Supplementary Material

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

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

About 500 g of the dried powdered seed of hadrami date palm (*Phoenix dactylifera* L.) were extracted exhaustively with 3 L of *n*-hexane and the filtrates were evaporated under reduced pressure by using a rotary evaporator (Buchi Rota-Vapour R-200, Serial No. 0500009474, Germany) yielding a concentrated crude *n*-hexane extract and then stored in a dark container in a deep-freeze (-20°C) for the future use. The date palm oil (Oi-Y) is leached out seed extract which is separated and purified by filtration. Oi-Y was made with a yield percentage of roughly 5%, it took about 25 mL. The date palm seed powder (500 g) was extracted exhaustively with 5 L of methanol and the filtrates were concentrated by using a rotary evaporator to get a crude dark brown seed extract.

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. The tested human carcinoma cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were grown on RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum, 1% L-glutamine, and 50 µg/mL gentamycin. The cells were maintained at 37 ºC in a humidified atmosphere with a 5% CO_2 incubator (Shel lab 2406, USA) and were sub-cultured two to three times a week.

2.2.1. Experimental design

Seven cell lines tested in this study were used to evaluate the antineoplastic effects of DPSE and Oi-Y. All the cells were treated with different concentrations (1.56-50 µg/ml) of extract for 24 h. After treatment, the anti-proliferative activities were determined using a 3-(4,5-dimethylthiazol-2-yl),5-biphenyl tetrazolium bromide (MTT) assay. Untreated control sets were run under identical conditions. All the experiments concerning the cytotoxicity evaluation were performed and analyzed by the tissue culture unit at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

2.2.3. Cytological examination

For antitumor assays, the tumor cell lines were suspended in the medium at a concentration of 5.0×10^4 cells/well in Corning® 96-well tissue culture plates and then incubated for 24 hr. The tested compounds were then added to 96-well plates (six replicates) to achieve seven

concentrations for each compound. Six vehicle controls with media or 0.5 % DMSO were run for every 96 well plates as a control. After incubating for 24 hr, the number of viable cells was determined by the MTT test. Briefly, the media was removed from the 96 well plates and replaced with 100 µL of fresh culture RPMI 1640 medium without phenol red then 10 µl of the 12.0 mM MTT (Sigma) stock solution (5.0 mg of MTT in 1 mL of PBS) to each well including the untreated controls. The 96 well plates were then incubated at 37 °C and 5% CO_2 for four hr. An 85 µl aliquot of the media was removed from the wells, and 50 µl of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37 °C for 10 min. Then, the optical density was measured at 590 nm with the microplate reader (Sunrise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as; [1-(ODt/ODI)] × 100%. Where it is the mean optical density of wells treated with the tested sample and ODI is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC_{50}), 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].

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

This experiment was performed as previously described in the procedure for antitumor activity. After the end of the treatment, the plates were inverted to remove the medium, the wells were washed three times with 100 µl of phosphate-buffered saline (pH of 7.2) and then the cells were fixed to the plate with 10% formalin for 15 min at room temperature. The fixed cells were then stained with 100 µl of 0.25% crystal violet for 20 min. The stain was removed and the plates were rinsed using deionized water to remove the excess stain and then allowed to dry. 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.

2.3. DPPH radical scavenging activity

Freshly prepared 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical in methanol (0.004%, w/v) solution was prepared and stored at 10 °C in the dark. 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 measurements were recorded immediately with a UV-visible spectrophotometer (Milton Roy, Spectronic 1201). The decrease in absorbance at 515 nm was determined continuously, with data being recorded at 1 min intervals until the absorbance stabilized (16 min). The absorbance of the DPPH radical without antioxidant (control) and the reference compound ascorbic acid were also measured [36]. All the determinations were performed in three replicates and averaged. The percentage inhibition (PI) of the DPPH radical was calculated according to the formula:

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

Where AC is the absorbance of the control, and AS is the absorbance of the sample [36]. The IC_{50} is defined as the concentration required for 50% DPPH radical scavenging activity and was estimated from graphic plots of the dose-response curves using GraphPad Prism software (San Diego, CA, USA). Ascorbic acid was used as the standard [17].

2.5. Statistical analysis

The mean ± SD was used to summarize and show the data. An Independent Student's *t*-test was used to conduct the significance of the association between extract and reference drug at different concentrations using SPSS program version 21. Two way ANOVA test was used for statistical analysis of differences between groups. 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 IC_{50} values were calculated from linear regression analysis. Results for DPPH and anticancer activity were analyzed by Pearson correlation coefficient.

