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chemical properties and active compounds of date pressing residues using high-performance chromatography (HPLC) analysis

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Abstract:

For routine needs like food and medications, humans grow plants. There are countless types of crops and goods made from plants, such as cotton, fruits, vegetables, cereals, pulses, and oils. Humans have utilized a range of seed oils as dietary supplements and as feedstock for biofuel production. One such plant that is utilized to produce oil is the date palms (Phoenix dactylifera L.). A commercial fruit, date palms are mostly grown in Iraqi nations. Date palm fruit is enjoyed all throughout the world, as are its processed goods including syrup, jams, and jellies. However, after processing, quantities of seed waste are produced, and inadequate methods for disposing of this trash cause environmental issues. According to scientific sources, date palm seed oil is rich in minerals, quercetin, B7, B12, and apgenin extract. The content of apgenin was 16250 µg/mg, the concentration of B12 was 606061 ug/mg, and the chromatograph of B7 extract analysis by HPLC Both the concentration and the quercetin concentration were 10699 µg/mg and 15658 µg/mg, respectively. These bioactive compounds have important pharmacological properties, including hepatoprotectivity, antidiabetic, anti-inflammatory, antioxidant, and antibacterial properties. This study outlines the phytochemicals found in date palm seed oil, talks about the fatty acid composition of different date palm cultivars around the world, and emphasizes the pharmacological properties of date seed oil, its efficient use in the food and pharmaceutical industries, and the use of seed oil byproducts in the production of biofuel. The manufacturing of pharmaceuticals and biofuel offers a great chance to increase the value of date palm farming and generate profits. The examination of antioxidant-interesting active components from date palm seeds, such as quercetin, B7, B12, and apgenin extract, begins with this work. A wide variety of phenolic compounds were identified through the extraction of organic fractions and biochemical characterisation utilizing high-performance chromatography (HPLC) analysis.

Key words: high-performance chromatography (HPLC), chemical properties**,** *Phoenix Dactylifera L***,** date extracts**,** antioxidant**.**

Introduction

Phoenix Dactylifera L. is the scientific name for the date palm fruit, which is a member of the Arecaceae family. Vitamins, minerals (including calcium, phosphorus, magnesium, and potassium), and sugar are all abundant in date fruit [1]. Because date fruit is quickly digested and its nutrients are readily absorbed by the body, it has significant cultural importance in Middle Eastern countries where Muslims utilize it to break their fast during Ramadan [2]. One of the most significant commercially farmed fruits in the Middle East, North Africa, and the Arabian Peninsula is date fruit. The Holy Qur'an mentions date fruit as an agricultural crop, and contemporary literature highlights its ongoing economic significance to the area [3]. Egypt is the nation that produces the most dates, with Iran coming in second and Saudi Arabia, Algeria, and Iraq coming in third [4]. The date fruit is regarded as both a medicinal plant and a complete diet [5]. The date fruit includes anthocyanins, phenols, sterols, carotenoids, and flavonoids, according to chemical analyses of its constituent parts. In the Middle East and Africa, date fruit has also been utilized in traditional medicine. It has been reported to have anti-aging, constipation, cancer, type 2 diabetes, anti-atherosclerosis, stomach ulcers, neuropathy, male fertility, and wound healing properties [6]. The chemical makeup of date seeds, including fatty acids, amino acids, fiber, phenolics, minerals, and vitamins, is presented in this study in order to explore the significance of dates, specifically date seeds or stones [7]. We also discuss several methods for analyzing these elements. Additionally discussed is the use of these chemicals in a variety of fields and industries, including aquaculture, clinical settings, nutraceuticals, and cosmeceuticals. Date seeds' essential chemical components, including fiber, phenolics, fatty acids, amino acids, vitamins, and minerals, are meticulously ascertained using a range of analytical techniques [8]. Amino acid content can be determined using methods like High-Performance Liquid Chromatography (HPLC) [9]. Proteins can be separated for additional examination using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) [10]. Atomic Absorption Spectrometry also explores mineral concentrations, providing a thorough comprehension of key components. This wide range of analytical techniques makes it possible to thoroughly investigate the complex chemical makeup of date seeds [11,12].

