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IDENTIFICATION OF PHENOLIC COMPOUNDS EXTRACTED FROM POMEGRANATE PEELS AND GRAPE JUICE RESIDUES USING HPLC TECHNOLOGY

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ABSTRACT

The current study included determining the optimal conditions for extracting phenolic compounds from pomegranate peels and remnants of black grape juice using aqueous extraction, alcoholic extraction and extraction using microwave power (400 watts). It found that the highest yield of phenolic compounds was (43.7) g/100 gm for aqueous extraction of pomegranate peels. While the lowest yield was (33.33) g/100 gm for extraction using the microwave energy of the remnants of grape juice, with a clear superiority of the pomegranate peel remnants over the remnants of grape juice in the total outcome of the phenolic compounds. The results indicated that the highest content of total phenolic compounds was (61.79) mg/g for aqueous extraction of grape juice residues. While the lowest content was (43.41) mg/gm for extracting using microwave energy from pomegranate peels. HPLC results showed that the concentration of phenolic compounds extracted from pomegranate peels for compounds such as (Gallic acid, Catechin, Caffeine, Ferulic acid, and P-Coumarin) reached (132, 37.2, 14.2, 1.1, 4), (185, 34, 15, 0.33, 2.1), (136, 35, 11, 0.33, 3) micrograms/ml while the grape juice residue was (31.3, 31, 8.5, 7.3, 5), (32, 10, 11, 0.3, 9), (108, 35, 9.2, 8, 9) g / ml for aqueous extraction, alcohol extraction and microwave extraction respectively.
Keywords: phenolic, pomegranate peels, grape juice, HPLC technology

Introduction

In recent years, attention has directed to the waste products of food processing that are thrown out by food factories, as these factories throw vast quantities of fruit seeds and agricultural waste by-products. One of the statistics indicated that the by-products resulting from the manufacture of wine from the fruits of grapes (which include seeds and husks in addition to Stems) amount to approximately 7 million tons per year (Pinelo *et al*., 2006).

Studies have proven the great importance of phenolic compounds for health. For this reason and the abundance of its sources, the interest of researchers and industrialists increased after 1990 in these compounds. Several studies have proven that there is a clear and inverse relationship between foods containing these compounds and heart disease, diabetes, and cancer. It also found that these compounds differ in their vital preparation and thus differ in their biological effects within the human body (Williamson *et al*., 2005).

The plant parts contain many polyphenolic compounds, including simple polyphenolic acids, such as phenolic acids, and complex such as tannins in different quantities. Moreover, these compounds often associated with other plant components such as carbohydrates and protein within the cell wall, while the simple ones located in the vacuoles within the structure of the plant cell (Fereidoon *et al*., 2016).There is no general extraction method for the extraction of the polyphenols. Before the extraction process, the plant part is chosen, where it is either air-dried or lyophilized (*et al*., 2005) (Abascal). The freeze-drying process of both strawberry and corn (before the extraction process) contributed to obtaining more massive quantities of Polyphenolic compounds compared to drying with air (Asami *et al*., 2003). The size of the plant fractions after grinding is an essential factor in enhancing the amount of the polyphenol compounds extracted (Pinelo *et al*., 2006). The choice of the solvent used in the extraction is of great importance in Increase the yield of the extraction process according to the polarity of these polyphenolic compounds, as different solvents and methods used in the process of extracting phenolic compounds. One of the most common methods of extraction is the use of water with some organic compounds (ethanol, methanol, ethyl acetate) and these determined according to the type of polyphenolic compounds present in the raw material. The researcher Chew (2011) *et al*. Indicated that the efficiency of the extraction process depends on the temperature and time. As the extraction time increases, it can affect the effectiveness of the extracted compounds. Thus, it reduces its efficiency and increases the extraction of solvent loss, which leads to a decrease in the recovery process of the used solvent and increases the toxicity of the resulting compounds. The extraction process with organic solvents is the most common method for extracting polyphenolic compounds due to its ease of use and being economical. There is a wide range of application, depending on the type of solvent used in the extraction (Stalikas, 2007). The organic solvents used in the extraction include (Methanol, Ethanol, Ethyl acetate, Diethyl ether, and Chloroform), and often mixed with different proportions of water (Xu & Chang, 2007). The extracts are for medicinal or nutritional purposes, so they need additional purification steps in order to get rid of the remnants of organic solvents.