Table S1. Semi-quantitative grading system of cellular proliferative features among cell line types.

Cell line	Group	Loss of Cellularity	Loss of Adhesions	Chromatin Condensation	Chromatin Fragments	Apoptotic Bodies	Membrane Blebbing
A-549	**Control**	-	-	+	-	-	-
	Oi-Y 10	+	+	+	+	+	-
	Oi-Y 100	++	++	++	++	+++	+
	Oi-Y 500	+++	+++	++	+++	+++	++
HeLa	**Control**	-	-	+	-	-	-
	Oi-Y 100	+	+	++	-	-	-
	Oi-Y 500	+	+	++	-	+	+
HepG-2	**Control**	-	-	-	-	-	-
	Oi-Y 10	+	+	+	+	+	+
	Oi-Y 100	+	++	+	+	++	+
	Oi-Y 500	+++	+++	+++	+++	+++	++
HCT-116	**Control**	-	-	-	-	-	-
	Oi-Y 10	-	-	+	+	+	-
	Oi-Y 100	++	++	++	++	++	++
	Oi-Y 500	+++	+++	+++	+++	+++	+++
PC-3	**Control**	-	-	-	-	-	-
	Oi-Y 10	+	+	+	+	+	+
	Oi-Y 100	++	++	++	++	++	++
	Oi-Y 500	+++	+++	+++	+++	+++	+++
HEP-2	**Control**	-	-	-	-	-	-
	Oi-Y 10	-	-	-	-	-	-
	Oi-Y 100	-	-	-	-	-	-
	Oi-Y 500	++	++	++	++	++	-
MCF-7	**Control**	-	-	-	-	-	-
	Oi-Y 10	-	-	+	-	+	-
	Oi-Y 100	+	+	++	-	+	-
	Oi-Y 500	+	+	++	-	+	+

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, -: no alterations, +: mild to moderate, ++: moderate, +++: severe up to 70%).

