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Phytochemical Profiling of Almond Shells: Comparative Analysis of Different Solvent Extracts

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Abstract

This study investigates the phytochemical profiles, UV-Vis spectrophotometry, IR spectroscopy, and HPLC analyses of local almond shells extracts using different solvents, including deionized water (DW), ethanol, and a mixture of DW:ethanol (1:1). All extracts contained flavonoids, alkaloids, glycosides, carbohydrates, coumarins, phlobatannins, phytosterols, phenols, quinones, resins, saponins, terpenoids, and triterpenoids, with different concentrations. The DW:ethanol mixture extract also showed the presence of anthraquinones, proteins, and amino acids, which were absent in the other extracts. The DW extract exhibited the highest total flavonoid content (117 mg QE/g), whereas the total polyphenol concentration was greatest in the mixture extract (711 mg TAC/g). The FTIR spectra revealed distinct functional peaks for aromatic rings, unsaturated hydrocarbons, esters, carboxylic acids, ketones, phenols, and alcohols in all extracts. HPLC analysis identified seven prominent phenolic compounds in all extracts, namely benzoic acid, cinnamic acid, gallic acid, ferulic acid, quercetin, kaempferol, and naringenin, with varying concentrations. This study highlights the potential of almond shells as a significant source of bioactive compounds that can function as capping and reducing agents in the environmentally friendly synthesis of nanomaterials. The potential of compounds like flavonoids suggests that almond shell extract is a promising candidate for developing antioxidant drugs.

Keywords: almond shells extract; phytochemicals; total phenol flavonoid contents; spectroscopy; HPLC

1. Introduction

The almond (*Prunus dulcis*), a widely cultivated nut species, belongs to the Rosaceae family and is a stone fruit with a hull, kernel, skin, and shell [1]. Biomass nut shells, high-yield agricultural byproducts accounting for 35-75% of the fruit weight, are often discarded, polluting the environment and wasting significant resources [2]. The large volume of shells holds significant practical and economic value, and research into their optimal utilization is gaining increasing attention. Generally, biomass nutshells are being researched for potential uses such as producing activated carbon, magnetic nanosorbents, and composites [3]. They have been used for water purification, as nanosorbents for heavy metals, to improve soil fertility, to replace fossil fuels, and to decrease greenhouse gas emissions [2].

Numerous bioactive compounds have been reported in almond shells, such as cellulose, lignin, tannins, polyphenols, polysaccharides, and fatty acids [1,4,5]. Generally, the extraction of phytochemicals and bioactive compounds from plant sources is strongly influenced by the solvent used; polar solvents, such as water and ethanol, are more effective for polar substances like

polysaccharides, sugars, flavonoids, alkaloids, and some phenolic compounds [6]. Different solvents can influence the types and amounts of bioactive compounds extracted, thereby affecting the observed biological activities, such as antioxidant and anti-inflammatory properties [6,7].

It has been reported that the water extract of almond shells contains high levels of hydrocarbons and ester compounds, in addition to phenols and terpenes, which are vital components of fragrances [2]. The almond shell contains bioactive compounds with antioxidant and anticancer properties. A recent study indicates that selecting almond varieties based on their bioactive compound profiles can enhance their health benefits and applications in the pharmaceutical and food sectors [8]. Geographical location is another critical factor influencing the phytochemical composition of plants, with factors like climate, soil composition, and seasonal changes, which can have significant implications for their medicinal and nutritional value [9]. For instance, warmer climates cause plants to synthesize more secondary metabolites, such as phenolic compounds [10].

