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Soxhlet-Extracted Date Kernel Oil from *Phoenix dactylifera* L.: Physicochemical Characterization and Ultra High-Performance Liquid Chromatography Phytochemical Profiling with Antioxidant and Selective Antimicrobial / Antifungal Activities

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Abstract

Date kernel is considered a waste product very rich in proteins, fibers and phenolic compounds with antioxidant, antibacterial, anti-inflammatory and antidiabetic properties. Oil was extracted from powdered kernels by hexane using Soxhlet. Physicochemical characterization was defined through index measurements, ultraviolet and gas chromatography. However, phyto-chemical composition was defined through total phenolic content, ultra high-performance liquid chromatographyand antioxidant capacity (1,1-diphenyl-2-picrylhydrazyl / ferric reducing antioxidant power). The evaluation of the antibacterial activity was carried out by the method of diffusion on a disc with cultures of 18-24 h at 37 °C in sterile distilled water and the measurement of the inhibition zones with a ruler relative to seven reference strains from the Pasteur Institute. Hence, hexane-extracted date kernel oil exhibited acceptable physicochemical characteristics (9.25% yield), with the high content of oleic acid and a unique phytochemistry profile characterized by phenolic acids and flavonoids, suggesting good antioxidants properties. It showed significant antibacterial activity against Gram-positive *Staphylococcus aureus* and *Bacillus cereus* and antifungal activity against *Candida albicans* (*ATCC 26790*). Lastly, date kernel oil comprises an Oleic-rich lipid matrix with bioactive phenolic constituents, which renders appreciable antioxidant and selective antibacterial effects featuring phytochemicals promising for functional food and natural antimicrobial purposes.

INTRODUCTION

Date palm provides substantial agricultural residues, including date kernels, which are often considered as waste despite their high lipid and phenolic contents. Valorization in the agro-industrial sector has the potential to reduce waste from by-products, yield valuable edible oils and bioactive extracts. Date kernel oil (DKO) exhibited important antioxidant, antibacterial and antifungal activities, with a capacity to inhibit certain foodborne pathogens and Gram-positive bacteria whilst showing high radical-scavenging potential in 1,1-diphenyl-2-picrylhydrazyl and ferric reducing antioxidant power (DPPH / FRAP) tests that would affirm its ability as a functional ingredient capable of functioning as a natural preservative [1]. Relevant to this, DKO has been identified as a potentially high-quality oil derived from an underexploited source that can be used in food, cosmetic and nutraceutical applications. Currently, most studies on *Phoenix dactylifera* kernel oil focus on solvent-based extraction techniques creating an oil yield of 6–12% depending on the cultivar and conditions used in extraction like Soxhlet [2]. The use of the principles of the circular economy, the recovery of residual waste in the agri-food sector and the more sustainable use of agro-industrial biomass by converting date kernels into oil not only contribute to reducing waste, but also to creating local business opportunities and adding value to date production chains via the recovery of functional oils and bioactive ingredients [3]. DKO and extracts have been subjected to analysis resulting in identification of phenolic acids, flavonoids, sterols, and tocopherols which are bioactive compounds from the analytical point of view. These compounds are directly associated with antioxidant and potential anti-inflammatory activity thus increasing the functional interest of the oil [4]. Physicochemical characteristics such as low acidity, peroxidation value with relatively moderate RI of DKO, also measured saponification and IV of DKO reflects potential merits in food and cosmetics

application. Despite this, integrated studies that combine detailed phytochemical analysis with physicochemical and fatty acid characterization followed by biological activity assessment are still limite especially for Algerian date varieties from the same region thus the actual work seeks to extract oil by Soxhlet using hexane followed to its composition, physicochemical properties and antioxidant and antibacterial activities evaluation within the valorization framework of date–kernel residues in agro-food industry [5]. The chemical composition of DKO is characterized as a balanced content of the saturated and unsaturated fatty structure with oleic acid (C18:1 n-9) being the major unsaturated fatty acid component, followed by significant proportions of lauric, myristic and palmitic acids. This type of profile imparts the stability associated with saturated fatty acids and health benefits related to monounsaturated fats which makes DKO an appealing option for lipid-rich food systems [6]. The aims of this research work are to extract DKO from powdered kernels within the Soxhlet using hexane, quantify its yield and characterize the extracted oil in terms of some physicochemical properties include refractive index, peroxide index, acid index, iodine index, saponification index as well as ultraviolet absorbance (UV) in order to determine oil quality and oxidative stability. Additionally, to be characterised the phytochemical composition (polyphenols, flavonoids, condensed tannins and carotenoids), identification of bioactive compounds by ultra high-performance liquid chromatography (UHPLC), and determination of fatty acids profile by gas chromatography focused on quantification of relative proportion between saturated and unsaturated fatty acid especially oleic, lauric, palmitic as well myristic acid linoleic and stearic acids. Lastly, the aims of this study were to determine the antioxidant capacity of oil using DPPH and FRAP assays and to evaluate antibacterial and antifungal activities against selected Gram-positive and Gram-negative bacteria and Candida albicans strains, suggesting that hexane-extracted DKO may serve as a potential functional food ingredient or nutritional antimicrobial agent.

MATERIALS AND METHODS

Preparation of Sample and Extraction of DKO

Date palm tree (*Phoenix dactylifera* L.) is a traditional tree for producing fruit in arid areas the date kernel used in this study was collected from Biskra province located in the northeastern of Algeria (Arid region). The kernels were soaked in water, rinsed to remove inert date tissue and air-dried before testing. They were then oven dried for 24h at 60°C, ground and sieved through a 1-mm mesh sieve and stored at −20°C until used. This study investigates extraction method for DKO using Soxhlet with n-hexane as the solvent to do solid–liquid extraction. For this purpose, 30 g of ground kernels were added to an extraction thimble and subsequently placed in a Soxhlet extractor (SOXTHERM, Gerhardt, Germany) as per the process reported by Mrabet [7]. During this time, 250 mL of n-hexane was pumped into the system at reflux state for 6 h, by gradually increasing the temperature to 180°C corresponding with optimum conditions. The solvent was evaporated by rotary evaporation under vacuum at 40–50 degree temperature after extraction. The obtained oil was filtered, transferred into an amber glass vial, and stored at 4°C until phytochemical analysis and evaluation of its antioxidant properties were performed.

Phytochemical Screening of DKO

A phytochemical screening of DKO was conducted to detect the presence of various classes of secondary metabolites. Results were interpreted using the following qualitative scale: +++ (strongly positive), ++ (moderately positive), + (weakly positive), − (negative), and ND (not determined) [8]. Total phenolics were detected by adding a few drops of 2% (w/v) alcoholic ferric chloride ($FeCl_3$) solution to 2 mL of extract. The formation of a color ranging from dark blue–black to green indicated the presence of polyphenolic compounds, with color intensity proportional to polyphenol concentration. This reaction is specific to polyphenolic derivatives [9]. Flavonoids were identified by treating 1 mL of extract with a few drops of concentrated hydrochloric acid (HCl) and 0.5 g of magnesium turnings, followed by 3 minutes of incubation, pink or red color appearance is the sign of flavonoids presence [10]. Ored condensing tannins by adding several drops of 1% $FeCl_3$ solution (dissolved in methanol) to 1 mL of extract and color change after shaking. Gallotannins are indicated by a color shift from green to dark blue, and catechic tannins produce a characteristic brown–green tint that allows the two types of tannin to be differentiated based on the spectra [11].