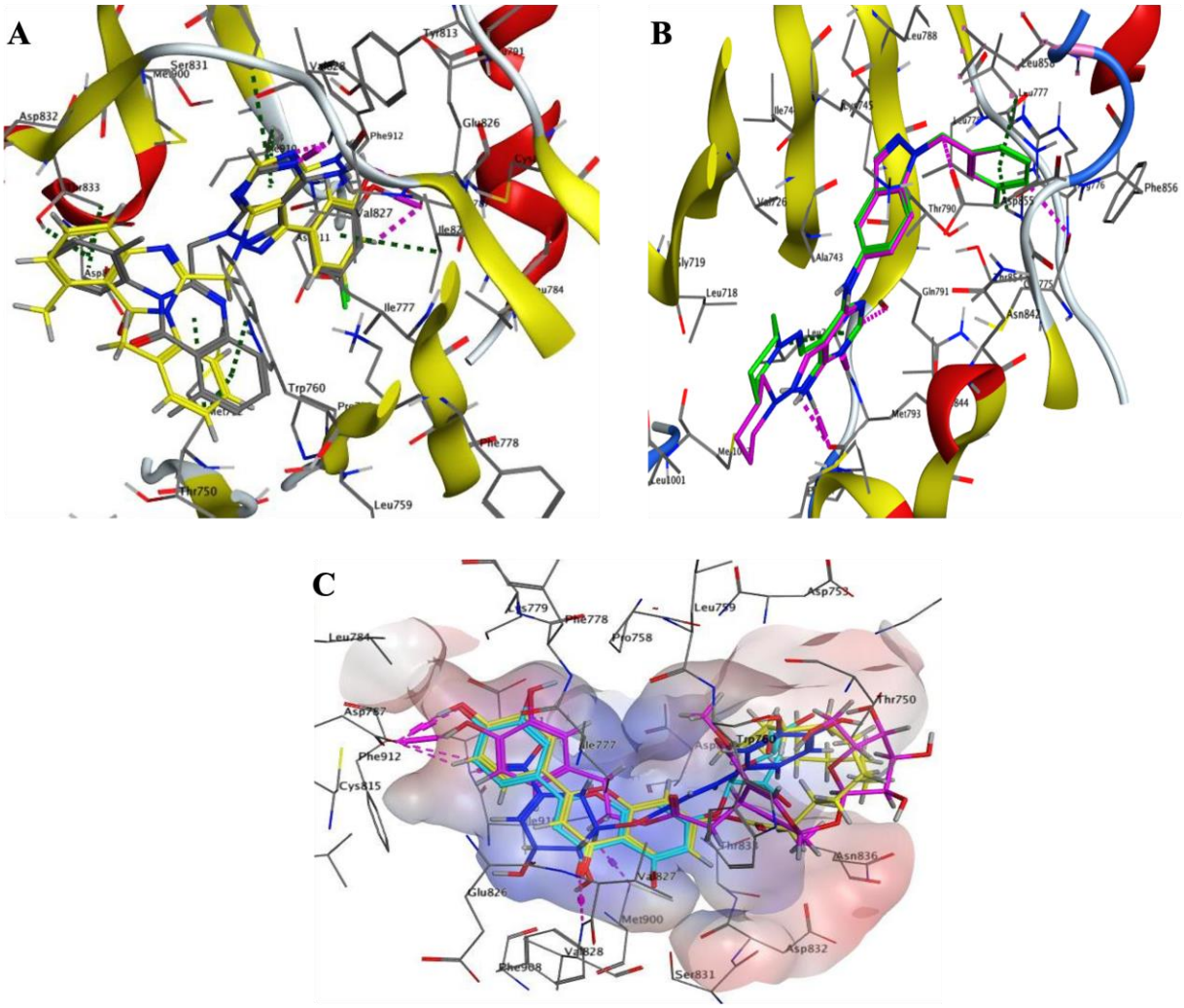
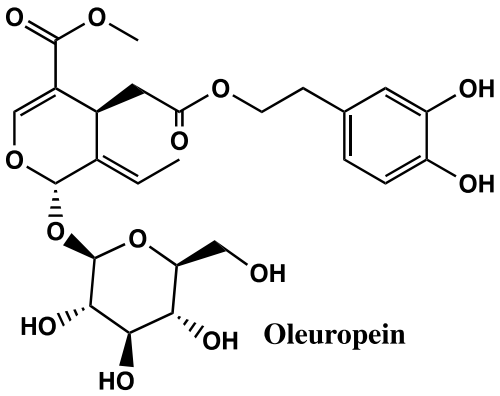
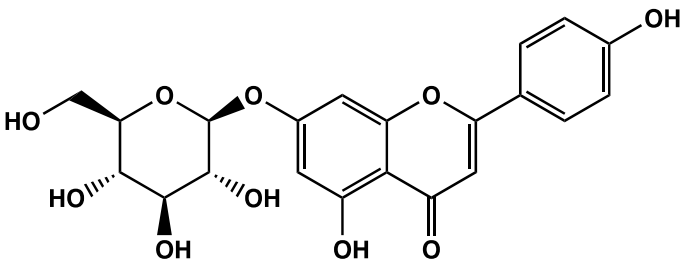


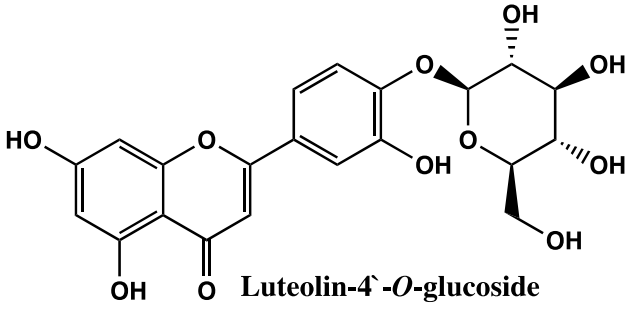
Figure S1. Docking validation of co-crystalized ligands: **A**. Grey color stand for the x-ray co-crystal SW13 and yellow denotes after redocking with PI3K (RMSD = 1.91 Å). **B**. Green color stand for the x-ray co-crystal HYD inhibitor and magenta denotes after redocking with EGFR (RMSD = 1.24 Å). **C**. Selected ligands fit to PI3K ATP site.



Oleuropein



Apigenin-7-*O*-glucoside



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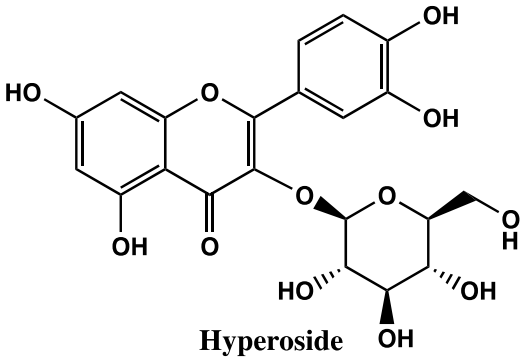
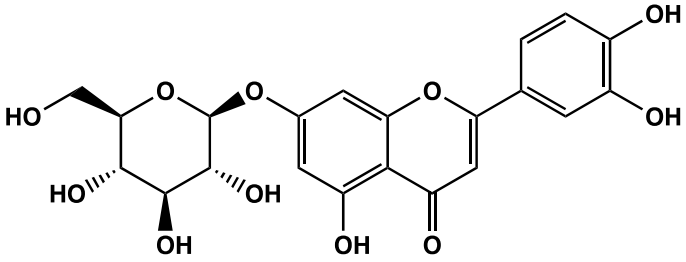