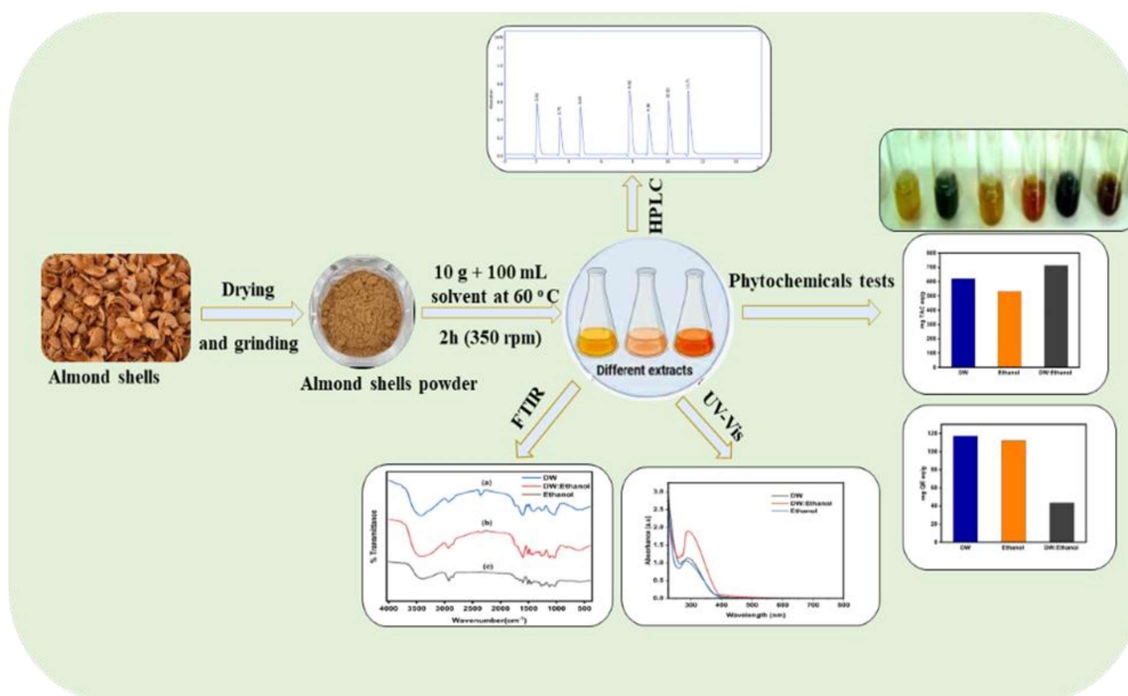

Figure 1. Monitoring results of different solvent extracts from almond shells.

Biomass waste can be transformed into valuable resources, reducing environmental impact and providing sustainable solutions. This study investigates the chemical composition of almond shells using various solvent extracts, including deionized water (DW), ethanol, and a mixture of DW:ethanol. This work differs from previous studies by focusing on almonds cultivated in Duhok city (a city in the Kurdistan Region, Iraq). Plants from different geographical areas can have significantly different chemical compositions, even if they are the same species. The aim of this work is to explore the preliminary phytochemical screening, flavonoid concentration, total phenolic content, UV-Vis spectrophotometry, IR spectroscopy, and HPLC analyses of various solvent extracts of the local almond shells, highlighting their potential as an eco-friendly bio-resource for applications such as the green synthesis of nanomaterials, developing antioxidant drugs, and others. Figure 1 illustrates the experimental work.

2. Materials and Methods

2.1. Materials

Fresh almond fruit was collected from the Bagera area around Duhok city in the Kurdistan region of Iraq in August 2024 and dried for the next 10 days. Tannic acid and picric acid were purchased from Fluka, USA. Hydrochloric acid, sodium hydroxide, acetic anhydride, and ninhydrin were purchased from Merck, Darmstadt, Germany. Ammonia solution, chloroform, potassium acetate, glacial acetic acid, and sodium carbonate were bought from ROTH, Karlsruhe, Germany. Sulphuric acid and ethanol were obtained from Scharlau, Barcelona, Spain. Ferric chloride and aluminum chloride were purchased from Labpak chemicals LTD, Mill Lane Fillongley, UK. Lead acetate was obtained from Alpha Chemika, Mumbai, India, and methanol was bought from Analar normapure, France. Alcoholic α-naphthol was purchased from CDH, New Delhi, India. Quercetin and Folin-Ciocalteu reagents were purchased from Sigma-Aldrich, USA.

