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Ultrasonic extraction of bioactive compounds from almond peels and assessment of their antioxidant activity

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Abstract

The antioxidant activities of almond peels extracts were evaluated and compared with the antioxidant Butylated Hydroxy Toluene (BHT) using the following tests: reducing power, beta-carotene bleaching, hydrogen peroxide scavenging activity, and F.TC.. almond peels were extracted with different polarities solvents (methanol , ethanol, ethyl acetate, chloroform and hexane). The properties of extraction solvents significantly affected Extraction yield , total phenolic compound and antioxidant activity of peels extract, methanol (80%) as extraction media give highest extracted yield of crude extract from peels, also the methanolic extract showed highest content of total phenolic compounds compared with other extraction solvents used in study .The methanolic crude extract of peels has better antioxidant activity compared with samples treated with extracts by other extraction solvents . The antioxidant activities can be arranged as follow: BHT > Extracted with methanol> Extracted with ethanol >Extracted with ethyl acetate > Extracted chloroform > Hexane extracted.

Key words**:** Antioxidant activity, Almond peels, BHT, Phenolic compounds, Solvent extraction

I. Introduction:

Oxidative rancidity of vegetable oils and the foods containing them leads to rancid taste, non-enzymatic browning, reduced nutritional value, and unsafe products for human consumption (Kasaai, 2025). Synthetic antioxidants are added to inhibit oxidative rancidity in oils and products containing them, most notably Butylated Hydroxyanisole (BHT), Butylated Hydroxy Toluene (BHA), and Tetrabutyltin Hydroxy Quinin (TBHQ) (Barzegar *et al*., 2026). Antioxidants are defined as chemical substances that significantly slow down or prevent the oxidation of certain compounds and oxidative reactions when present in small quantities, even though they themselves are frequently oxidized. Free radical reactions, through the generation of peroxides and their derivatives, are fundamental to the oxidative destruction of fats, which can lead to the development of undesirable tastes in foods (Shahidi & Hossain 2022).

Almond (*Prunus amygdalus*) is a tree species within the family Rosaceae, mostly cultivated for its edible seeds. The world production of almonds reached 3.5 million lots in 2019, with the main producers being the USA, Australia, Iran, and Italy (Pang *et al.,* 2026). Almond production produces a large spinoff in almond shell size

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(Prgomet *et al.,* 2017), ranging from 35% to 62% of the fresh weight of almonds. Almond peel can be classified as a low-value by-product, used in general as animal feed, despite their high content of total phenolic compounds and their potential applications in functional food product development (Kahlaoui *et al*., 2019). Almond husks are also a source of antioxidants and have demonstrated free-radical scavenging properties (Wijeratne *et al*., 2006). Ultrasonic extraction (UAE) has been optimized for total phenolic content (TPC) extraction from almond husks using response surface methodology (RSM). The response variables were total phenolic content (TPC), reducing power activity, beta-carotene shortening, hydrogen peroxide degradation inhibitory activity, and FTC (Khan *et al*., 2022). Author: The food industry uses almond husk extract as a functional food ingredient and a naturally occurring preservative (Caeiro *et al*., 2025).

The best use of agricultural by-products are taken to minimize the environmental pollution of organic waste and improve the economic efficiency. Foods contain various phenolic compounds, and the conditions for extracting these phenolic compounds depend on extraction parameters, including the solvent type, temperature, sample-to-solvent ratio, and extraction time, all the above factors directly impact the extraction efficiency of phenolic antioxidant activity. The classical extraction techniques are time-consuming and require high temperatures and large volumes of solvents, but their impacts on the heat-labile bioactive compounds are negative. Thus, ultrasonic extraction and other modern extraction techniques have gained popularity as they are more efficient and require less solvent in less temperature within a shorter time. This technique (ultrasonic-assisted extraction) is based on acoustic cavitation, which destroys the structure of plant cell walls, and releases the intracellular contents into the extraction medium to enhance the extraction of targeted compounds. It is simple, inexpensive, and can also be developed on an industrial scale (Alharbi and Ghonimy 2025; Pang *et al.,* 2026).