Physicochemical Properties of DKO

DKO was physiochemically characterized using recommended analytical methods. For refractive index (RI), was measured at 40°C according to NE ISO 6320 (2017). In short, a filtered oil sample was dropped between the prisms of a refractometer that had been calibrated at 20°C and allowed to reach thermal equilibrium; the RI measured through its glass faces provided information on oil purity and identity. Acid value (AV) was determined according to NF EN ISO 660 (1999) and expressed in mg of KOH required to neutralize free fatty acids contained in 1 g of oil. For this analysis, 1 g of oil was diluted with a 50:50 (v/v) mixture of ethanol and diethyl ether, phenolphthalein was added as an indicator, and the solution was titrated with 0.05 N alcoholic KOH until the alloying point turned pink for at least fifteen seconds. The peroxide value (PV), a measure of primary oxidation, was determined in accordance with method NE 1.2–50 (1985). Two grams of the oil was weighed, dissolved in acetic acid and chloroform, and: subsequently 1 mL of potassium iodide (KI) solution is added. The released iodide was then titrated in the dark with 0.002 N sodium thiosulphate (Na2S203) using starch solution as indicator after an incubation time. The content of unsaturation was analyzed by measurement of the iodine value (IV) according to the Wijs procedure (NE I.2–96, 1988). Previous studies had already revealed the nature of these chemical bands between adsorbed oil and silica, their dependence on solvent types and molecular configurations,78–80 which indicated that a particular mole number of carbon atoms (Cm) was needed to chemically dissolve a known mass of oil in carbon tetrachloride followed by reaction with iodine monochloride (ICl) in acetic acid (Wijs reagent). After 2 h, KI was added to release excess iodine, followed by titration with 0.1 N sodium thiosulfate using starch as an indicator. Saponification value (SV) was determined by the method based on NF ISO 3657. Then, 5 g of oil were reacted with 10 mL of KOH–methanol (0.5 M) under refluxing conditions for 30 min. Phenolphthalein was subsequently added and the undissociated KOH excess was titrated with 0.5M HCl after cooling. The SV is defined as the mass of

KOH consumed to saponify and neutralize, in milligrams of KOH per 1 g of oil. Last, UV absorbance was measured according to International Olive Council methods [12]. Absorbance was measured at 232 nm and 270 nm using a cuvette with a pathlength of 1 cm by dissolving approximately 0.25 g oil in cyclohexane in a volumetric flask (25 mL). The extinction coefficients (K_{232} and K_{270}) were estimated to assess the diene and triene formation indicating oil oxidative condition.

Extraction (Liquid–Liquid Extraction) and Estimation of Phenolic Compounds

Phenolic compounds were extracted from DKO according to the method of Pirisi et al [13]. In short, 2 g of oil was placed in a test tube and added to 1 mL n-hexane and then there were added 2 mL of a solution containing 60% (v/v) methanol. The prepared mixture was subsequently centrifuged at 3000 rpm for 5 min to separate the phases and collect the upper phase, which contained all of the phenolic compounds. This process was repeated two additional times for complete recovery of phenolics. The supernatants were pooled, concentrated to complete dryness at 40°C in vacuo and further reconstituted with 1 mL of 50% (v/v) methanol in preparation for antioxidant assays.

Estimation of Total Polyphenol Content

Total phenolic content was measured using the Folin–Ciocalteu method [14] and quantified using gallic acid as standard. In brief, 0.3 mL of sample extract was mixed with 1.5 mL Folin–Ciocalteu reagent (previously diluted to 1:10 by mixing 1 mL of the reagent with 10 mL distilled water) and saturated sodium bicarbonate solution (7.5%, w/v; 1.2 mL). The reaction mixture was incubated at room temperature in the dark for 1 h, and the absorbance was spectrophotometrically measured at a wavelength of 760 nm. Using gallic acid as a calibration standard, total phenolic content was expressed as mg GAE/g DW.

Estimation of Flavonoid Content

The flavonoid content of the DKO extracts was determined according to the method introduced by Kumazawa et al [15]. In short, to be specific whatever the sample we take is if we nail down only 1 mL of each sample and mix it with 1 mL of an equimolar (2% w/v) aluminum chloride ($AlCl_3$) solution prepared in absolute methanol. Absorbance was then measured at 430 nm against a reagent blank after 10 min incubation in RT. The flavonoid concentration was determined by interpolation using a standard calibration curve prepared with quercetin as the reference compound.

Condensed Tannin Content Estimation

The content of condensed tannins was determined with the method from Sun et al [11]. Acid-catalyzed depolymerization of tannins in the presence of hydrochloric acid, followed by reaction with vanillin to

yield red anthocyanidolic compounds. All these derivatives, in turn, were spectrophotometrically quantified at 510 nm. In short, 50 µL of extract was combined with 1.5 mL of 4% (w/v) vanillin solution and 0.75 mL of concentrated hydrochloric acid (HCl). The absorbance was measured at 510 nM against a reagent blank after complete homogenization and incubation for 15 min at room temperature. Data were expressed as milligrams of catechin equivalents per gram of oil (mg CE/g oil) according to a catechin calibration curve (0–500 µg/mL) for the quantification of condensed tannins.

Carotenoid Content Estimation

Extraction of carotenoids following the protocol described by Sass-Kiss et al. In brief, 5 g of DKO was treated with 20 mL of hexane/acetone/ethanol (2:1:1, v/v/v) mixture. The upper phase was harvested following assemblage for 30 min. A second extraction was performed using 10 mL of hexane, and the two organic phases were combined. Total carotenoid content was quantified spectrophotometrically at 450 nm [16].

UHPLC-Based Characterization of the Antioxidant Capacity of DKO

The polyphenolic profile of DKO was determined using an ultra-high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UHPLC-ESI MS/MS) system, specifically a Shimadzu 8040 equipped with ultra-high sensitivity UFMS technology and a binary pump Nexera XR LC-20AD (Openlab13, Tlemcen, Algeria). Chromatographic separation was performed on a Restek Ultra C18 column (3 µm, 150 × 4.6 mm) with mobile phases consisting of (A) water containing 0.1% formic acid (B) and methanol, delivered at a flow rate of 0.2 mL/min. A 5 µL aliquot of the oil sample was injected and separated using a gradient program (time in min; %A/%B): 0.0–0.2 (98/2), 0.2 2.5 (25/75), 2.5–4.0 (0/100), 4.0–10.0 (0/100), 10.0–10.1 (98/2), followed by column re equilibration. Electrospray ionization was conducted in negative mode with the following optimized parameters: CID gas pressure at 230 kPa, conversion dynode voltage at 6.00 kV, drying line (DL) temperature at 250°C, nebulizing gas flow at 3.00 L/min, heat block temperature at 400°C, and drying gas flow at 10 L/min. Detection and quantification were achieved in multiple reaction monitoring (MRM) mode, targeting specific mass transitions for reference standards including catechin (m/z 291.1 → 161.1), myricetin (m/z 318.5 → 256.05), ferulic acid (m/z 195.0 → 44.7), and others, with retention times spanning 1.6–9.9 min; peak areas and heights were compared against calibration standards for accurate compound identification and quantification.

DPPH (1,1-Diphenyl-2-picrylhydrazyl) Free Radical Scavenging Activity Assay

The DPPH free radical scavenging activity assay assesses the ability of an extract to neutralize stable DPPH radicals. It is done by mixing the extract at different concentrations with a DPPH solution and incubating for 30 min. Then, the reduction of absorbance at 517 nm is measured. Antioxidant activity is represented as the percent inhibition of DPPH. The extract concentration at which the inhibition of 50%

of free radicals is achieved (IC_{50}) was taken from the graph produced by plotting scavenging capacity against extract concentration [17].