Materials and Methods:

The method for preparing date fibers involves manually removing the pits, then washing the date palm with plenty of hot water to remove sugars and impurities. The solid material is then dried at a low temperature (50–60°C) to reduce moisture content, and finally ground into a fine powder. The powder is stored in an airtight container to prevent moisture and heat. This method is effective in extracting high-quality microfibers and can be improved by adding steps such as chopping before drying or using natural preservatives. The resulting fibers may be used in the food or medical industries. [13].

One gram of residue powder was taken, and a suitable solvent (such as HPLC-grade methanol) was added to prepare the sample for HPLC analysis. After that, ultrasound was used to completely mix the material in order to improve extraction. After that, it was centrifuged at 4,000 rpm for ten minutes. Before being injected into the HPLC equipment, the solution was filtered through a 0.45 μm filter to exclude any solid particles. This technique maintains the integrity of the fibers and active chemicals while guaranteeing the production of exact, chromatographically analyzable molecules [14].

Chemical Tests and HPLC Tests

Preparation Method:

Proximate Analysis

All chemical analyses were carried out according to standard methods recommended by the Association of Official Analytical Chemists [15].

Moisture Content

Moisture content was determined using the oven-drying method. Approximately 5 g of the sample was weighed into pre-weighed dishes and dried in an oven at 105°C until a constant weight was obtained. Moisture percentage was calculated as follows:

Moisture (%) = (Initial weight − Final weight) / Initial weight × 100 [15]

Carbohydrates

Carbohydrate content was calculated by difference as follows:

Carbohydrates (%) =
100 − (Moisture + Protein + Fat + Ash) [15]

Crude Fiber

Crude fiber was determined by sequential digestion using 1.25% sulfuric acid followed by 1.25% sodium hydroxide. The residue was filtered, washed, dried at 105°C, and then ashed at 550°C. Fiber content was calculated from the weight difference before and after ashing [15].

Ash Content

Ash content was determined by incinerating the samples in a muffle furnace at 525°C for 6 h until a light gray or white ash was obtained. The ash percentage was calculated gravimetrically (AOAC, 2019).

Crude Fat

Crude fat was determined using a Soxhlet extraction apparatus with petroleum ether as a solvent for 4–6 h. The solvent was evaporated, and the residue was dried at 80°C. Fat content was calculated based on weight difference [15].

Crude Protein

Crude protein content was determined using the Kjeldahl method. About 0.3 g of the sample was digested with concentrated sulfuric acid, followed by distillation and titration using hydrochloric acid (HCl). The protein content was calculated by multiplying the nitrogen content by a conversion factor of 6.25 [15].

Figure 1. Chemical analyses of the sample

Chemical composition	%
Humidity	84.61%
Protein	4.375%
Fats	0.6%
Ashes	2.32%
Carbohydrates	75.705g
Fibers	7%

HPLC Tests

Vitamin B12

Vitamins were analyzed using High-Performance Liquid Chromatography (HPLC) according to the method described by [15]. Samples were extracted using both aqueous and alcoholic solvents, filtered through a 0.22 µm membrane filter, and directly injected into the HPLC system.

0.01mg was weighed and dissolved in methanol in a 20 mL volumetric flask to obtain a concentration of 1000 ppm.

B7 - Preparation Method:

0.01mg was weighed and a few drops of DMSO were added to dissolve the vitamin. Methanol was then added to a 20 mL volumetric flask to obtain a concentration of 1000 ppm. Ideal conditions: Quercetin/B12/Apgenin, Column: Hyper 0DS2 C18, Mobile phase: 50% Acetonitrile + 50% Methanol, Flow rate: 1.5 mL/min, UV Detector: 210 nm.

Apigenin - Preparation Method:

Plant sources (such as Adinandra nitida or possibly date palm extracts) can yield apigenin, a bioactive flavonoid, by extracting it with boiling water and then hydrolyzing the sugar moieties with acid. To obtain pure apigenin crystals, the resultant yellow precipitate is filtered and then recrystallized with ethanol.