Table 1. Protocols for the phytochemical screening study of almond shell extracts.

No	Test	Method	Indication
1	Alkaloids	Add 1 mL of Hager's reagent (a saturated solution of 2% picric acid) to a test tube holding 1 mL of all extracts.	Yellow precipitate [11]
2	Anthocyanins	To each 2 mL of extracts, 2 mL of ammonia and 2 mL of 2N hydrochloric acid were added.	The color shifts to blue-violet rather than pink-red [12]
3	Anthraquinones	A few drops of a 10% NH_3 solution were added to one milliliter of each extract.	Pink precipitate [13]
4	Carbohydrates (Molisch's test)	The extract was exposed to alcoholic α-naphthol drops. 0.2 mL of concentrated sulphuric acid was thereafter added with precision to the side of the test tube.	Appearance of a purple to violet ring at the boundary between the two liquids [14]
5	Cardiac glycosides	One drop of 5% ferric chloride and 2 mL of glacial acetic acid were combined with 5 mL of each extract. Then 1 mL of concentrated H_2SO_4 was added.	Violet ring [15]
6	Coumarins	2 mL of each extract was combined with 3 mL of 10% sodium hydroxide.	Yellow color [16]
7	Flavonoids	A small quantity of 10% lead acetate solution was added to each extract.	Yellow precipitate [17]
8	Glycosides	5 mL of diluted 10% H_2SO_4 was mixed with 1 mL of extract in a test tube, and then placed in a water bath for 15 minutes. After cooling, 20% sodium hydroxide was introduced, followed by the addition of 5 mL of Fehling's reagent, and the mixture was heated for 5 minutes.	Reddish precipitate [18]
9	Phlobatannins	Boil each extract with diluted 1% HCl.	Crimson precipitate [19]
10	Phytosterols	1 mL of each extract was taken, coupled with 1 mL of acetic anhydride and chloroform. After that, three drops of concentrated H_2SO_4 were added, and it was thoroughly shaken.	Pink hue that later turned to bluish-green [20]
11	Proteins and amino acids	2 mL of each extract was combined with two drops of a 2% ninhydrin solution.	Purple color [21]
12	Phenolics	One or two drops of 5% $FeCl_3$ were added to 1 mL of each extract. Gently stir the contents of the test tube.	Color shift appearing, such as turning blue, green, purple, or red [11]
13	Quinones	Concentrated sulfuric acid was combined with an equal volume of the extract, followed by gentle mixing.	Red color [22]
14	Resins	1 mL of each extract was combined with a few milliliters of acetic anhydride, then 1 mL of concentrated H_2SO_4 was added.	Orange to yellow color [19]
15	Saponins	3 mL of each extract was amalgamated with 3 mL of distilled water, and the mixture was agitated briskly for approximately one minute following the addition of three drops of olive oil to the mixture, which was then thoroughly agitated	Formation of an emulsion [23]
16	Tannins	1 mL of each extract should be mixed with 1 or 2 drops of 5% ferric chloride. Stir the contents of the test tube gently.	The appearance of a hue shift, such as red, blue, purple, or green [11]
17	Terpenoids	Combined 0.5 mL of each extract with 2 mL of chloroform and 3 mL of sulfuric acid.	Appearance of a reddish-brown hue [24]
18	Triterpenoids	Two milliliters of each extract were individually mixed with 1 mL of sulfuric acid and one milliliter of chloroform.	The color turns purple or red [17]