Ferric Reducing Antioxidant Power (FRAP) Assay of Fe^{3+} Ions

This test measures the reducing power of ferric (Fe^{3+}) ions by extracts, and is an indicator of antioxidant capacity. This mixing consists of potassium ferricyanide solution and phosphate buffer with the extract. Following incubation, trichloroacetic acid is added and the mixture centrifuged. The mixture is then collected and combined with distilled water as well as ferric chloride ($FeCl_3$) solution. Measurement of absorbance at 700 nm. Higher absorbance suggests a higher reducing power of the extracts. The absorbance values obtained were used to calculate the percentage of ferric reducing power [18].

Determination of Fatty Acid Composition by CPG

Fatty acid composition was identified by gas chromatography (GC) using 6890 HP series GC (Agilent, USA), which injected a sample of 1 µL of hexane containing fatty acid methyl esters into an Agilent H2 column with capacity of detection at 30m × 0.32mm i.d., 0.25µm INNOWAX column. The split injector (1:80) setup was maintained at 250°C, while helium was only as the carrier gas at a flow of 1.5 mL/min. Flame ionization detector temperature was performed at 270°C. Oven temperature programming was initial 150°C for 3 min, ramp of 3°C/min to 220°C (hold time:9 min; run-time: total time on the chromatogram to be equal to the column run). Identification of peaks compared to retention times from FAME standards (olive, sunflower and palm oils analyzed under the same conditions). Reproducibility was ascertained by injection in triplicate under the same settings.

Antimicrobial and Antifungal Activity of DKO

The paper disc diffusion method described by Balouiri et al. was used to test the antibacterial and antifungal activity of the fixed oil using seven reference microbial strains obtained from Pasteur Institute (Table 1). Young cultures generating isolated colonies were obtained by streaking each strain onto appropriate agar plates then incubating them at 37°C for 18–24 h and subsequently suspended in sterile distilled water to make standardized inocula which should provide the same initial cell densities [19]. Each oil-impregnated discs is detected by ruler, that is used to quantify the antibacterial activity in terms of diameter of inhibition zones. Antimicrobial activity of DKO was evaluated by using agar disc diffusion method on Mueller-Hinton agar (for bacteria) and Sabouraud agar (for yeasts/fungi), according to CLSI guidelines [20, 21]. Sterile 6 mm Whatman No. 3 paper discs were infused with 10 µL of oil at a concentration of 50 mg mL^{-1} using multiple deposits, dried in the air and placed on inoculated plates obtained by swabbing standardized microbial suspensions (optical density 0.08–0.1 at 620 nm for bacteria; ranging from 0.12 to 0.15 nm wave length 530 constant measure 340554 nm650; corresponding to a McFarland value equal to or lower than) As positive controls for anti-bacterial and anti-fungal activity, discs for ampicillin (10 µg/disc) and cycloheximide (10 µg/disc), respectively, were employed; aqueous solutions were used as negative controls. Plates were kept at 4°C for 2 h to allow

diffusion, subsequently incubated at 37°C (bacteria) or 35°C (yeasts/fungi) for 24 and 48 h respectively, zone diameters measured, an observable zone was considered positive regardless of size (Table 1).

Table 1
Reference Microorganisms Used for Antimicrobial and Antifungal Activity Testing

Test Microorganisms	Reference	Appropriate Media
Gram-positive bacteria		
Bacillus cereus	ATCC11778	Müller-Hinton
Staphylococcus aureus	ATCC 25923	Chapman
Gram-negative bacteria		
Escherichia coli	ATCC 25922	MacConkey
Klebsiella pneumoniae	ATCC 70603	MacConkey
Pseudomonas aeruginosa	ATCC 27853	Gélose cétrimide
Yeasts/fungi		
Candida albicans	ATCC 10231	Sabouraud
Candida albicans	ATCC 26790	Sabouraud

*ATCC: American Type Culture Collection

Statistical Analysis

Values are shown as means ± standard deviation. Variables with non-normal distributions were logarithmically transformed. Comparisons between pairs of groups were conducted using Tukey post hoc test.

RESULTS

Determination of the DKO Extraction Yield and Secondary Metabolite Detection

The extraction yield was determined by calculating the percentage of extracted oil relative to the initial mass of plant material. The oil from date kernels was successfully extracted using hexane as the solvent in a Soxhlet apparatus for 5 h under heating conditions. The yield obtained from this hot extraction was high, reaching 9.25 ± 1.155% (w/w), and it was consistent with literature values reported for the efficiency of extracting unused oils from date pits.

The phytochemical screening of DKO showed that it comprises various bioactive secondary metabolite classes in different levels. Employing the qualitative scale used, DKO tested highly (++) for total polyphenols which was supported by a high colorimetric reaction at 30 min with ferric chloride (FeCl3) suggesting high levels of polyphenolic derivatives. The presence of polyphenols indicates a strong free-radical scavenging activity, which is well-known to reduce oxidative stress and improve the lipid stability in oils. Moderate levels of total flavonoids were detected (++) indicated by showing pink–red color after treatment with hydrochloric acid and magnesium. These results suggest that significant levels of flavonoid compounds, linked to antioxidant, anti-inflammatory and antimicrobial effects which can provide potential biological properties for DKO. Moreover, you found traces of condensed tannins (+) evidenced by change in color after adding ferric chloride in methanol. Additionally, phenolics related with tannins also appeared, reinforcing antioxidant capacity and possibly also providing protection from microbial growth and lipid peroxidation. Carotenoids were only weakly positive (+), indicating that their concentrations were relatively lower compared to the phenolic classes. In lesser abundance, carotenoids also play a role as antioxidants and natural pigments that can prevent the damage caused in biological systems by singlet-oxygen and other reactive-oxygen-species mediated toxicity.

Physicochemical Characterization of Date Kernel Oil (DKO) Extract

The physicochemical characterization of DKO was performed by measuring the RI, PV, AV, IV, SV, and UV absorbance (Table 2). The RI, which is an indicator of sample purity at 40°C, was 1.358 ± 0.003, suggesting high purity and negligible oxidation. The PV, a marker of primary lipid oxidation, was 1.30 ± 0.05 meq O_2/kg, indicating a low level of oxidative alteration. The AV was 1.44 ± 0.27 mg KOH/g, corresponding to 0.7% free fatty acids, while the IV, expressed as grams of iodine absorbed per 100 g of fat, was relatively low at 54.88 ± 1.65 g I_2/100 g, reflecting a moderate degree of unsaturation. The saponification value,

Table 2
Physicochemical characteristics of DKO extract

Chemical index / UV Absorbance	DKO extract Value
Refractive index	1.358 ± 0.3
Acid value (mg KOH/g)	1.44 ± 0.27
Peroxide value (meq O_2/kg)	1.30 ± 0.05
Iodine value (g I_2/100 g)	54.88 ± 1.65
Saponification value (mg KOH/g)	173.94 ± 0.00
Acidity (%)	0.70 ± 0.00
Absorbance at 232 nm (K_{232})	1.98 ± 0.00
Absorbance at 270 nm (K_{270})	0.53 ± 0.00

Values are expressed as mean ± standard deviation (SD) of three independent replicates.

DKO: Date kernel oil

defined as the milligrams of KOH required to saponify 1 g of oil, was 173.94 mg KOH/g, which indicates a high content of medium-chain fatty acids. UV absorbance values were 1.98 at 232 nm and 0.53 at 270 nm, reflecting both the oxidation status of the oil and its conjugated diene/triene content.