The gap includes the small number of studies dealing with extraction of bioactive compounds from almond husks using ultrasound technology and furthermore the comparative study of antioxidant activity of these extracts with the extracts prepared by use of other polar solvents. For this reason, this research aimed to extract bioactive compounds obtained from almond husks by ultrasound, analyzing the content of total phenolic compounds and antioxidant activity of the extracts obtained using polar solvents.

II. Materials and Methods

Almond peels were used in this study, almonds are sourced from the markets in Duhok, and a number of end results are as uniform as possible in color and size. This involved soaking the fruit in lukewarm water and peeling the seeds by hand. The well was dried with a cabinet dryer at 40 °C until completely dry. The wells have since been ground to powder in a day mill. It was lyophilized, sealed in plastic containers, and stored at −20°C until use.

The extraction of almond peels powder crude extracts was carried out using a kind of polar solvent such as 80% methanol, 80% ethanol, ethyl acetate, chloroform, and hexane in accordance with the technique carried out through Ozsoy *et al* (2008) with some approaches. A total of 8 grams of dry powder is combined with 200mlof solvent

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and extracted for four hours in a high-speed (40 °C) ultrasonic device. Then, the solvent was removed in a rotary evaporator under reduced voltage at 40 °C and the extraction yield was determined as weight

The antioxidant activity of the crude extract of almond husk has been investigated in several studies where one decreases in potency Check out the step-by-step method given through Seneviratne and Tharmarajah,(2017). For testing, 2.5 ml of the crude extract (0.2 mg/ml) was tested with 2.5 ml of phosphate buffer (200 mM, pH6.6) and 0.5 ml of ferric cyanide solution (1·5%). This mixture is incubated in a 50 °C water bath for 20 min. Ten percent trichloroacetic acid (2.5 mL) was then administered and allowed to rest. Finally, 5 milliliters of the compound is converted to a trapped form and it is mixed with 5 ml of distilled water and 1 ml of ferric chloride Ans. The absorbance was 700 nm. Beta-Carotene Inhibition Assay: The extract was evaluated for its ability to inhibit beta-carotene oxidation mainly on the basis of Lino-oxidation The antioxidant activity was calculated using the following equation:

$$\% \text{ for antioxidant activity} = 1- \frac{s\,0 - s\,120}{x\,0 - x\,120} \times 100$$

Where s_0 and s_{120} are the absorbances of the test sample at time 0 and at 120 min and x_0 and x_{120} are the absorbances of the comparison sample at these two times (not necessarily equal time intervals) (Ozsoy et al. 2008).

different concentrations of the crude extracts (0–1 mg/ml) followed by a combination of 0.6 ml of hydrogen peroxide solution and 4 ml of rat extracts at the different extract concentration and 10 minutes incubation at 25 °C, and finally measured the absorption at a wavelength of 320 nm (spectrophotometer) from the samples (BHT) Was used as a control sample. The percentage of inhibition of hydrogen peroxide was calculated using the following equation:

% inhibition hydrogen peroxide = 1 – (absorbance of sample extract/absorbance control (BHT) sample) × 100.

Ferric thiocyanate (F.TC.) assay was conducted as previously described by Akinpelu et al.(2010) The free radical inhibition capacity was measured according to the method of mixing 2.5 mL of linoleic acid emulsion with 0.2 mL of crude extract (0.02) and phosphate buffer pH 7 and incubating at 37°C, then adding ammonium thiocyanate solution, followed by ferric chloride solution, measuring the absorbance at 500 nm every 24 hours for three days. The positive control sample was BHT and distilled water was used as negative control sample.

Statistical analysis

The tests were conducted with three replications. The results were statistically analyzed using the SAS (2012) statistical analysis system, and Duncan's test was performed to compare the average values of the studied tests.