2.2. Plant extraction

After hulling and shelling, the almond shells were thoroughly rinsed with flowing tap water, distilled water, and deionized water (DW) to eliminate contaminants, followed by air drying at 25°C for 24 h, grinding into small particles using an electrical grinder, and sieving (38 µm, 400 mesh), resulting in a powder that was stored in a refrigerator (4°C) for future use. To prepare the extracts, 10 g of powdered almond shell was dissolved in 100 mL of different solvents (pure ethanol, DW, and a mixture of DW:ethanol (1:1)), followed by heating at 60°C on a magnetic heating stirrer for two hours with constant stirring (350 rpm). Subsequently, each extract was subjected to filtration utilizing Whatman filter paper No. 1, followed by centrifuging for 20 min at 8000 rpm to obtain pure extract, which was stored at 4°C for future usage.

2.3. Phytochemical screening

The phytochemical evaluation was performed on three extracts using the standard protocols outlined in Table 1.

2.4. Total phenolic content estimation

The total phenolic content in all extracts was measured using the Folin-Ciocalteu method with tannic acid as a standard calibration curve (50-500 µg/mL) [25]. Almond shell extracts were prepared by dissolving 10 mg of the dried extract in 5 mL of each solvent (water, ethanol, and DW:ethanol). A calibration curve was established by combining 0.5 mL aliquots of each tannic acid solution concentration and the produced extract in a test tube, followed by the addition of 2.5 mL of ten-fold diluted Folin-Ciocalteu reagent, and then 2 mL of 7.5% Na_2CO_3, mixing thoroughly, and then incubating for half an hour at 40 °C. The absorbance was measured at 760 nm using a UV-Vis spectrophotometer at 760 nm. The tannic acid equivalent (TAC eq) was determined using the calibration curve equation (n = 3). The following formula was employed to ascertain the total phenolic content (TPC) in mg/g of each extract:

$$TPC = \frac{cV}{m} \quad (1)$$

TPC is expressed in milligrams of tannic acid equivalent per gram of extract (mg TAC eq/g), where c is the phenolic concentration in mg/mL derived from the calibration curve, V is the volume of the extract in mL, and m is the weight of the extract in grams.

2.5. Estimation of total flavonoid content

Quercetin (QE) was used as a standard to evaluate total flavonoid content (TFC), employing a calibration curve at concentrations of 10, 20, 30, 60, 90, 120, 150, and 180 µg/mL. The extracts were prepared by dissolving 10 mg of each dried extract in 10 mL of methanol. Subsequently, 0.5 mL of each extract, diluted quercetin standard, 1.5 mL of methanol, 0.1 mL of 1 M potassium acetate, 0.1 mL of 10% aluminum chloride, and 2.8 mL of distilled water were individually added to test tubes. The mixtures were carefully mixed and allowed to equilibrate at 25 °C for 30 minutes. Thereafter, the absorbance was measured at 415 nm. For the blank, the 10% of aluminum chloride solution was substituted with distilled water. Quercetin equivalents were determined using the regression equation of the curve (n = 3) [26].

$$TFC = \frac{cV}{m} \quad (2)$$

TFC is expressed in milligrams of quercetin equivalent per gram of extract (mg QE eq/g), where c is the flavonoid concentration in mg/mL obtained from the calibration curve, V is the volume of the extract in mL, and m is the weight of the extract in grams.

3. Results and Discussion

3.1. Preliminary phytochemical analysis

The qualitative phytochemical screening examination of almond shells using different solvents is displayed in Table 2.

Table 2. Qualitative phytochemical screening analysis of almond shell extracts.

No	Test	DW	DW:Ethanol	Ethanol
1	Alkaloids	++	++	+
2	Anthocyanins	-	-	-
3	Anthraquinones	-	+	-
4	Carbohydrates	+	++	++
5	Cardiac glycosides	+	++	++
6	Coumarins	+	+	+
7	Flavonoids	++	++	++
8	Glycosides	-	++	+
9	Phlobatannins	++	++	++
10	Phytosterols	++	++	++
11	Proteins and amino acids	-	+	-
12	Phenolics	+	++	+
13	Quinones	++	++	++
14	Resins	+	++	++
15	Saponins	++	++	+
16	Tannins	+	+	-
17	Terpenoids	+	++	++
18	Triterpenoids	+	++	++

(++) presence of high concentration, (+) presence in moderate concentration, (-) absent.