Determination of phenolic compounds and carotenoids

The DKO extracts also revealed the presence of several phytochemical classes, including polyphenols, flavonoids, carotenoids, and condensed tannins, as confirmed by three qualitative tests and subsequent quantitative analysis. The total polyphenol content, determined using a gallic acid calibration curve (y = 0.572x + 0.073 ; R^2 = 0.998), was 43.34 ± 0.171 mg GAE/g extract (Fig. 1A). The total

flavonoid content, quantified with a quercetin standard curve (y = 0.106x + 0.122 ; R^2 = 0.996), reached 42.361 ± 4.29 mg QE/g extract (Fig. 1B). The condensed tannin content, calculated from a catechin calibration curve (y = 0.0007x + 0.0006 ; R^2 = 0.999), was 0.896 ± 1.34 mg CE/g extract (Fig. 1C). Finally, the total carotenoid content, estimated using a standard curve (y = 0.537x + 0.059 ; R^2 = 0.941), was 20.637 ± 2.307 mg/kg (Fig. 1D). These values highlight the substantial abundance of phenolic and flavonoid compounds, underscoring the strong bioactive potential of the DKO extract.

The phytochemical profile of DKO by UPLC-ESI-MS/MS

The phytochemical profiling of DKO was performed using ultra-performance liquid chromatography coupled with electrospray

ionization tandem mass spectrometry (UPLC-ESI-MS/MS). Based on more than

50 individual MRM transitions, together with retention times (RT) and peak areas, 20 bioactive compounds were identified among 40 targeted metabolites. Ferulic acid was the dominant analyte (RT 5.394 min; peak area 5,161,201; height 256,390), followed by catechin (RT 6.614 min; area 1,435,258) and myricetin (RT 5.705 min; area 687,533), which together represented approximately 92% of the total detected peak intensity in the corresponding chromatograms. Phenolic acids were the predominant chemical class, accounting for 60–65% of the relative abundance. Within this group, hydroxybenzoic acids (C_6–C_1 skeleton) included gallic and salicylic acids, while hydroxycinnamic acids (C_6–C_3 skeleton) were represented by ferulic, sinapic (RT 1.767 min; area 676,598), and caffeic acids. Several flavonoids, including myricetin, quercetin (RT 5.191 min), apigenin (RT 5.124 min), luteolin, and catechin, were also identified, reflecting their antioxidant potential. In addition, triterpenoids such as oleanolic acid (RT 6.015 min) and carotenoids such as β-carotene (RT 9.905 min) were detected, highlighting the distinctive phytochemical profile of date palm by-products (Fig. 2).

Antioxidant activity assessment of DKO extract

Complementary DPPH and FRAP assays were conducted to evaluate the antioxidant capacity DKO extract (Table 3).

Table 3
Antioxidant Capacity of DKO Extract

Extract / Standard	DPPH (IC_{50}) (µg/mL)	FRAP (EC_{50}) (mg Fe^{2+}/mL)
BHA	30.84 ± 0.69	ND
Vitamin C	ND	0.21 ± 0.13

DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric Reducing Antioxidant Power. IC_{50}/EC_{50}: Values represent the half-maximal inhibitory/effective concentration. ND: Not Detected.

In the DPPH radical scavenging assay, the extract showed a mean IC_{50} of 45.445 µg/mL, corresponding to an antiradical power of 0.022, which indicates moderate activity compared with the positive control BHA, which exhibited an IC_{50} of 30.84 µg/mL and an antiradical power of 0.032. These results suggest the presence of antioxidant compounds with scavenging ability, although their activity is lower than that of BHA. The FRAP assay, which measures the ability of the extract to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) in the $K_3Fe(CN)_6$ system at a wavelength of 700 nm, was expressed in mM $FeSO_4$ equivalents per mg of extract. In terms of ferric reducing power, the DKO extract showed lower values than ascorbic acid, with an EC_{50} of 2.9 mg/mL compared with 0.21 mg/mL for ascorbic acid, suggesting that the bioactive compounds present in the oil possess antioxidant protective potential.

Fatty Acid Profile of DKO

The fatty acid profile of DKO was characterized by a predominance of saturated fatty acids, with lauric acid at 17.95 ± 0.52%, myristic acid at 9.85 ± 0.64%, palmitic acid at 10.98 ± 0.89%, and stearic acid at 3.34 ± 0.33%. Among the unsaturated fatty acids, oleic acid was the major component, reaching 43.21 ± 1.74%, which reflects a high proportion of monounsaturated fatty acids and likely contributes to the nutritional value and oxidative stability of the oil. Linoleic acid was the principal polyunsaturated fatty acid, with a content of 11.53 ± 3.06%, indicating the presence of essential fatty acids, although at a lower level than oleic acid. Overall, the oil exhibited a lipid profile dominated by saturated and monounsaturated fatty acids, with a moderate contribution from polyunsaturated fatty acids (Fig. 3).

Antimicrobial and antifungal activity of DKO against microorganisms

The antimicrobial screening results showed that DKO was active only against Gram-

Values are expressed as mean ± standard deviation (SD) of three independent replicates. DKO: Date kernel oil. FID: Flame Ionization Detector.Cx" (Carbon Chain Length): The number before the colon represents the total number of carbon atoms in the fatty acid chain. C18:1, C18:2: Indicates an unsaturated fatty acid, meaning it contains one or more double bonds.C12:0, C14:0, C 16:0, C 18:0: Indicates a saturated fatty acid, meaning there are no double bonds in the carbon chain. n-9 (or ω-9) means the first double bond is located at the 9th carbon from the methyl end. Cis" vs. "trans": This refers to the geometric orientation of the hydrogen atoms around the double bond.

Table 4
Antimicrobial and Antifungal Activity of DKO

Test Microorganisms	**Inhibition zone diameters (mm)**
Gram-positive bacteria	
Bacillus cereus ATCC11778	07 ± 1.04
Staphylococcus aureus ATCC 25923	09 ± 0.61
Gram-negative bacteria	
Escherichia coli ATCC 25922	-
Klebsiella pneumoniae ATCC 70603	-
Pseudomonas aeruginosa ATCC 27853	-
Yeasts/fungi	
Candida albicans ATCC 10231	-
Candida albicans ATCC 26790	12 ± 0.84

*ATCC: American Type Culture Collection

Each value is expressed as mean ± standard deviation (SD) from experiments performed in triplicate

positive bacteria and one yeast strain, whereas no inhibitory effect was observed against the two Gram-negative bacteria tested. As presented in Table 4 and Fig. 4, only a small inhibition zone was observed for *Bacillus cereus* and *Staphylococcus aureus*, also exhibited a limited inhibition zone (Fig. 4. A, B).

In contrast, all Gram-negative bacterial strains tested were completely resistant to the oil, with no inhibitory effect detected at any concentration. Regarding yeasts, the two *Candida albicans* strains displayed different responses: no inhibition zone was recorded for *Candida albicans ATCC 10231*, while a marked inhibition zone was observed for *Candida albicans ATCC 26790* (Fig. 4. C). Overall, the DKO showed susceptibility ranging from weak to strong in three of the seven tested microorganisms, namely the two Gram-positive bacteria and *Candida albicans ATCC 26790*.