General Extraction and Preparation Method (Plant Source Example):
Extraction: Two times, dried leaves (or plant material) are extracted using boiling water (e.g., 1500 mL water per 150g of raw material) for an hour. Filtration comes next. Hydrolysis: To turn glycosides into pure apigenin, the extract is acidified (for example, using sulfuric acid in a 1:50 volume ratio) and heated.

Quercetin - Preparation Method:

• Date fruit (or its waste) can be used to make quercetin by extracting flavonoids with alcoholic solvents (ethanol or methanol), then breaking down quercetin glycosides into aglycone quercetin through acidic hydrolysis. Extraction, concentration, acid hydrolysis (HCl or H_2SO_4), purification (crystallization), and drying are the usual steps in the process. To obtain crude flavonoids, dried, powdered date fruit is treated with an alcoholic solvent (such as ethanol) using Soxhlet or ultrasound-assisted extraction.

• Hydrolysis: To hydrolyze quercetin glycosides, the crude extract is heated (refluxed) after being treated with 10% sulfuric acid (H_2SO_4) or concentrated hydrochloric acid (HCl).

Results

As seen in Figure 1, apgenin was examined under established ideal circumstances with a retention period of 2.25 minutes. Chromatograph examination of Apgenin standard material.

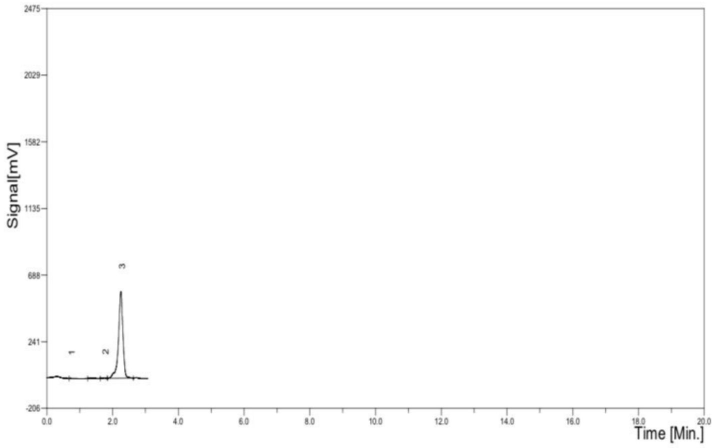

Figure (1) Chromatograph of the HPLC analysis of the reference material Apgenin with a 2.25-minute retention period

Apgenin was found at peak number five when the extract was analyzed as depicted in Figure (2). By dividing the peak area of the standard material by the peak area of the extract, quantitative analysis was carried out. Apgenin had a concentration of 16250 μg/mg

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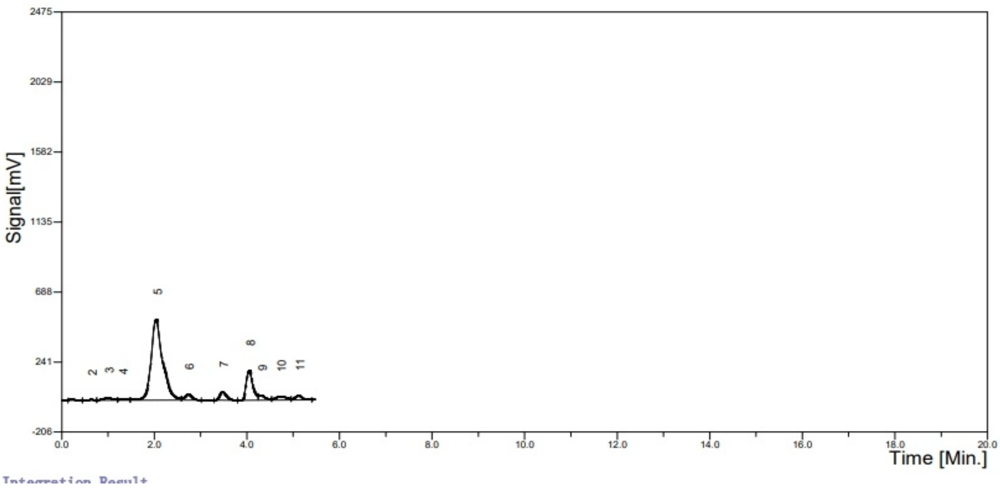