The findings indicated that a high concentration of these bioactive compounds, including alkaloids, flavonoids, phlobatannins, phytosterols, quinones, and saponins was present in the deionized water (DW) extract. Additionally, secondary metabolites such as coumarins, carbohydrates, phenols, cardiac glycosides, resins, tannins, terpenoids, and triterpenoids, appear less frequently. Even so, the DW extract lacked proteins, amino acids, glycosides, anthocyanins, and anthraquinones due to their low water solubility [28]. Conversely, the mixed extract of DW:ethanol showed that the majority of bioactive compounds were present in high concentrations, including alkaloids, carbohydrates, cardiac glycosides, flavonoids, glycosides, phlobatannins, phytosterols, phenolics, quinones, resins, saponins, terpenoids, and triterpenoids. It also confirmed the presence of anthraquinones, coumarins, tannins, proteins and amino acids in moderate amounts. However, anthocyanins were absent from the mixed extract.

In the ethanol extract, secondary metabolites such as carbohydrates, cardiac glycosides, flavonoids, phlobatannins, phytosterols, quinones, resins, terpenoids, and triterpenoids were present in high concentrations. Bioactive compounds including alkaloids, coumarins, glycosides, phenolics, and saponins, were present in moderate amounts. While anthocyanins, anthraquinones, tannins, proteins, and amino acids were absent. The results indicate that anthraquinones, which are hydrophilic compounds, are present in the balanced polarity mixture extract and absent in the other extracts. This finding is corroborated by other studies, including those by Dulo *et al*. and Olabanji [29,30], which demonstrate that anthraquinones exhibit moderate polarity due to their structure. Multiple studies indicate that water, due to its greater polarity compared to ethanol, is more effective at extracting bioactive compounds, particularly those with high solubility in aqueous environments. Also, aqueous-alcoholic solvents containing ethanol and water can be more efficient at extracting a wider range of bioactive compounds [31], such as carbohydrates, organic acids, and amino acids [32,33]. Water molecules interact with the polar groups of amino acids and proteins, while the ethanol interacts with less polar groups. Plant phytochemicals, such as alkaloids, flavonoids, phenols, and tannins, are known to neutralize free radicals and are increasingly recognized as potential antioxidants by researchers and consumers [34]. The findings of this study evaluate the potential of almond shells as a source of natural drugs and contribute to understanding their traditional medicinal applications.

3.2. Total phenolic content

Phenolic chemical substances are essential plant constituents possessing redox characteristics that augment antioxidant action [35]. The phenolic and flavonoid constituents of plant extracts are thought to function as stabilizing and reducing agents in the biological synthesis of nanomaterials [36]. The Folin-Ciocalteu technique is often used to determine total phenolic content (TPC) by measuring electron transfer before more detailed analyses such as HPLC. This method is simple, fast, and produces precise results. It involves reducing the Folin-Ciocalteu reagent with phenolic chemicals in an alkaline environment, predicated on an electron transfer from phenolic compounds, resulting in a blue-colored complex with a maximum absorbance at 760 nm, directly proportional to the TPC, which is reported as tannic acid equivalent in milligrams per gram of dry extract (mg TAC eq/g) [37]. The concentration of tannic acid in each extract was determined by the equation of the calibration curve using their absorbance ($y = 0.0008x-0.0251$, $R^2 = 0.9858$).