DISCUSSION

The extraction yield obtained in the present study, 9.25 ± 1.155%, falls within the range reported for coconut milk-based conventional solvent extraction, although it remains lower than the yields obtained under optimized conditions. Conventional Soxhlet extraction with n-hexane is widely used as a reference method because it efficiently recovers non-polar lipids from date pits, but it is strongly influenced by extraction time, temperature, solvent-to-solid ratio, and particle size. A yield of 16.5% after 7 h at 70°C was reported in a recent optimization study, showing that longer extraction times and improved operating parameters can significantly increase oil recovery compared with standard Soxhlet conditions. Similar findings were also reported by other authors, who observed that extraction efficiency increased during the first hours of reflux and then gradually levelled off, confirming that the 5 h duration used here was sufficient to obtain a high, though not necessarily maximum, yield [22]. A relatively high extraction yield of 13.2% was also reported for the Shavi-Shirin variety from Qom, Iran [23]. This value was significantly higher than the 8.5% and 5% observed in Musaafti and Kabkab cultivars from Karaj, respectively. In comparison, Benyoussef et al. reported the lowest yield, with only 4.44% for the Allig variety from Tozeur in Tunisia [24].

Qualitative phytochemical screening of DKO revealed that it primarily contains phenolic compounds, and in particular total polyphenols, concentrated ω-3/cis-linolenic fatty acids and moderate levels of flavonoids and condensed tannins but has lower content of carotenoids. Such a profile is characteristic of the general phytochemical richness for all chosen extracts prepared from kernels of dates, which are well-documented sources of bioactive antioxidants [25]. The low amount of flavonoids also agrees with these recent studies that have shown that compounds related to flavonoids but not classified as such in date kernel materials exert antioxidant and anti-inflammatory activities. Flavonoids exist in relatively lower concentrations than total polyphenols, but they are extremely important because the hydroxylated structure of flavonoids allows for an electron donating free-radical scavenging buffering activity that may partially contribute to the antioxidant action observed in DKO. These results bolster the hypothesis that DKO's antioxidant activity is not due to a single compound class, but rather a mixture of phenolic

subclasses [26]. The fairly high concentration of condensed tannins also contributes substantially to the antioxidant status of DKO. High-tannin content, especially proanthocyanidin-type compounds have been indicated as the most common phenolic constituents in date kernels which are responsible for both radical-scavenging and antimicrobial activity. They might also increase oxidative stability in lipid-rich matrices by reducing lipid peroxidation; this is particularly relevant for oil-based goods [27]. Only traces of carotenoids were detected with the implication that while present they do not comprise a large component of the antioxidant fraction in DKO. This finding is in accordance with those indicating the presence of carotenoids from date-extracted materials, although at significantly lower concentrations than phenolic compounds. However, carotenoids may still provide benefit via anoher mechanism for antioxidant profile as singlet oxygen and other reactive oxygen species quenching and a nutritional and technological value as natural pigments even if at the low concentrations [28].

The RI of the DKO extract was close to that reported by Belabed for the H'mira variety (1.462) [29]. This value is consistent with Codex Alimentarius standards (1983). RI is one of the parameters used to assess oil quality and purity in bulk oil identification [30]. In general, a longer fatty acid chain length and a higher degree of unsaturation are associated with a higher RI [31]. The PV obtained in this study was well below the limits established by the International Codex Alimentarius (1983). Benmohamed reported a PV of 3.4 for Soxhlet-extracted oil from the Deglet Nour variety, whereas pressed oil had a value close to 1; after 7 months of storage in closed containers without exposure to light or oxygen, this value increased to just above 0.2, indicating that only low levels of peroxides were formed and that oxidation and fat degradation remained limited [32]. In comparison, Krichene et al. reported PV ranging from 7.8 to 15.2 meq O_2/kg for Tunisian olive oils, depending on cultivar [33]. The low PV observed here may be attributed to the presence of natural antioxidants in the oil, such as tocopherols, polyphenols, and carotenoids [34]. The AV of DKO was significantly lower than the Codex Alimentarius limits (1983) (2.2–7.26 mg KOH/g oil) and even lower than the total AV reported by Belabed for DKO derived from the Hmira variety (2.224 mg KOH/g oil) [29]. Low acidity is a clear indicator of oil purity and room-temperature stability [35], which is consistent with the profile of this oil. The measured acidity was 0.7%, well below the International Olive Council limit [36] of ≤ 2%. The AV also reflects the free fatty acid content, the edibility and industrial suitability of the oil [37]. Regarding the IV, the result obtained was below the normal range reported by Codex Alimentarius, 2009 for fresh oils (94–102 g I/100 g oil). Since IV is directly related to the total number of double bonds in fatty acids [38], it is an important index for evaluating the degree of unsaturation of an oil and consequently, its oxidative stability [39] which indicates that the DKO analyzed in this study was less unsaturated. The SV obtained in the present study fell within the normal range established by Codex Alimentarius in 1983. According to Soliman et al. SV of DKO from Saudi Arabian cultivars ranged from 206 to 215 mg KOH/g oil, which is higher than the value recorded in this study [40]. In this regard, Benrachou et al. stated that the SV is an important criterion for identifying oil composition and fatty acid type, since it indirectly estimates the average molecular weight and chain length of fatty acids [41]. Finally, UV absorbance is also a useful indicator of the oxidation level of oils. The specific absorbance of the studied DKO at 232 nm was within the range reported by Besbes et al. for Deglet Nour and Allig varieties, which ranged from 1.2 to 2.5. Thus, the value obtained for our sample was higher than

that of Deglet Nour but lower than that of Allig. By contrast, the K270 values reported for both varieties were lower but very close to that of the sample in question (0.5) [42].

Compounds such as polyphenols, flavonoids, carotenoids, and condensed tannins have also been reported in DKO extracts from similar varieties, including the Algerian Mech-Degla, where comparable qualitative assays routinely reveal the presence of these metabolite classes [43]. The total polyphenol content measured in this study (43.34 ± 0.171 mg GAE/g extract; calibration curve: y = 0.572 x + 0.073, R^2 = 0.998) was considerably higher than that reported for crude DKO in recent literature, where values range from very high levels in Mech-Degla oil (112.92 mg GAE/kg) to much lower values in other studied varieties (0.64–1.27 mg/g). Such a relatively high concentration suggests that the extraction process may have been more efficient, possibly due to the polarity of the extraction conditions, and it indicates a marked antioxidant potential, as previously shown for Tunisian kernel oil and Algerian samples [44]. The total flavonoid content (42.361 ± 4.29 mg QE/g extract; y = 0.106 x + 0.122 ; R^2 = 0.996) was much higher than the values reported for native oils, such as Mech-Degla (15.7 mg QE/kg, often below 50 mg/kg), and was close to those found in hydroethanolic enriched extracts (44.5 mg QE/mg DE), thereby enhancing the bioactive profile of the oil, as supported by studies on Moroccan and Algerian cultivars [45]. Moderate condensed tannin levels (0.896 ± 1.34 mg CE/g extract; y = 0.0007 x + 0.0006 ; R^2 = 0.999) were also detected, and although extracted separately, they still contributed significantly to the antioxidant activity. In addition, inter-varietal variation in total phenolic content has been shown to influence antioxidant capacity [46]. The carotenoid content (20.637 ± 2.307 mg/kg; y = 0.537 x + 0.059 ; R^2 = 0.941) was comparable to, or even higher than, reference oils (Mech-Degla: 125 mg β-carotene/kg; general range: 12–126 mg/kg), and may contribute to the characteristic yellow coloration and oxidative stability of the oil [47].