Among the unconventional methods used in extraction at present are ultrasound extraction and extraction using waves or microwave energy. One of the advantages of these methods is the reduction in time and amount of solvent and the increase in yield compared to conventional methods (aqueous extraction) which require longer time and higher energy consumption (Chemat *et al*., 2011).

The present study aimed to extract the phenolic compounds from the pomegranate peel and the remnants of the red grape juice by different extraction methods and to study the effect of the solvent on the yield of the phenolic compounds and calculate the concentration of some common phenolic compounds using HPLC technology.

Material and Methods

Raw materials used

The remains of the Iraqi red grape juice, a French type, represented by the peel and seeds, were selected after the pressing process. The Iraqi pomegranate peel was also selected as raw materials for extracting the phenolic compounds, as they are available and considered as residues that not used in manufacturing.

Extraction of phenolic compounds:

Aqueous extraction of phenolic compounds

The method suggested by Kanmaz *et al*. (2014) followed with some modifications. Ten grams of the powder of the raw material (grape peels, seeds and pomegranate peels) mixed with an appropriate amount of distilled water in a volume flask to 300 ml distilled water, then complete the volume. The extraction done at a temperature of 100 ° C. on an electric mixer with constant stirring for 30 minutes. Then filter the extraction solution with a soft cloth and then use the filter paper (Whatman-No.1) both separately. The extraction solutions concentrated using a rotary evaporator at a temperature of 70 °C. The concentrated product was placed in an electric oven at 45 °C for 24 hours. The product was collected, weighed, then placed in opaque, airtight containers and stored by freezing at -10 ° C until use.

Alcoholic extraction of phenolic compounds:

The method suggested by (Jemal *et al.*, 2017) was followed by mixing 30 gm of powdered raw material (grape peels, seeds and pomegranate peels) separately with an appropriate amount of 80% ethyl alcohol and complete the volume to 300 ml with the same alcohol. The prepared extraction solutions were placed on an electric mixer and the extraction was carried out at a temperature of 50 °C. for half an hour with continuous stirring, and the same steps were completed in the previous paragraph.

Aqueous extraction of phenolic compounds using microwave energy

The method suggested by (Alias and Abbas, 2017) followed with some modifications. Ten gram of the powder of the raw material (the remains of grape juice or pomegranate peel) mixed with an appropriate amount of distilled water in a flask and volume to 300 ml and complete volume with distilled water. Mix the prepared solution well by an electric mixer and perform the extraction using the microwave at a power level of 400 watts for 30 minutes at a temperature of 100 °C. Then complete the same steps mentioned in the previous paragraph.

Calculation of the final yield of phenolic compounds:

The final yield (g/100 g) of the phenolic compounds extracted from the remnants of grape juice and pomegranate peels estimated according to the method used by Ljiljana *et al*. (2009) according to the following equation:

Yield = (weight of extract (g) / weight of plant material (g)) * 100

Spectrophotometrically determination of phenolic compounds' concentrations:

The concentration of total phenolic compounds determined according to the method proposed by Kamtekar *et al*. (2014) using Folin-Ciocalteau's reagent.

Determination of some phenolic compounds by HPLC technology:

Some common phenolic compounds diagnosed using a high-performance liquid chromatography technique using SYKMA device of German origin according to the method proposed by (Gupta *et al*. 2012). The samples were prepared by dissolving the phenolic extract powder with distilled water at a concentration of 10 mg/ml, and injected, ten µl using the autoinjector under the conditions of the next separation in the column of separation. Column type used (5m) × 4.6 mm × C18-ODS (25cm), mobile phase (Acetonitrile: DW: Orthophosphoric acid) (5: 70) and UV detector It has a wavelength of 365 nm, at a temperature of 25 ° C and a flow rate of 1 mL/minute.