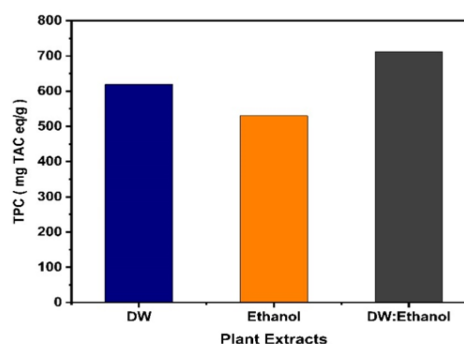

Figure 2. Total phenolic content of almond shell.

The results in Figure 2 illustrate that the maximum phenolic content was found in the mixture of DW:ethanol extract (711 mg TAC eq/g), followed by the DW extract (619 mg TAC eq/g), and the lowest in ethanol extract (530 mg TAC eq/g). Phenolic compounds, mainly hydrophilic antioxidants, indicate that a mixture of ethanol and water is the most effective solvent for extracting phenolic compounds from almond shells, owing to hydrogen bonding at polar sites. The presence of the ethyl radical reduced ethanol's effectiveness, leading to reduced solvation of phenol molecules. However, when mixed with water, solvation increases [38]. It has been reported that extraction efficiency can be improved by using solvent mixtures. Using a mixture of ethanol and water has a greater ability to extract phenolic compounds than ethanol alone [39]. An ethanol–water mixture yields maximum phenolic content due to the combination creates a solvent with an ideal polarity for dissolving the majority of these compounds.

3.3. Total flavonoid content

Flavonoids, a unique polyphenolic subclass of secondary plant metabolites, are crucial for human health due to their applicability in pharmaceutical, cosmetic, and nutritional sectors [40]. Total flavonoid content (TFC) assays quantify the amount of flavonoids in almond shell extracts, providing insights into potential antioxidant or biological activities [36]. These chemicals demonstrate diverse pharmacological effects, including antibacterial, antioxidant, antifungal, anti-inflammatory, and cytotoxic characteristics [41]. The aluminum chloride colorimetric test is a commonly used technique for determining the total flavonoid content (TFC) in plant extracts after solvent extraction. Flavonoid content was calculated from a quercetin calibration curve equation (y = 0.0019X - 0.0027 and R^2 = 0.9927), and the results of total flavonoid content for each extract were expressed as milligrams of quercetin equivalents (QE eq) per gram of extract.

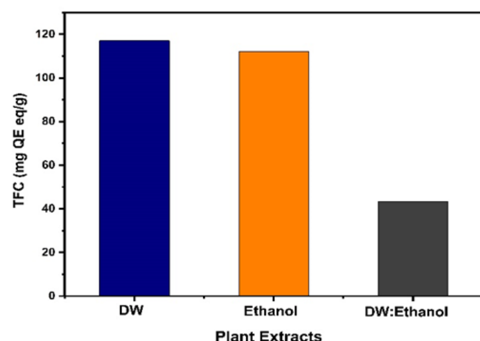

Figure 3. Total Flavonoid content of almond shell.

As shown in Figure 3, the results demonstrated that the highest flavonoid content was found in DW (117.05 mg QE eq/g) and pure ethanol extract (112.05 mg QE eq/g), and the lowest amount of total flavonoid content was found in the mixture extract (43.32 mg QE eq/g). The reduced flavonoid content in the mixture extract may result from the solvent's polarity and variations in flavonoid chemical composition, which influence solubility and extraction efficiency [42–44]. The optimal solvent extraction depends on the polarity and solubility of the targeted compound, as there are no standardized solvent compositions for all compounds [45]. It has been reported that ethanol and water are commonly used solvents for extracting flavonoids due to their capacity to inexpensively and environmentally solubilize moderately polar flavonoids [46].

3.4. UV-Vis analysis

All extracts prepared from almond shells were analyzed using UV-visible spectroscopy. All extracts were diluted 1:20 due to their high concentration. As illustrated in Figure 4, the UV-Vis spectra show that the mixture extract exhibits the highest absorbance at 287 nm, followed by the DW extract at 288 nm and ethanol at 283 nm. This can be explained by the bathochromic shift in DW and the mixture extract, indicating increased stabilization of the excited state of the almond shells due to solvent polarity.