Figure (2) Chromatograph of Apgenin sample analysis by HPLC, the concentration of Apgenin was 16250 µg/mg

As seen in peak 1 of Figure 3 (Chromatograph of B12 standard analysis), B12 was examined under established ideal circumstances, with a retention time of 2.04 minutes. The extract was examined as shown by peak 3 in Figure 4 (extract analysis chromatograph). By dividing the peak area of the extract by the peak area of the standard, quantitative analysis was carried out. The B12 content was 606061 µg/mg. Ideal circumstances for B12:

Column: Hyper 0DS2 C18

Mobile phase: 50% Acetonitrile + 50% Methanol

Flow rate: 1.5 ml/min

U.V. Detector: 220 nm

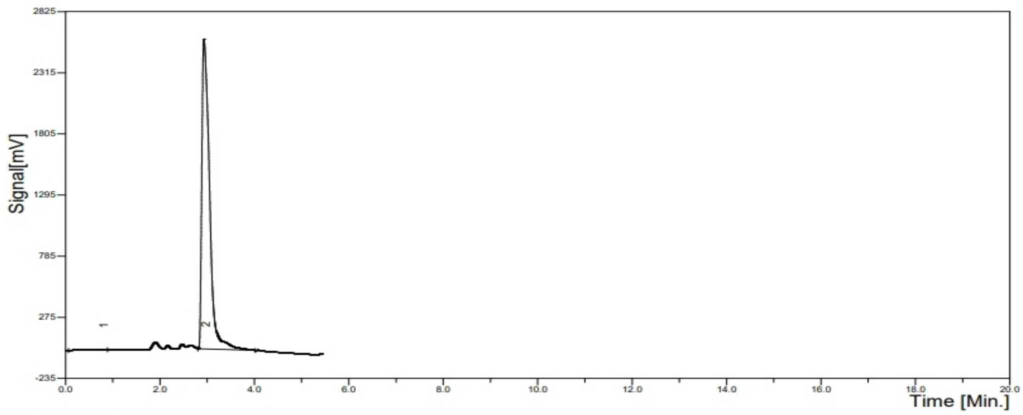

Figure (3) Chromatograph of standard substance B12 analysis by HPLC

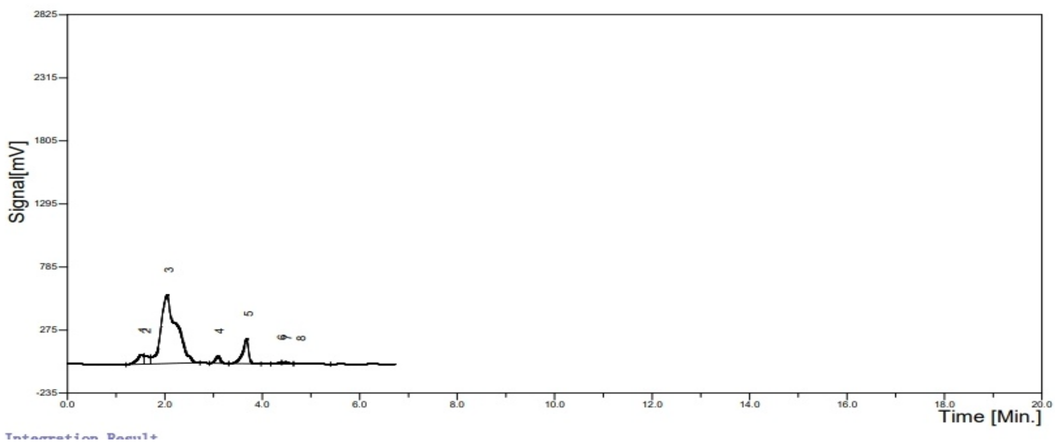

Figure (4) Chromatography of standard substance analysis B12 by HPLC, the concentration of B12 was 6060 ug/mg

As seen in Figure (5), B7 was examined under ideal circumstances with a retention time of 2.17 minutes. HPLC chromatograph of B7 Standard Analysis. B7 was found at peak number 3 after the extract was analyzed as depicted in Figure (6) at peak number 5. By dividing the peak area of the extract by the peak area of the standard, quantitative analysis was carried out (B7). 10699 µg/mg was the concentration.