Hyperoside



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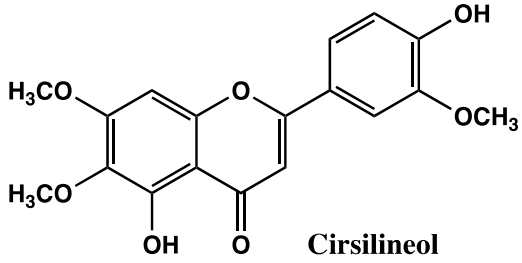
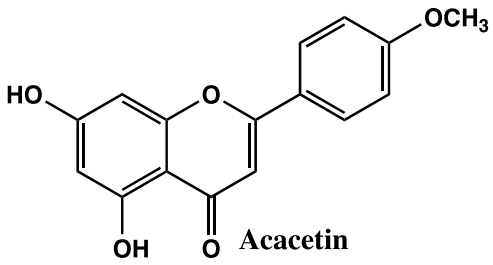
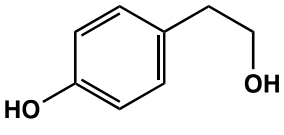


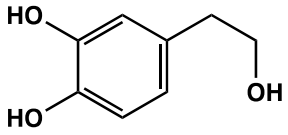
Cirsilineol



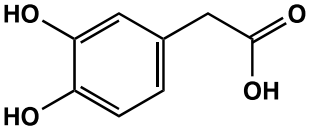
Acacetin



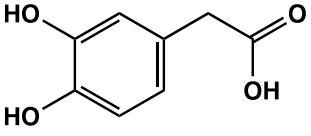
Tyrosol



Hroxyyrosol



Homoprotocatechuic acid



Homovanilic acid

Figure S2. Phenolic compounds reported in the date palm seed oil.