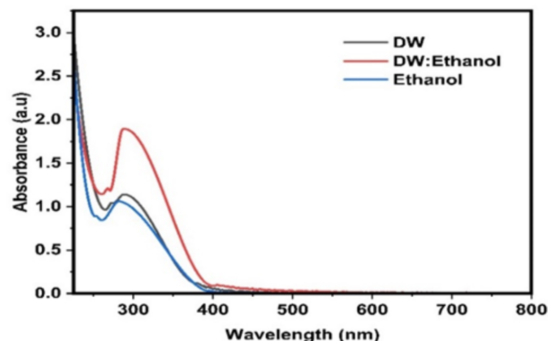

Figure 4. UV-Vis spectra for extracts of almond shell using different solvents.

Electronic transitions in benzene and its derivatives, which may include phenolics and other aromatic compounds, are responsible for absorption in the 270 to 290 nm range [47,48]. In general, such phenolic molecules share the feature of having an aromatic ring with one or more functional side chains and substituent hydroxyl groups**.** The result illustrates that in all three extracts, there is evident absorbance around 300 nm, indicating that an electronic $\pi \rightarrow \pi^*$ transition occurs due to the presence of phenolic, flavonoid, and other aromatic rings.

3.5 Infrared analysis

The Fourier transform infrared (FTIR) spectrophotometer was used to scan the three almond shell extracts in the range of 500 to 4000 cm^{-1}, as demonstrated in Figure 5.

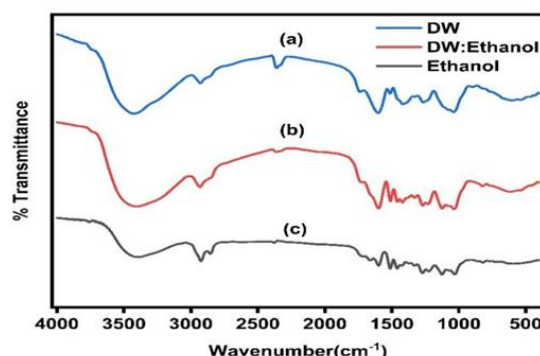

Figure 5. FTIR spectra for extracts of almond shell using different solvents.

A broad peak at 3427.51 cm^{-1} in DW extract (Figure 5a) corresponds to the hydroxyl group (-OH) stretching vibration in alcohols or carboxylic acid, peaks at 2927.94 cm^{-1} having saturated aliphatic (C-H) stretching vibration, a weak peak appears at 1734.01 cm^{-1} demonstrate the exist-

ence of a carbonyl group (C=O) stretching vibration from esters or ketones, and a peak at 1602.85 cm^{-1} indicates the presence of C=C of aromatic rings or alkenes, and a peak at 1039.63cm^{-1} belongs to the stretching vibration of C-O of ether or ester. In the spectrum of the extract of a mixture of ethanol and DW (Figure 5b), the stretching vibration of the O-H group corresponds to the absorption band at approximately 3406.29 cm^{-1}, often found within the range of 3200-3650 cm^{-1} for hydrogen-bonded O-H groups in phenols and alcohols.

Table 3. Quantity of identified phenolic compounds by HPLC analysis of the almond shells in three extracts.

