conceptualization; A.E., A.E.R.: Collection, preparation and the phytochemical analysis; G.M.E., N.M.I.M.E.: The biological studies; A.S.A.: Molecular docking study; N.M.I.M.E.: Statistic analysis; N.A.A.: Cytological and microscopic examination; N.M.I.M.E., A.E.R, A.S.A, G.M.E.: Writing original draft, editing and revision; All authors discussed, revised and approved the submitted final version.

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