III. Results and Discussion

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1-*Extraction yield and total phenolic content*

The extraction of almond peels was performed with several polar solvents as described in Table 1, being the percentage extraction yield evaluated. Methanol exhibited the highest extraction potential compared to other solvents, with corresponding extraction rates of 17.90% (dry weight). Recovery by extraction with ethanol, ethyl acetate, chloroform and hexane were 12.27%, 10.87%, 7.26% and 4.89% (dry weight) respectively. This is due to the presence of diverse compounds in crude extract, which vary with respect to polarity and chemical structure. It can be a solvent in which it is soluble, and thus the factors between them will vary solubility (soluble) or the amount extracted (extraction rate) depending on the type of solvent used. The more polar solvent extracts more polar compounds (Iqbal & Banger, 2007;Sulltan etal.,2009). This is a typical feature of phenolic compounds as they are polar due to hydroxyls that can stick better with more polar solvents . Based on the extraction solvents tested in the present study, we ranked solvent performances from most to least efficient as: methanol > ethanol > ethyl acetate >chloroform>hexane. The results of Queirós *et al.* (2020) regarding the yield of almond husk extraction may agree or disagree. The differences in results can be explained by plant genetic variability as well as extraction methods solvent types, conditions of extraction (temperature, time of infusion and raw material particle size), or even cultivation conditions (Manchanda *et al.* 2023; Khalil etal., 2007).

The effect of the solvent on the total phenolic content (TPC) of crude almond peels extract is shown in table (1). Quantities from methanol, ethanol, ethyl acetate, chloroform and hexane were measured. All the extracts showed a range of total phenolic contents from 103.46 to 40.53 mg gallic acid/g of the crude extract as shown in Table (1). This data demonstrates that the methanolic crude extract possessed much higher ($p < 0.05$) total phenolic content than the other crude extracts. It is generally due to more polar solvents, like methanol used in Sultana *et al* (2009) extracts phenolic compounds more efficiently than others. These variation levels are also related to what Shi *et al*. (2022) emphasized that variation in results depends on extraction methods (water or hydroalcoholic), the type of solvent, plant genetics, growth conditions and ways of expressing phenolic content.

Table (1): Extraction yield and total phenolic content of almond peels

Solvent	Total phenols	extraction yield
Methanol 80%	103.46a	17.90 a
Ethanol 80%	79.33 b	12.27 b
Ethyl acetate	70.05 c	10.87 c
Chloroform	46.25d	7.26 d
Hexane	40.53 e	4.89 e

2- *Antioxidant Activity of Crude Almond peels Extracts*

Reducing power, as measured by absorbance at 700 nm, of crude almond peels extracts in solvents used in this

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study as concentrations of 0.2, 0.4, 0.6, 0.8, and 1 mg/ml of the crude extract are shown in Table (2). The results indicated that the absorbance values of the methanolic extract for the examined concentrations were higher ($p \leq 0.05$) than other solvent extracts measured at the same concentrations. In 1mg/ml concentration, the average values of absorbance of methanol, ethanol, ethyl acetate, chloroform and hexane extracted samples were 1.76, 1.30, 1.20, 1.14 and 1.11. It is due to the higher phenolic content of the methanolic extract (which possess a greater ability of donating hydrogen atoms to unstable radicals converting them into stable groups). Consequently, extracts treated with this soil had a more significant reduction of ferric ions to ferrous ions than other extracts prepared in this study, whose solvents were used for the first time (Sultana *et al.,* 2009). As presented in the same table, the study showed that increasing the extract concentration in the various solvents led to a significant increase ($p \leq 0.05$) in the absorption values (higher reducing power). Moreover, the values of absorption for extracts prepared by methanol, ethanol, ethyl acetate, chloroform and hexane at the concentration of 0.2 mg/ml, were 0.61, 0.35, 0.31, 0.24 and 0.23. Thus, these finding suggest that reducing power of studied samples were concentration dependent as shown in the table. Higher extract concentrations are also associated with higher concentrations of the redox compounds. This agrees with Vaughan et al.,2017 When the concentration of extract was increased the absorbance values also increased so did the reducing power (more antioxidant activity).

Table (2): Reducing power of the sample extracts using different solvents compared to the standard BHT.