In this study, UPLC-ESI-MS/MS analysis of DKO enabled the identification of approximately 20 bioactive compounds among 40 targeted metabolites where phenolic acids predominated (60–65%), which is in line with recent untargeted metabolomic profiles of date kernels, where hydroxybenzoic acids (C_6–C_1 skeleton: gallic, salicylic, 4-hydroxybenzoic, and gentisic acids) and hydroxycinnamic acids (C_6–C_3 skeleton: ferulic, sinapic, and caffeic acids) typically account for 50–80% of phenolics detected by UHPLC-MS [43]. Ferulic acid, identified as the principal analyte (RT 5.394 min; peak area 5,161,201), followed by catechin (RT 6.614 min; area 1,435,258) and myricetin (RT 5.705 min; area 687,533; approximately 92% of total peak intensity), mirrors the abundances reported in Saudi and Egyptian cultivars (2023–2025), where ferulic and caffeic acids are major constituents in pulp and kernels, catechin and vanillin occur at 22–25 mg/kg, and myricetin-like flavonols are associated with antioxidant capacity [48]. The antioxidant potential of oil and flour extracts is positively correlated with flavonoids such as myricetin, quercetin (RT 5.191 min), apigenin (RT 5.124 min), luteolin, and catechin; these patterns are highly consistent with four varieties identified by UHPLC-MS as rich in hydroxybenzoates and flavonoids in kernels, with elevated caffeic and sinapic acids, although the accumulation of phenolic compounds generally decreases after maturation, as well as with studies on syrups and kernels

reporting 77 annotated metabolites, including numerous phenolics and flavonoids across syrup and kernel samples [49].

Overall, the phenolic-dominated phytochemical profile of DKO, with ferulic acid, catechin, and related phenolics accounting for about 92% of total peak intensity, underlines its potential as an important bioactive source with antioxidant and plant defense relevance. This profile also suggests higher antioxidant activity than some crude oils, although it remains dependent on variety and extraction conditions, particularly the fatty acid ratio. These observations are consistent with recent literature documenting phytochemical variability and nutraceutical applications of date kernel-derived products without overlap with earlier citations (2022–2025), and they align well with the DPPH and FRAP results used to strengthen the discussion [47].

The moderate antioxidant activity observed in both the DPPH and FRAP assays is consistent with recent studies on similar kernel oils and extracts, which have also shown antiradical capacities lower than those of synthetic or standard controls such as BHA and ascorbic acid [50]. The DPPH IC_{50} value of 45.445 µg/mL (antiradical power = 0.022) indicates moderate scavenging activity and is close to that reported for cold-pressed Mech-Degla kernel oil extracts (55.91% DPPH for polar extracts), while remaining lower than BHA (30.84 µg/mL). This confirms the presence of phenolic and other bioactive reducing agents, although without reaching the activity level of commercial antioxidants. This observation is also in agreement with the high polyphenol content reported in Tunisian and Algerian kernel oils, where such compounds were associated with moderate DPPH activity (30–56% inhibition) [43]. As noted by Mansouri et al. lower IC_{50} values correspond to higher antioxidant capacity [51]. Given the good reproducibility of the antioxidant response of the DKO extract [52], the FRAP assay was also used to further assess its reducing power. Although the EC_{50} value obtained in the FRAP assay (2.9 mg/mL, based on Fe^{3+} to Fe^{2+} reduction at 700 nm) was lower than that of ascorbic acid (0.21 mg/mL), it still indicates a noteworthy reducing capacity linked to the presence of protective compounds such as phenolics and carotenoids. This result is comparable to UAE-NADES extracts from date fruits and kernels, for which FRAP values ranged from 60 to 704 mg AAE/100 g DM and EC_{50} values were approximately 1–5 mg/mL [50]. Although iron is essential for oxygen transport during respiration and for many enzymatic functions in the body, it can also behave as a reactive metal capable of catalyzing oxidative damage in living cells and tissues [53]. To our knowledge, historical data have not previously been reported for DKO using the FRAP assay at the EC_{50} concentration.

These results are consistent with recent studies on the lipidomics of *Phoenix dactylifera* kernel oils, which also reported a predominance of saturated fatty acids, including lauric, myristic, palmitic, and stearic acids, together with a major monounsaturated fraction represented by oleic acid. This fatty acid composition is considered a fingerprint of stable kernel oils and is associated with low pro-oxidation potential, largely due to the high oleic acid content balanced by stable saturated chains [54]. The observed oleic acid level (43.21%) and the lower contribution of linoleic acid (11.53%) in the present sample are also in agreement with comparative studies suggesting that such a lipid matrix is suitable for food applications from a shelf-life perspective. Although linoleic acid occurred in much smaller amounts

than monounsaturated fatty acids, its presence still supports the nutraceutical value of the oil and may justify its use as a functional ingredient in lipid-based food fortification. In addition, the high SFA/MUFA ratio, together with the presence of lauric and myristic acids, contributes to the stability of the oil and supports its characterization as an underexploited source of functional lipids with potential industrial value, particularly in food applications where more stable oils are needed [55]. The nutritional value of this oil, linked to its unsaturated fatty acids and antioxidant compounds, also makes it a promising renewable resource for cosmetic and pharmaceutical uses, especially for lipid-based product stabilization [5]. Moreover, the balance between saturated fatty acids such as lauric, myristic, and palmitic acids and unsaturated fatty acids such as oleic and linoleic acids provides not only oxidative stability but also the potential to preserve bioactive nutrients, thereby enhancing the nutraceutical interest of the oil [56]. Detailed analyses further confirm that DKO is distinguished by its high thermal and oxidative stability, mainly associated with its elevated oleic acid content (42–52%) and abundance of tocols, including tocopherols and tocotrienols, supporting the view that date kernels represent a promising and sustainable alternative to traditional palm or vegetable oils [57].

The selective sensitivity observed for the Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) and the complete resistance of the Gram-negative strains are consistent with previous reports on the antimicrobial activity of date kernel derivatives [1]. The activity against *S. aureus* and *B. cereus* is generally attributed to the synergistic action of phenolic compounds, such as gallic and ferulic acids, together with fatty acids present in the oil, which can destabilize the bacterial plasma membrane. The limited inhibition zones obtained in the present work may be explained by the lower concentration of active constituents or by the extraction procedure, which may yield products with weaker antimicrobial potential than essential oils derived from more concentrated kernels [58]. The resistance of Gram-negative bacteria, such as *E. coli*, is often associated with the structure of their outer membrane, which contains a lipid-rich layer that limits the penetration of hydrophobic compounds, including lipid-based oils. Nevertheless, such discrepancies may also reflect differences in experimental methodology between studies, including microdilution versus agar diffusion, the microbial strains tested, and cultivar-specific phytochemical composition [3]. The different responses observed for the two *Candida albicans* strains (*ATCC 10231* and *ATCC 26790*) highlight the importance of strain-specific susceptibility in antifungal testing. This variation is frequently reported in studies involving natural products, where efficacy can differ markedly between clinical isolates because of intrinsic resistance genes [59]. Overall, these findings indicate that DKO exerts a narrow-spectrum antimicrobial activity that appears to be isolate-specific and may result from the synergistic action of secondary metabolites, including phenols, flavonoids, and triterpenes. In light of the discrepancies reported in the literature,

where some studies have shown activity against Gram-negative bacteria using microdilution methods while others have not using agar diffusion, it is reasonable to suggest that these results might be improved through more sensitive analytical methods or by testing higher concentrations of the oil [60].