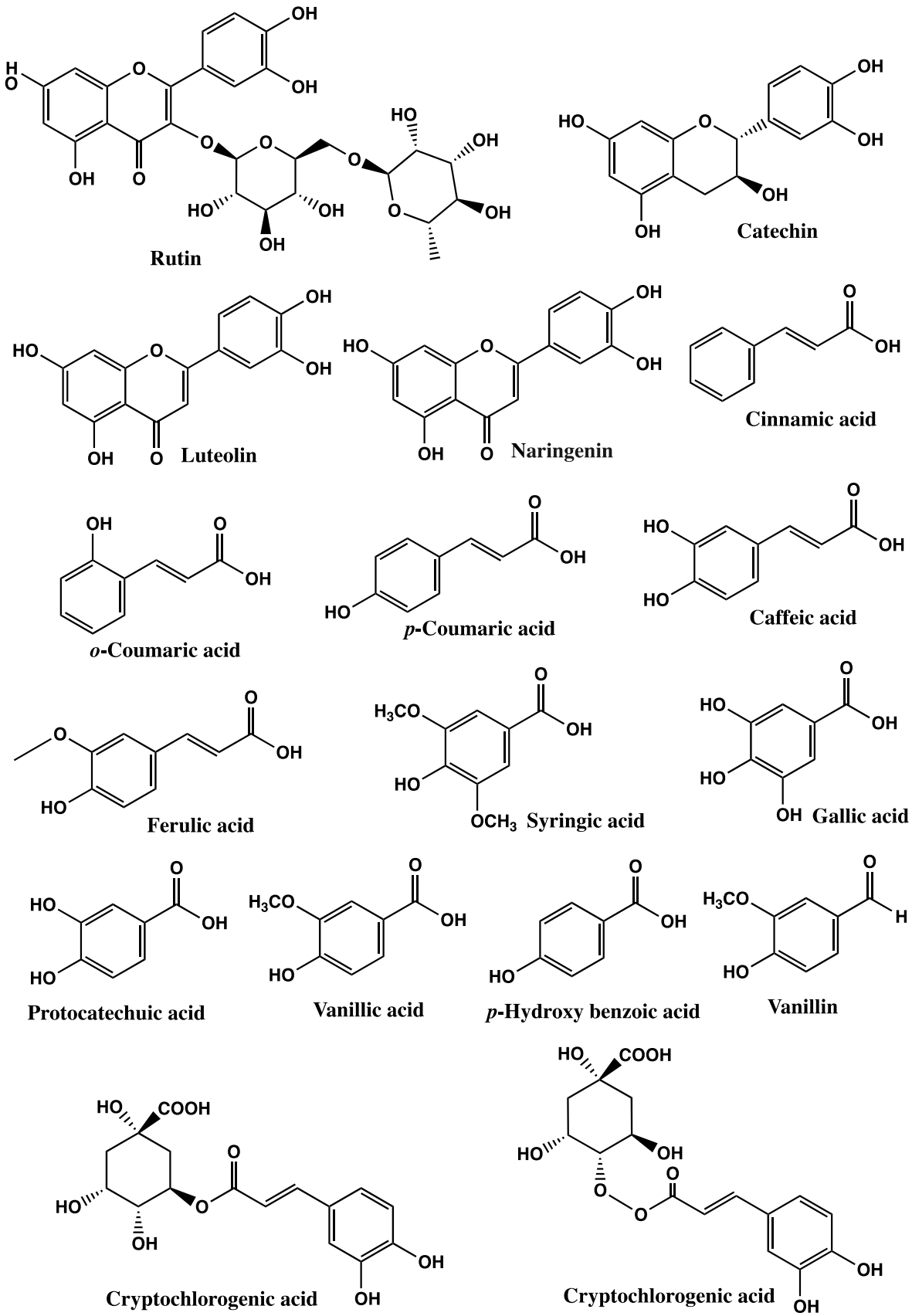


Figure S3. Phenolic compounds reported in the date palm seed oil and total extract.