Concentration	BHT	methanol 80%	ethanol 80%	ethyl acetate	chloroform	Hexane
	Absorbance values					
0.2	3.10 d	0.61 o	0.35 r	0.31 s	0.24 t	0.23 t
0.4	3.50 c	0.70 n	0.31 s	0.30 s	0.31 s	0.20 u
0.6	3.90 a	1.38 f	0.82 l	0.40 q	0.41 q	0.50 p
0.8	3.90 a	1.31 g	1.01 k	0.79 m	1.00 k	0.81 l
1	3.80 b	1.76 e	1.30 g	1.20 h	1.14 i	1.11 j

3- ***Evaluating the effectiveness of bleaching beta-carotene pigment***

Results from Table (3) reveal the potential of crude almond peel extracts of the solvents applied in this study to retard beta-carotene pigment bleaching by linoleic acid oxidation products. The test found that the percentage of antioxidant activity in the negative control sample and the samples treated with the crude extracts at a concentration of 0.2 mg crude extract/1 ml decreased progressively throughout the incubation periods from 0 to 120 minutes, with the values of BHT (the positive control samples) remaining almost stable. The activity of antioxidants decreased most in the samples of the negative control and lowest in the samples with the addition of BHT. The obtaining outcome once more demonstrated that all the samples to which individual crude peel concentrates were added indicate an obviously more noteworthy cancer prevention agent movement ($p \leq 0.05$) than

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the negative control test. This allows to infer that the mixed solvent of crude peel extract samples have the potential to inhibit positive organic compounds and hydroperoxides from conjugated dienes produced from Linoleic acid Oxidation (Tepe *et al.,* 2005), though the values are lower compared to samples using among others BHT. This is due to the fact that crude extracts of the peels are a mixture of compounds of different composition that caused a clash with antioxidant compounds such as phenolic compounds and others, and thus reduced their antioxidant effect compared to BHT, which acts as a pure phenolic compound, which gave higher antioxidant activity (Bello *et al.,* 2023). It has been noted by the results that sampled treated with the almond peels crude extracts with the these solvents used alone showed a high value of the antioxidant activity which are leading up to bleaching the beta-carotene pigment. As was the case in this study, the high phenolic content in the crude methanolic extract of almond peels was significantly higher($p \leq 0.05$) than in the phenolic content of other extracts used in the study and was also in agreement with the data of Sultana *et al.* (2009) conducted a study carried out to compare the effect of methanol and ethanol extraction on antioxidant potential of some medicinal plants.

Table (3): Ability of the almond peels extracts to bleaching beta-carotene using different solvents compared to the standard BHT.

Time	Negativity	BHT	methanol 80%	ethanol 80%	ethyl acetate	chloroform	Hexane
0	97.0 Aa	97.0 Aa	97.0 Aa	97.0 Aa	97.0 Aa	97.0 Aa	97.0 Aa
15	60.5 Al	97.0 Aa	80.5 Ae	73.5 Af	69.5 Ag	67.5 Ahi	64.5 Ai
30	50.0 Au	97.0 Aa	73.5 Af	66.5 Ai	61.5 Akl	58.5 Am	57.5 Amn
45	40.5 AzBa	95.0 Ab	68.5 Agh	61.5 Akl	55.5 Aop	53.5 Aqr	49.5 Auv
60	29.5 Be	95.0 Ab	62.5 Ak	56.5 Ano	52.5 Ars	46.5 Aw	43.5 Ax
75	20.5 Bg	95.0 Ab	58.5 Am	51.5 Ast	45.5 Aw	42.5 Axy	39.0 Bb
90	15.5 Bh	92.0 Ac	55.5 Aop	48.5 Av	42.5 Axy	38.5 Bb	34.5 Bc
105	8.5 Bi	90.0 Ad	54.5 Apq	45.5 Aw	39.5 Bab	35.5 Bc	31.5 Bd
120	6.5 Bj	89.0 Ad	50.5 Atu	41.5 Ayz	35.5 Bc	31.0 Bd	27.5 Bf

4- ***The ability of almond peel extracts to inhibit the decomposition of hydrogen peroxide into toxic hydroxyl radicals and singlet oxygen.***

The crude almond peel extracts (using the solvents used in the study) ability to inhibit the decomposition of hydrogen peroxide into toxic hydroxyl radicals and singlet oxygen is illustrated in table (4). Results showed that all samples of the almond shell extracts, treated with methanol, ethanol, ethyl acetate, chloroform or hexane and in the concentrations used, inhibited hydrogen peroxide decomposition. The percentage of inhibition of hydrogen