Peak no	Rt. Of the standard solution (min)	Name of compound	Concentration (ppm)		
			DW	Ethanol	DW: Ethanol
1	2.05	Cinnamic acid	63.52	79.80	58.99
2	3.78	Naringenin	30.22	38.98	20.65
3	5.32	Ferulic acid	65.70	71.05	60.11
4	8.00	Gallic acid	99.80	114.58	92.66
5	9.35	Benzoic acid	37.80	43.58	30.22
6	10.30	Kaempferol	70.44	82.11	74.15
7	11.78	Quercetin	90.77	95.05	84.56

Although the broad peak is more characteristic of alcohols or carboxylic acids, it can also be attributed to asymmetric and symmetric N-H stretching vibrations in the presence of proteins and amino acids. There is a slight difference in the intensity of other peaks compared to the DW extract (Figure 5a), notably at at peaks 1598.99 cm^{-1}, which is sharper, indicating the presence of C=C stretching vibration for an aromatic ring or unsaturated hydrocarbons, with sp^2 hybridization [49]. The absorption band at peak 1124.50 cm^{-1} is attributed to the (C-O) group, corresponding to the presence of ester or ether. In the ethanolic extract (Figure 5c), a weak, broad peak appears at 3394.72 cm^{-1}, corresponding to the hydroxyl (OH) stretching vibration in alcohols or carboxylic acids. The most distinct differences appear in the absorption bands at 2924.09 and 2854.65 cm^{-1}, due to stronger, sharper C-H stretching peaks. Whereas peaks at 1597.06 and 1124.50 cm^{-1} were attributed to C=C and C-O groups, which are related to aromatic rings or unsaturated hydrocarbons, and to esters or ethers, respectively.

3.6. HPLC analysis of standard phenolic compounds

HPLC analysis was performed to detect and quantify seven phenolic compounds: benzoic acid, cinnamic acid, gallic acid, ferulic acid, quercetin, kaempferol, and naringenin, in all extracts under identical chromatographic conditions. The phenolic compounds and flavanols, which are groups of phytochemicals, are secondary metabolites typically present in almond shells and exhibit antioxidant properties due to their redox potential, functioning as reducing agents [50–52]. These compounds have diverse physiological properties, including antibacterial, anti-inflammatory, analgesic, and vasodilatory effects. Figure 6 displays chromatograms indicating the presence of phenolic chemicals, characterized by peaks with varying retention times in each extract. The identity of each peak was validated by comparing its retention time (Rt) with that of the standard solution (5 ppm) for each constituent, as presented in Table 3, along with the concentration of each compound in three extracts. The elevated levels in the ethanol extract are mostly attributable to ethanol's polarity and its ability to dissolve these compounds from almond shells. Due to the existence of these phenolic compounds, almond shells may be considered a potential natural antioxidant source, serving as a promising option for the development of antioxidant pharmaceuticals for the management of several oxidative stress-associated illnesses.

4. Conclusion

This study explores an environmentally friendly approach to recycling almond shells waste, focusing on almonds cultivated in Duhok city, investigating the presence of biologically active compounds of almond shell in three extracts, namely deionized water (DW), ethanol, and a mixture of DW:ethanol (1:1). The qualitative phytochemical examination of each of these extracts revealed that the mixture of DW:ethanol extracts exhibited a significantly higher response rate in almost every assay than the DW and ethanol extract. Whereas the quantitative test results demonstrate that the DW extract has higher total phenolic content, a mixture of DW and ethanol extract shows higher total flavonoid content. UV-visible and FTIR spectra showed better results with a mixture extract. HPLC analysis confirmed the presence of seven phenolic compounds, namely benzoic acid, cinnamic acid, gallic acid, ferulic acid, quercetin, kaempferol, and

naringenin, in all extracts, with the highest concentrations in the ethanol extract. The present study revealed that almond shells, especially in a mixture of DW:ethanol extract, contain a variety of bioactive compounds, indicating their potential for green synthesis of nanomaterials, and may serve as a promising source for antioxidant applications and other fields.

Author contributions

Bjeen Hakim Hasan conducted the practical work, data collection and drafted the manuscript, while **Nidhal Meena Sher Mohammed** oversaw the final revisions, data validation and project coordination. Both authors approved the manuscript for publication

Conflicts of interest

The authors affirm that they have no conflicts of interest in the publication of this work.

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