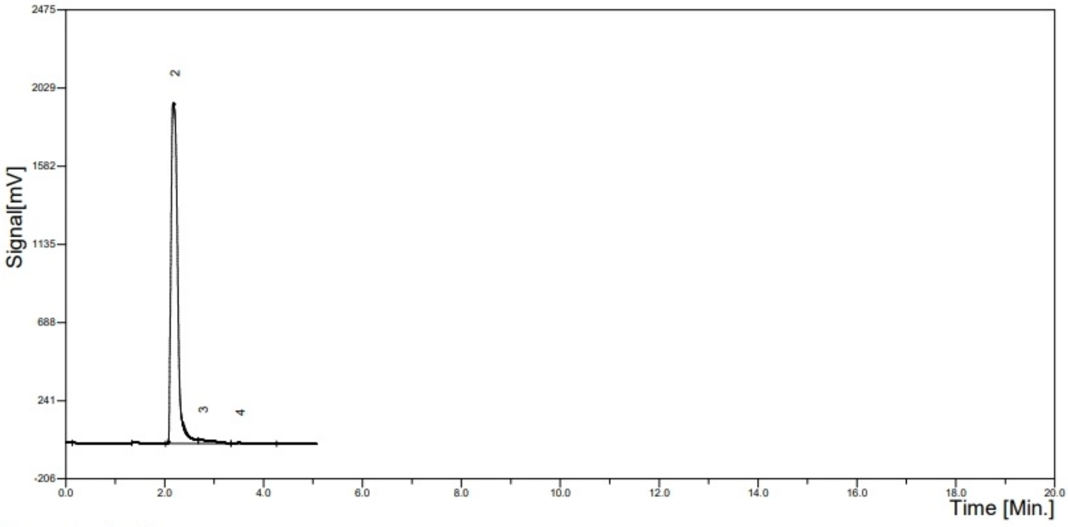

Figure (5) Chromatography of standard substance analysis B7 by HPLC

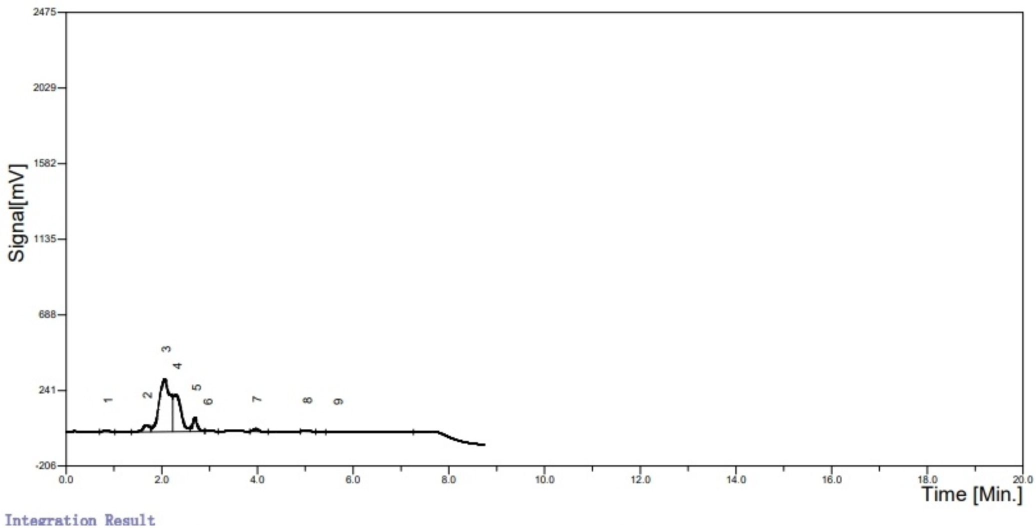

Figure (6) Chromatograph of B7 Extract Analysis by HPLC The concentration was 10699 µg/mg