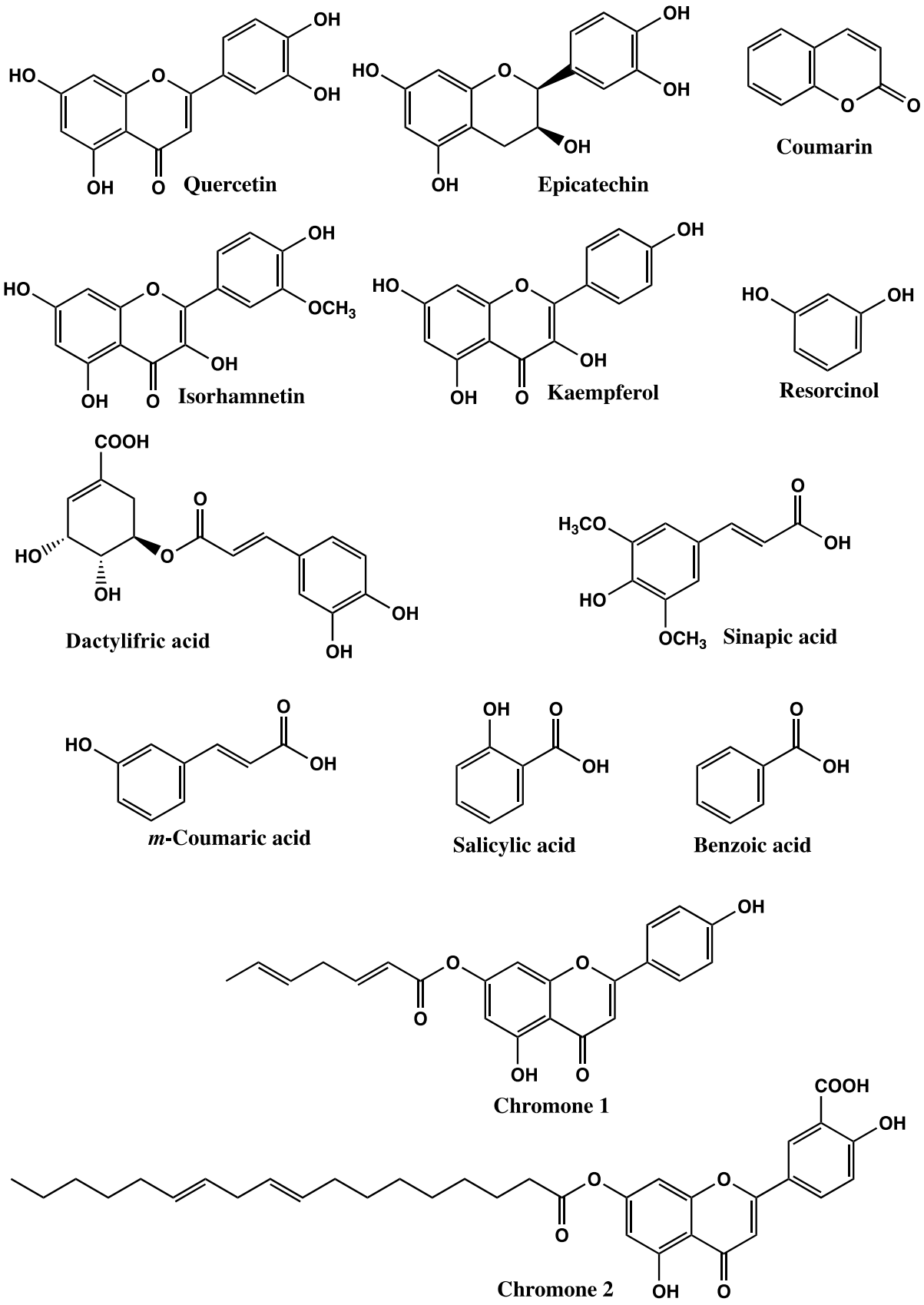


Figure S4. Phenolic compounds reported in the date palm seed total extract.

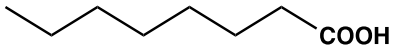
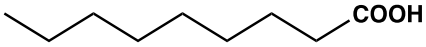
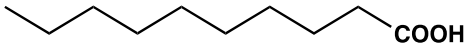
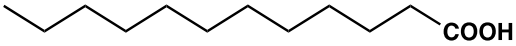
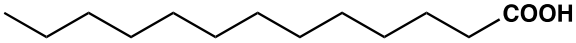
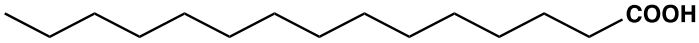
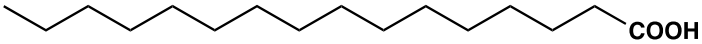
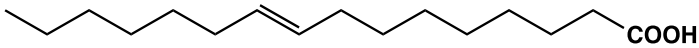
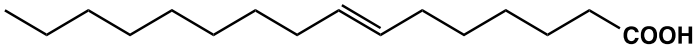
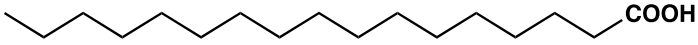
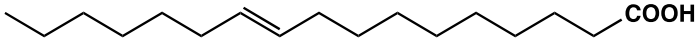
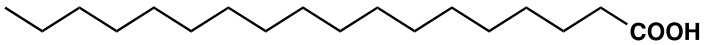
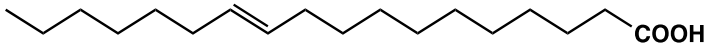
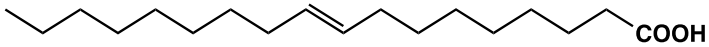
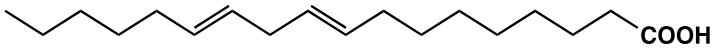
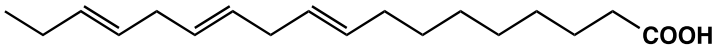
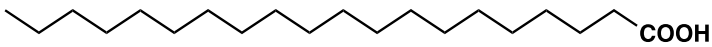
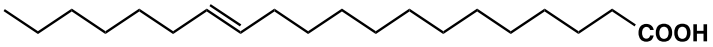
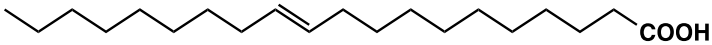
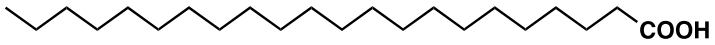