CONCLUSION

This study demonstrated the potential of DKO as a high-value matrix for bioactives. The lipidomic study revealed a favorable fatty acid profile—characterized by predominance of oleic and lauric acids—which, in combination with also high levels of phenolic acids (mainly ferulic acid) and flavonoids endows the oil with important functional properties as well as excellent oxidative stability. The presence of these chemical characteristics, and the selective antimicrobial activity of the oil only against Gram-positive pathogens and certain yeasts, render it a sustainable natural alternative in the food cosmetic/and pharmaceutical industries. While differences in antimicrobial sensitivity highlight the necessity of standardized analytical methodologies, these findings support the potential utility of date kernel by-products as a source for industrial stabilisers and nutraceuticals. The future direction should be centered on upscaling extraction and stabilization technologies such as microencapsulation, as well as detailed toxicological assessment of DKO to further promote its incorporation in commercial applications and facilitation of the sustainable circular bio-economies.

Declarations

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Figures

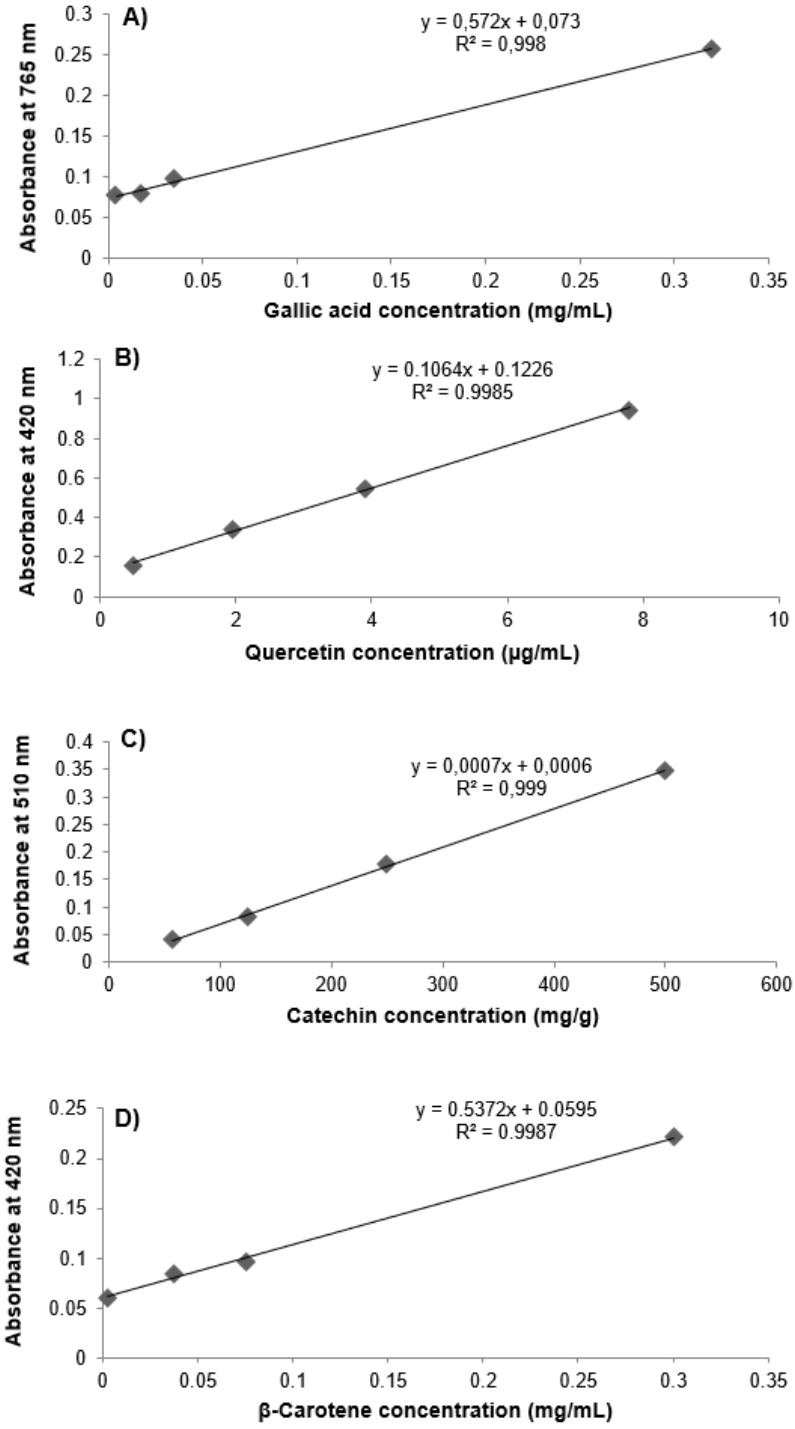


Figure 1

Calibration curves for the determination of total polyphenols, total flavonoids, total condensed tannins, and total carotenoids. y: is the response variable estimated from x. R^2: value shows that the linear model explains % of the variation in y, which indicates an excellent fit to the data.