As seen in Figure 7, quercetin was examined under ideal circumstances with a retention period of 5.5 minutes. Quercetin standard chromatograph analysis was carried out at peak 2 using HPLC. Quercetin was discovered at peak 3 when the extract was examined, as seen in Figure 8. By dividing the peak area of the extract by the peak area of the standard, quantitative analysis was carried out.

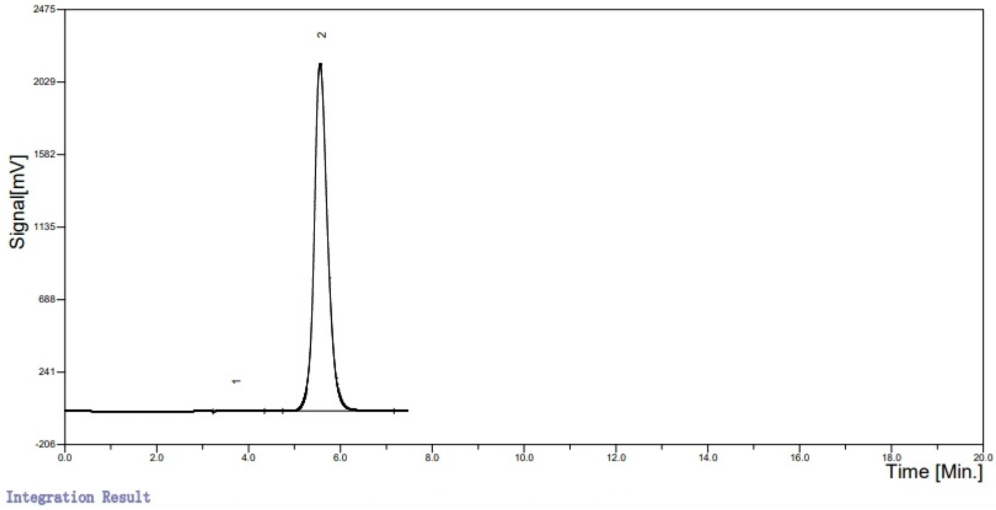

Figure (7) Chromatograph of the analysis of the standard substance Quercetin by HPLC

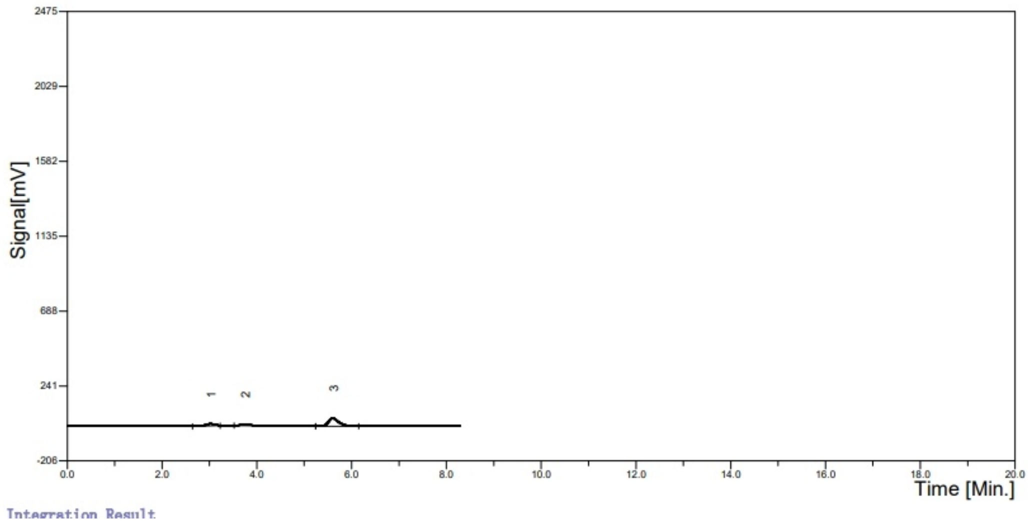

Figure (8) Chromatograph Analysis of Quercetin Extract by HPLC, the concentration of quercetin was 15658 µg/mg.