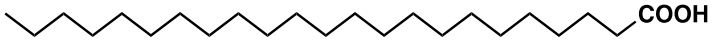
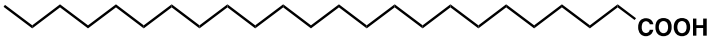
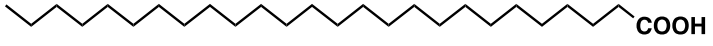
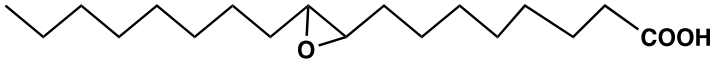
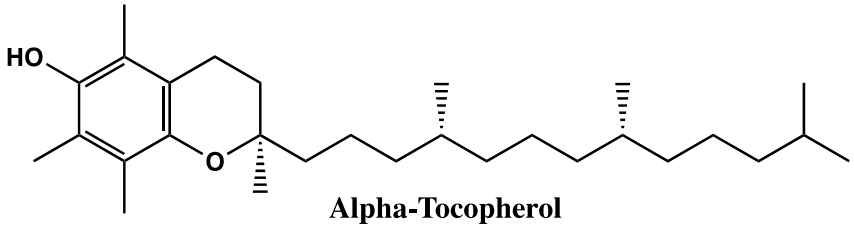
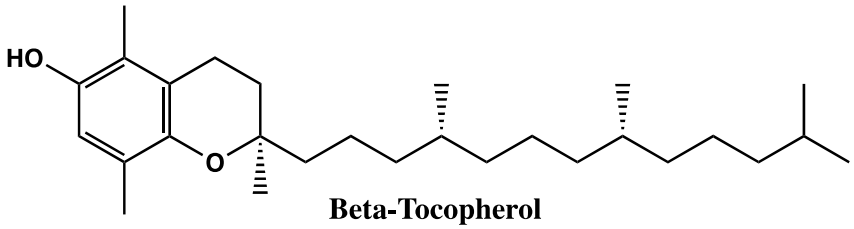
ω-characteristics	Structure	Δ-characteristics
Caprylic 8:0		C8:0
Pelargonic 9:0		C9:0
Capric 10:0		C10:0
Lauric 12:0		C12:0
Tridecylic 13:0		C13:0
Myristic 15:0		C15:0
Palmitic 16:0		C16:0
Palmitoleic 16:1, ω-7		C16:1 Δ9
Hexadecenoic 16:1, ω-9		C16:1 Δ7
Margric 17:0		C17:0
Heptadecenoic 17:1, ω-7		C17:1 Δ10
Stearic 18:0		C18:0
Vacenic 18:1, ω-7		C17:1 Δ11
Oleic 18:1, ω-9		C18:1 Δ9
Linoleic 18:2, ω-6		C18:2 Δ9, 12
Linolenic 18:3, ω-3		C18:3 Δ9, 12, 15
Arachidic 20:0		C20:0
Eicosenoic 20:1, ω-7		C20:1 Δ13
Gondoic 20:1, ω-9		C20:1 Δ11
Behenic 22:0		C22:0
Tricosylic 23:0		C23:0
Lignoceric 24:0		C24:0
Cerotic 26:0		C26:0
9,10-Epoxystearic 18:0		C18:0