Result and Discussion

The effect of extraction methods on the yield of phenolic compounds

Figure (1) shows the effect of aqueous and alcohol extraction and microwave-assisted extraction on the final yield of the phenolic compounds. The results indicate that the yield of the aqueous extract of the phenolic compounds of the pomegranate peel powder and the remains of the grape juice was (43.70 and 40.30) g/100 g, respectively. In comparison, the alcoholic extract was by (40.00 and 33.33) g/100 g, respectively, while the yield was (37.33 and 35.00) g/100 g, respectively when using microwave power (400 W) for aqueous extraction. It noticed that pomegranate peel powder exceeded the remains of grape juice in terms of the yield of phenolic compounds and for all methods of extraction.

The results of the statistical analysis showed that there were no significant differences ($P < 0.05$) between the yield of the phenolic compounds extracted from the pomegranate peel powder and the remains of the grape juice by aqueous extraction. Also, there were no significant differences ($P < 0.05$) between the yield of phenolic compounds when extracting with ethanol solution (80%) and when using microwave power (400 watts), with the yield of phenolic compounds extracted from pomegranate peel powder significantly greater. The results of the statistical analysis also showed that there were significant differences ($P < 0.05$) between the extraction methods used for one extraction source, with a clear significant superiority for the aqueous extraction of pomegranate peelings and grape juice residues, which gave a higher yield of phenolic compounds.

The variation in the yield of phenolic compounds in both methods of extraction is due to the polarity of the phenolic compounds, as water is of the highest polarity of alcohol, so the high amount of phenolic compounds in aqueous extraction is due to their being compounds of high polarity (Cai *et al*., 2004).

These results were close to what was reached by Salah *et al*. (2012) when assessing the outcome of the phenolic compounds extracted from pomegranate peels.

Hong *et al*. (2001) found that the yield of phenolic compounds extracted from grape seed using microwave power (300 watts/3.5 minutes) was 13.50% and that the total phenolic compounds content reached 392 mg / g. It also found that the polarity of methanol used in the extraction increased by adding 10% distilled water increased the yield of these compounds, which rose to 15.20% and the total content of them increased to 429 mg / g.

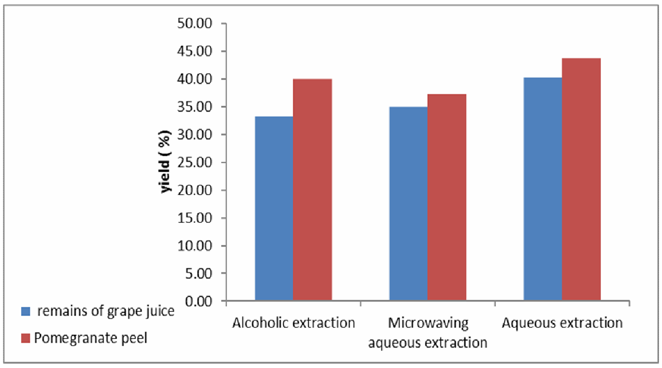

Fig. 1 : The result of the phenolic compounds from the aqueous and alcoholic extracts of the pomegranate peel and the remains of the grape juice.

Total phenolic compounds content in pomegranate peel extract and grape juice residue:

Figure (2) shows the total content of the phenolic compounds in the aqueous extract, the alcohol extract, and the microwave extract (400 watts) for each of the pomegranate peel powder and the remains of the grape juice. The total content of these extracts of pomegranate peel powder was 52.36, 52.17 and 43.41 g / mg, while the total content of these extracts from grape juice residues was 61.79, 59.05 and 51.23 g / mg for the three extraction methods, respectively.

The results showed that the total phenolic compounds content was the highest in the aqueous grape juice residue extract, which reached 61.79 µg / mg, and the lowest was 43.41 µg / mg with a microwave energy extraction of pomegranate peel powder. The results of the statistical analysis showed that there were no significant differences in the content of the total phenolic compounds extracted from the remains of grape juice between the aqueous extraction and the alcoholic extract, which differed significantly with the content of the total phenolic compounds extracted from the microwave energy of the same source. While the results of pomegranate peelings did not differ by aqueous extraction and alcoholic extraction, these results differed significantly with the results of the microwave energy extraction, which gave the lowest content. We notice through the results the superiority of the aqueous extract over the alcohol extract and the extract with microwave energy for each of the phenolic compounds extracted from the remains of grape juice and pomegranate peel. The variation in the content of phenols from different sources is due to the nature of those sources, the chemical composition of the plant and the age of the plant, as well as the difference in the polarity of the phenolic compounds and the polarity of the solvents used in the extraction (Jauhar *et al*., 2018).