Discussion

The identification of chemical constituents in date extracts via HPLC analysis has been the subject of numerous investigations. In fact, the study by Djaoudene *et al.* (16) evaluated the levels of Quercetin, B7, B12, and Apgenin in the methanolic extracts of cultivars of Phoenix Date palm (Phoenix dactylifera L.) growing in Iraq and found that the chromatographic analyses of the Iraqi variations of Phoenix dactylifera L. demonstrated the predominance of the carotenoid pigments, which are represented by β-carotene and lutein. Date fruit and its byproducts as a potential source of bioactive components. The geographical origin of the dates' extracts and the extraction technique can be blamed for the discrepancies between these and the study's findings. Furthermore, differences in the date extract's phenolic constituents may be caused by the solvent (17). The DPPH and reducing power tests were used to assess and compare the improved extract's antioxidant activity. RP-HPLC-PAD-ESI-MS/MS assays that characterize the metabolic profile of date extracts (18). This study demonstrated dates' interest in the context of technological exploitation and provided scientific validation for their usage in traditional medicine. Future research will concentrate on the large-scale extraction and optimization of the chosen phytonutrients from dates and their application as food additives (19).

The value of the remaining date palm tree parts, which are lost and thrown away throughout the harvesting, storage, commercialization, and conditioning processes, is not taken into account by the strong demand for date fruits (20). These components are referred to as "by-products" or "co-products." The manufacturing of date seed coffee or its use as animal feed or soil fertilizer (compost) are some traditional use for date by-products. It is important to note that the MENA region produces more than 2.7 million tonnes of garbage annually, with a single date palm tree yielding about 26 kg (21). Leaves, leaflets, mesh, midribs or rachis, spadix stems, midrib base or petiole, and pits are examples of these leftovers. The majority of them are burned or just dumped in landfills, which causes serious environmental issues. In fact, the growing number of articles on "agro by-products" and "date by-products" in Scopus indicates a significant interest in agro-industrial by-products, particularly date palm by-products (22). When comparing various date by-product components at the time of review, "date leaves" had the most scientific articles, followed by "date seeds," "date pollen," and "date press cake." Date seeds and leaves are becoming more and more popular since they are readily available and rich in a variety of bioactive and/or functional components, such as phenolics and dietary fibers. Date palm seeds do, in fact, have a high polyphenolic content. Protocatechuic acid, p-hydroxybenzoic acid, caffeic acid, and some flavanols, such as catechin, epicatechin, and procyanidins B, are predominant. Additionally, because of its high lignocellulosic matter content, date leaves are regarded as a valuable waste (23). Date variety, growing conditions, geographic origin, climate, and extraction technique all have a significant impact on the type and quantity of these active chemicals (24). Numerous research have demonstrated how the extraction method and process factors (solvent type, date by-product-solvent ratio, temperature, time, and number of extractions) affect the extraction yield of phenolic compounds (25). Conventional extraction techniques, such as the traditional maceration process in organic solvents (26), or novel extraction techniques, referred to as "green," like ultrasonication assisted extraction (UAE), subcritical water extraction (SCWE), and supercritical fluid extraction (SFE), have been used to extract bioactive compounds, primarily phenolic compounds (27).

Conclusion

The biochemical compositions and antioxidant qualities of the active compounds in date seed oils made from Iraqi date palm cultivars are thoroughly described in this work. The province, which is Iraq's most productive area for the production of dates and their byproducts, is home to the majority of the investigated kinds. The chromatographic analysis of the date seed oils was followed by the antioxidant properties of quercetin, B7, B12, and apgenin extract.

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Authors' contributions
All the aspects of this study were fully the responsibility of each author and they were involved in data analysis, writing and editing of the article.

Conflict of interest

All authors declare that there are no competing interests.

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