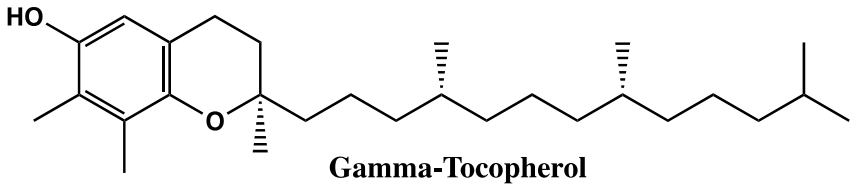
Figure S5. Fatty acids reported in the date palm seed oil.



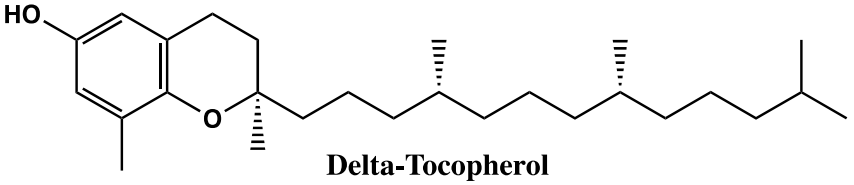
Alpha-Tocopherol



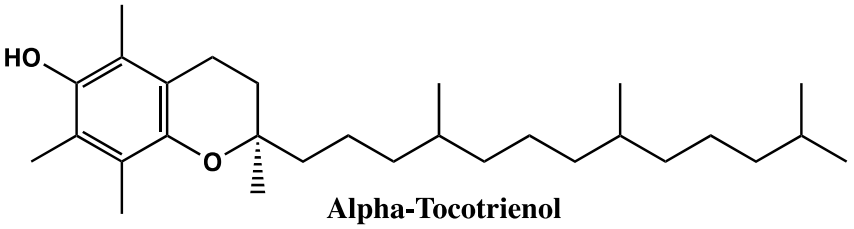
Beta-Tocopherol



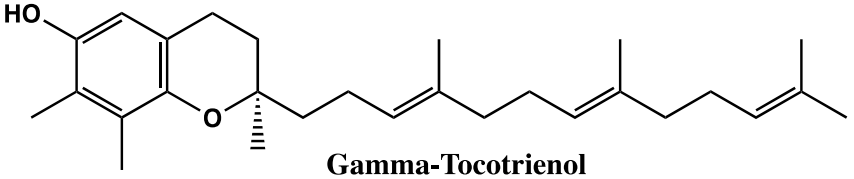
Gamma-Tocopherol



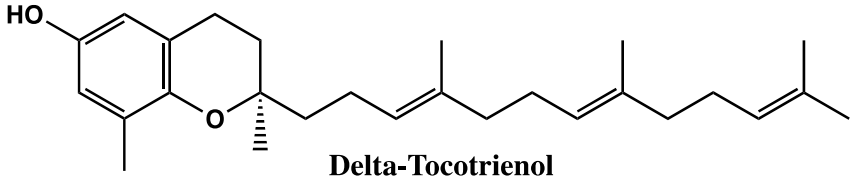
Delta-Tocopherol



Alpha-Tocotrienol



Gamma-Tocotrienol



Delta-Tocotrienol

Figure S6. Vitamin E isomers reported in the date palm seed oil.

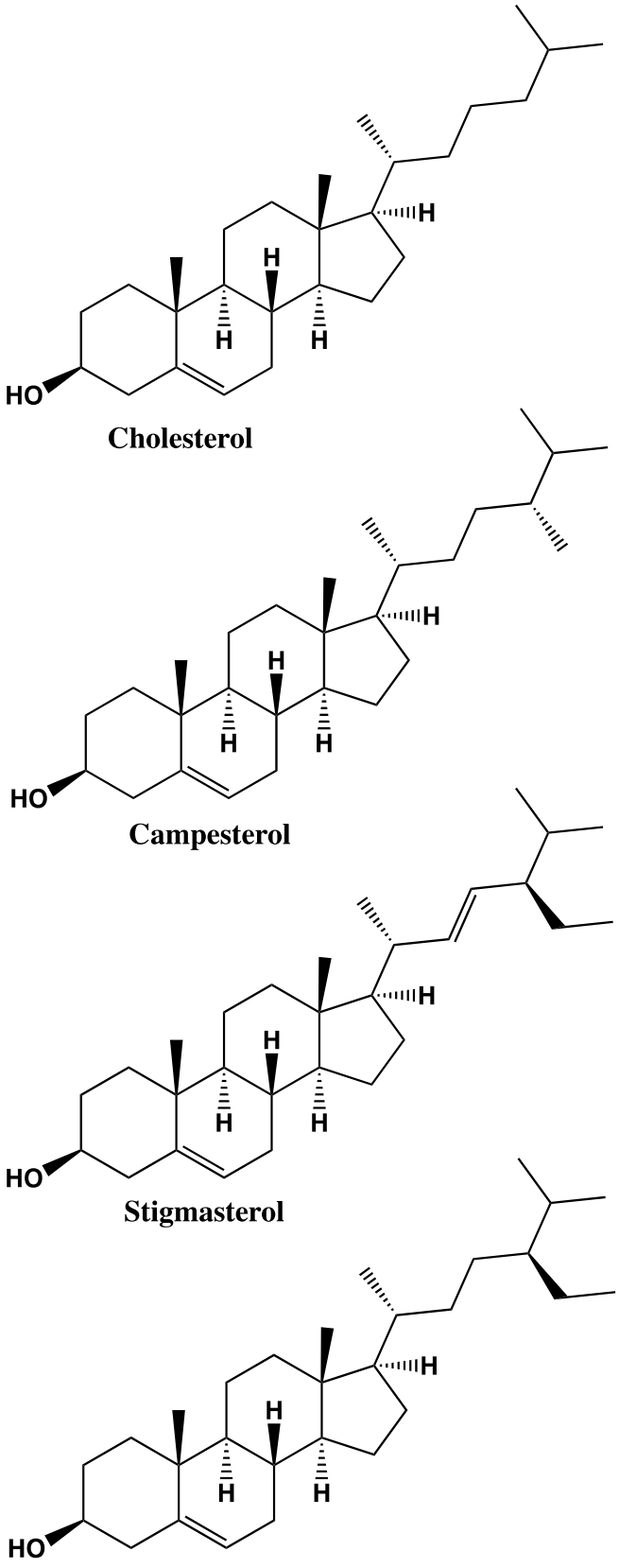


Figure S7. Sterols reported in the date palm seed oil.