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peroxide decomposition for methanolic extract of the different samples was more significant ($p \leq 0.05$) than the percentages obtained with the other solvents. This is due to the fact that the methanolic extract has greater content of phenolic compounds, compared to other extracts, which had lower contents of these compounds (Table 1). The results in the same table also show that the increase in percentages of inhibition of hydrogen peroxide decomposition on the tested samples for all the concentrations used resulted from increasing the concentration of the different extracts that were added. This indicates that extracts containing higher antioxidant concentrations are in general starts with high concentrations of the antioxidants. The other types of crude extracts had a greater effect on the inhibition of the breakdown of hydrogen peroxide into active oxygen and hydroxyl radicals, which are powerful oxidizing agents (Senvirathin *et al.,* 2017). As can be seen in the same table, the results showed that the positive treatment sample (BHT) provided a higher inhibition capacity for hydrogen peroxide decomposition with regard to the inhibition rates for hydrogen peroxide decomposition for all samples treated with the crude extracts and solvents used in this study. This is because the crude extracts of almond shells contain a complex composition of phenolic and non-phenolic compounds, presence of such compounds affected the antioxidant activity of the phenolic compounds compared to (BHT) as a pure phenolic compound, (BHT) was used in the test as a positive control sample.

Table (4): Effectiveness of the sample extracts in inhibiting hydrogen peroxide decomposition using different solvents compared to the standard BHT.

Concentration	BHT	methanol 80%	ethanol 80%	ethyl acetate	chloroform	Hexane
0.2	92.0 a	17.5 q	13.5 r	9.5 s	4.5 t	1.5 u
0.4	92.0 a	50.5 i	42.5 l	38.5 m	23.5 p	14.5 r
0.6	92.0 a	65.5 f	55.5 h	48.5 j	36.5 n	23.5 p
0.8	93.0 a	78.5 c	67.5 e	63.5 g	46.5 k	32.5 o
1	93.0 a	82.5 b	69.5 d	62.5 g	51.5 i	36.5 n

5- ***The ability of almond peel extracts to inhibit linoleic acid oxidation and reduce the number of peroxides during the first stage of lipid oxidation***

Table (5) illustrates the role of various almond shell extracts, (by the solvents used in the study) in suppressing linoleic acid oxidation and reduction of peroxides at the first phase of lipid oxidation in the presence of ferric thiocyanate (F.T.C). This was calculated based on the measurement of color absorbance of the extracted sample at a wavelength of 500 nm. The results indicate that the negative control presented higher absorbance values($p \leq 0.05$) than those obtained with samples treated with the different crude extracts of the peels, at a concentration of 0.2 mg of crude extract/1 ml. These results indicate that these extracts with their various solvents,

can inhibit oxidation and decrease the percentage of peroxide formation in both the first phase of the catalytic phase and the multiplication phase. However, this methanolic crude extract had the lowest absorbance values ($p \leq 0.05$) in the same table compared to the other samples treated with the different extracts. This is due to more phenolic compounds present in the methanolic extract, a property that provides the methanolic extract to terminate the chain reaction. This results in the production of free radicals compared to the other extracts with the solvents used in the study. Similar results and the same table show that the samples treated with BHT showed absorption values significantly lower ($p \leq 0.05$) than the absorption values of extract samples with solvents used and for all concentrations. This is because, as discussed above, the extracts, whether antioxidants or not, are just a crude mixture of compounds. These findings are similar with those work of Peschal *et al.* (2006) studied the antioxidant activity of some secondary wastes of food factories.

Table (5): Effect of the sample extracts on inhibiting linoleic acid oxidation using different solvents compared to the standard BHT.

Time	Negativity	BHT	methanol 80%	ethanol 80%	ethyl acetate	chloroform	Hexane
24	0.535 c	0.075 p	0.130 mn	0.115 o	0.185 k	0.255 i	0.265 i
48	0.595 b	0.085 p	0.135 m	0.120 no	0.225 j	0.330 g	0.355 f
72	0.685 a	0.085 p	0.155 l	0.195 k	0.285 h	0.375 e	0.435 d

Conclusion:

This study concludes that the crude extracts of dry almond peels and solvents have an antioxidant activity, also indicate that methanolic extract gained the highest antioxidant activity, and can be considered a source of natural antioxidants which could serve as alternatives for synthetic/ artificial ones during food manufacture.

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