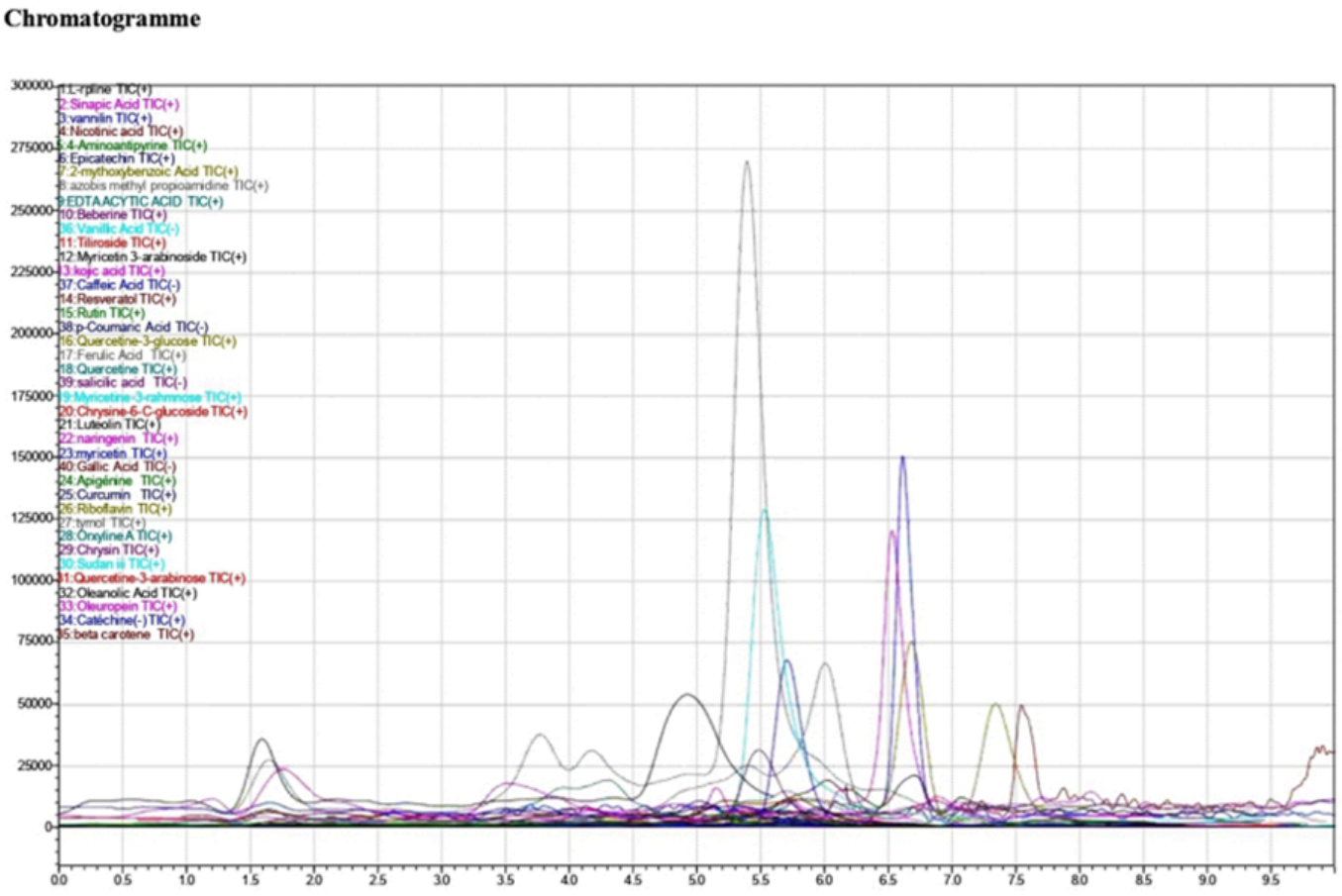


Figure 2

Bioactive metabolite profile of DKO determined by UPLC-ESI-MS/MS.

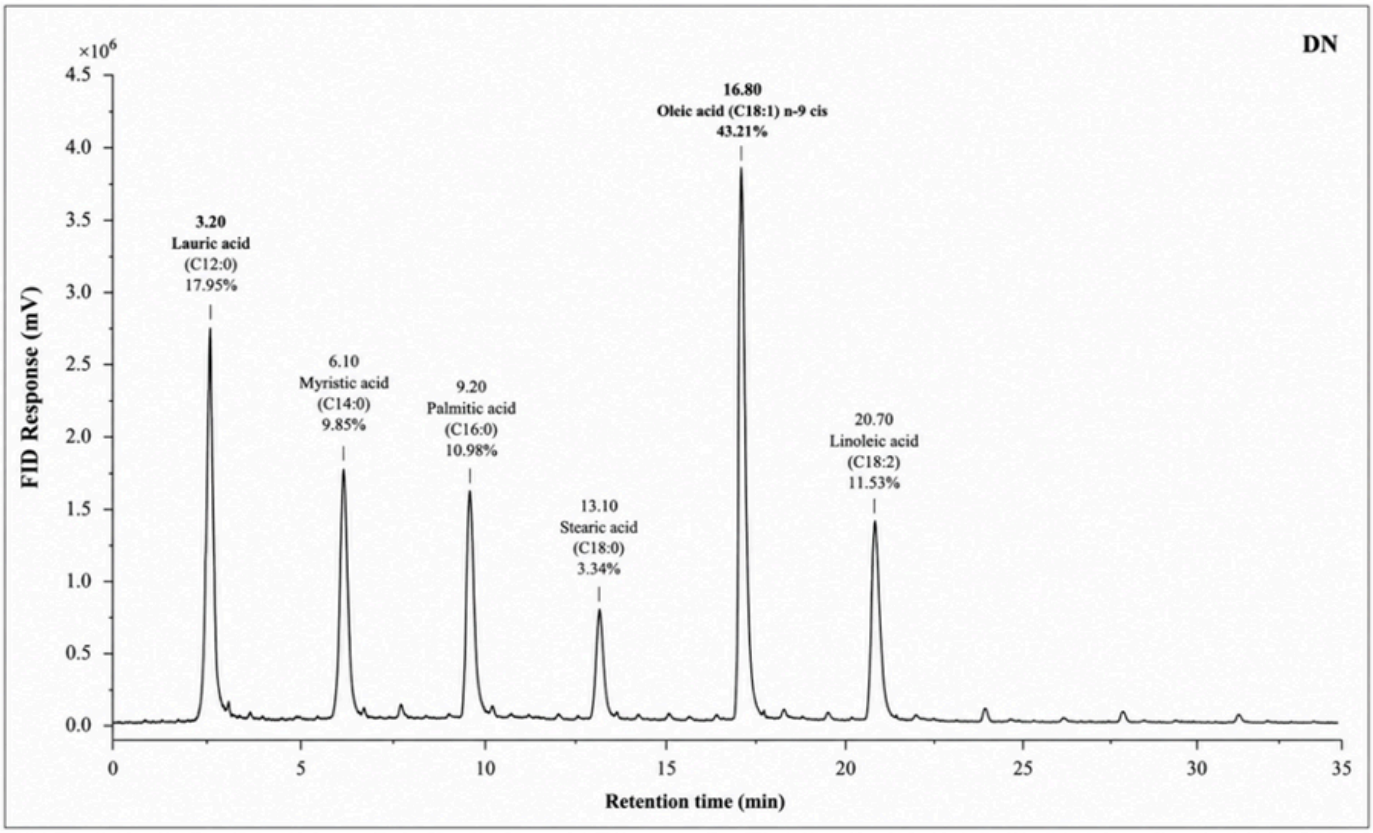


Figure 3

Fatty Acid composition of DKO

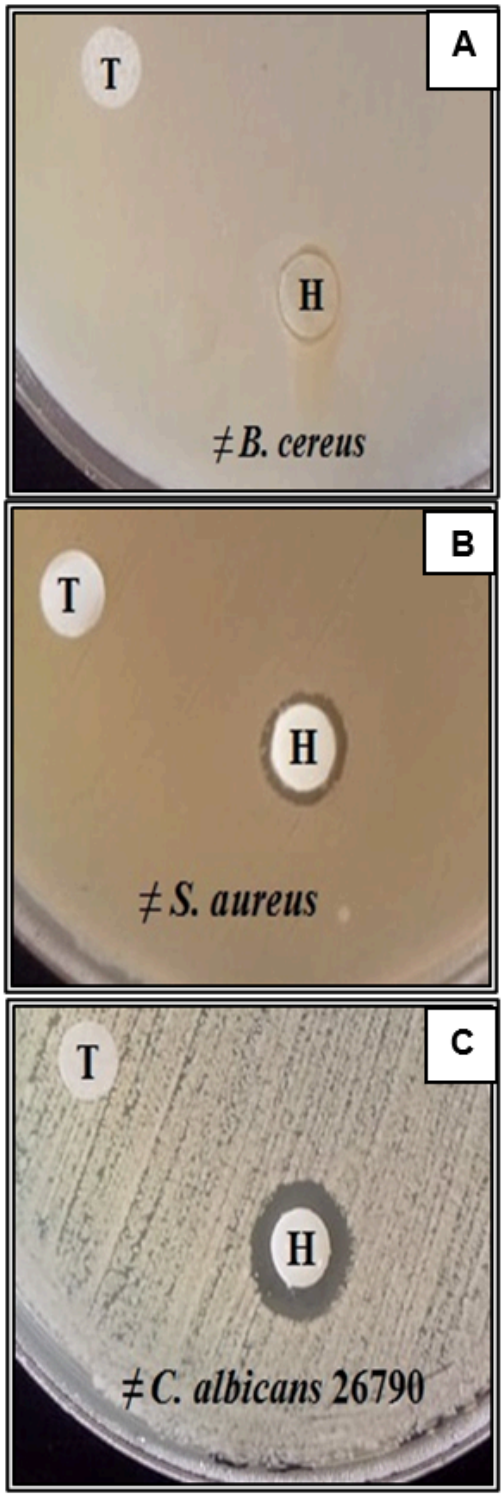


Figure 4

Antimicrobial and Antifungal Activity of DKO. Inhibition zone: T: Test ; H: Oil