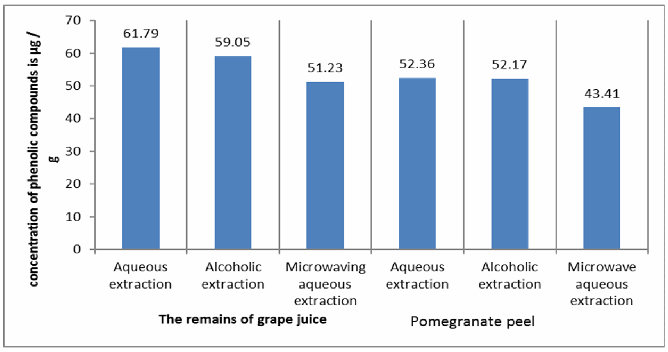

Fig. 2 : Concentration of total phenolic compounds in pomegranate peel extract and grape juice residue (µg / mg).

Determination of the concentration of some phenolic compounds in the crude extracts with HPLC technology:

Table (1) shows the concentration of some polyphenols extracted from the pomegranate peel powder and the remains of grape juice by HPLC technology, as shown in figure (1). It found that the concentration of phenolic and flavonoid compounds such as Gallic acid, Catechin, Caffeine, Ferulic acid and P-Coumarin in pomegranate peel powder was (132, 37.2, 14.2, 1.1, 4) micrograms/ml for the aqueous extract, while it was (136, 35). 3, 0.33, 11, 0.33 µg / ml for aqueous extract using microwave power (400 watts), while it was (185, 34, 15, 0.33, 2.1) micrograms/ml for the alcoholic extract, respectively. While the concentration of the same phenolic compounds in the remains of grape juice was (31.3, 31, 8.5, 7.3, 5) micrograms/ml for the aqueous extract, while it was (108, 35, 9.2, 8, 9) micrograms/ml for the aqueous extract using energy the microwave was (400 watts). At the same time, it reached (32, 10, 11, 0.3, 9) micrograms/ml in the alcohol extract, respectively. It is noted from the results that the content of the phenolic compounds extracted from the pomegranate peel is high in calic acid for all extracts, with a clear superiority of the alcoholic extract (185 micrograms/ml) with the approximate percentages of the remaining compounds for the extracts themselves with the superiority of the aqueous extract. It noted that the aqueous extraction was superior to the microwave energy in the content of calic acid (108 µg / ml) extracted from the remains of grape juice with the approximate percentages of the remaining compounds to the extracts themselves, with the aqueous extract being superior to the microwave energy. Between Middha *et al*., (2013), the content of aqueous extracts and alcoholic extracts of some phenolic compounds of pomegranate peel powder, as it found that the content of corsetin, rutin, calic acid, and ellagic acid was high in the alcoholic extract, and there were no significant differences from their content in the aqueous extract of pomegranate peel powder. He mentioned that the reason for this might be due to the different polarity of the polyphenols as well as the different polarity of the solvents used in the extraction.

Table 1 Concentration of phenolic compounds (micrograms/ml) extracted from pomegranate peel powder and grape juice residues, calculated by HPLC technology.

Sample	Extraction method	Gallic acid	Catechin	Caffeine	Ferulic acid	P-Coumarin
Pomegranate peel powder	Aqueous extract	132	37.2	14.2	1.1	4
	Aqueous extract using microwave power	136	35	11	0.33	3
	Alcoholic extract	185	34	15	0.33	2.1
Ggrape juice residues	Aqueous extract	31.3	31	8.5	7.3	5
	Aqueous extract using microwave power	108	35	9.2	8	9
	Alcoholic extract	32	10	11	0.3	9

Conclusions

Pomegranate peels and grape juice residues have a good yield of phenolic compounds that can be extracted aqueous. These compounds form a cheap source and therefore have good economic feasibility. The extraction conditions represented by the extraction method and the type of solvent are an essential factor in obtaining the highest extraction rate. The aqueous extraction method, with a mixing ratio of 30: 1 and a temperature of 100 °C for 30 minutes, has proven the most appropriate conditions for the extraction of phenolic compounds.

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