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Evaluation Of Ellagic Acid Content In Different Extracts Of Pomegranate Peel To Recycling.

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ABSTRACT

Recently, the recycling of food-waste to produce a green substance has been interested subject in the world. Ellagic acid is one of the major antioxidants in different medicinal plants including pomegranate. The peel of pomegranate that considered as waste may cause some problems in the environment, so this research comes to evaluating the level of ellagic acid in pomegranate by using more different extraction methods (stirring extraction and ultrasonic extraction) with different solvents (99% methanol, 50 % v/v water in methanol, 10 % v/v water in methanol) and comparing the results with standard extract obtained by soxhlet extraction reported in previous articles to recycling this peel. Results revealed that the highest percentage of ellagic acid was yielded when pomegranate peel extracted with dDW at 60 °C and achieved 81.38% extraction of ellagic acid. This may lead to a conclusion that this method is simple, cheap and with high yield value.

Keywords: Ellagic acid, pomegranate, extraction, antioxidant,

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INTRODUCTION

Pomegranate (*Punica granatum* L. family, *Punicaceae*) peel, a byproduct of pomegranate juice (about 67% of the weight of the fruit), is a rich source of antioxidants, specially polyphenols, , such as ellagic acid (Figure 1) (1), these antioxidant substance which counter act free radicals and prevent the damage due to oxidants by crumbling them before they react with biological targets , preventing chain reaction or preventing the activation of oxygen to highly reactive products (2).

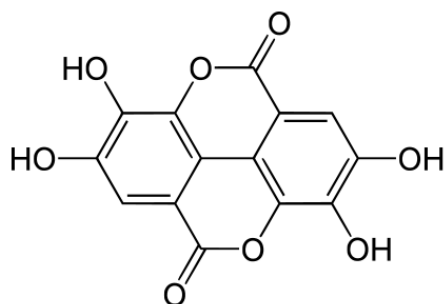


Figure 1. Structure of ellagic acid, is a natural phenol antioxidant found in pomegranate and numerous fruits and vegetables.

Ellagic acid – rich pomegranate fruit peel extract (EARPPE) has antioxidant, anti-inflammatory, anti-mutagen and anti-cancer properties. Studies have shown the anti-cancer activity on cancer cells of the breast, esophagus, skin, colon, prostate and pancreas. EARPPE has also antiviral and antibacterial activities (3). Extraction of pomegranate peel and obtain the extract rich in ellagic acid is the most important steps in pomegranate extract which possess effective medicinal and pharmacological properties. Most of the extraction process are time consuming involves lengthy operation technique, bulk amount of solvents and ultimately thermal decomposition of the target molecules at continuous high temperature.

Stirring extraction methods have been applied for the extraction of biological active compounds (4). Ultrasonic extraction uses high frequency sound to deliver the target compound from the plant materials. Recently, there have been several reports on the application of ultrasonic methods in the extraction of various phytochemicals (5). Soxhlet extraction by using 10% v/v water in methanol and partitioning the extract between ethyl acetate and 2% aqueous acetic acid was capable of increasing the ellagic acid content of the extract from 7.06% w/w to 13.63% w/w (6). The aim of this study was to extract ellagic acid from pomegranate peel using different methods and different solvents for this purpose and comparing these results with a standard method reported by other researchers.

MATERIALS AND METHODS

Plant materials and reagents

Pomegranate was provided from local market in Al-Najaf city, Iraq (September 2018). The outer layer of pomegranate peeled and the rind was dried at 50C in incubator for 48 hours, grinder (First Austria, China) and mill powdered the dried material by 250 micrometer sieve (60 mesh size). Methanol 99% (Poicha, UK), Acetic acid (Sigma Lab., UK), Ethyl acetate (Thomas Baker, India) were all of analytical grade. De-ionized Distilled water (dDW) obtained from Millipore apparatus (Simply UV Millipore, USA). Ellagic acid standard was purchased from (Shenyang Bowente Import and Export trading Co., Ltd., China), Ellagic acid melting point was measured (350 C) and the spectra of standard Ellagic acid measure by FTIR (Shimadzu, Japan), Figure (2).

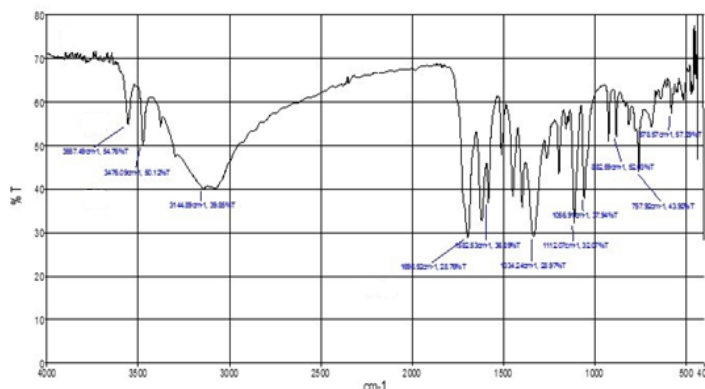


Figure 2. Spectra of standard ellagic acid measure by FTIR (Shimadzu, Japan)

Stirring extraction method

Stirring extraction was carried on magnetic stirrer (Stuart, UK) equipped with temperature sensor. The stirring speed was at point five and solvent temperature controlled while stirring at 60 °C. Pomegranate peel was extracted with dDW and three different concentration of methanol (99% methanol, 50% v/v water in methanol, 10% v/v water in methanol). Pomegranate peel powder (20 g) was put into 250 ml flask and added appropriate solvent (80mL). The pomegranate peel powder was extracted at different solvent for 60 minute at 60 C. After extraction the content of the flasks were filtered through filter paper (Whatman No.1, UK), the filtrate partitioning between ethyl acetate and 2% aqueous acetic acid four times (400mL each) , then collecting ethyl acetate fractions and drying by air drier to obtain extract powder (table 1)

Ultrasonic extraction method

Ultrasonic extraction was carried in an ultrasonic bath (Raypa, Spain) and working frequency was maximum. (20 g) of pomegranate peel powder extracted with (80 mL) of dDW and three different concentration of methanol (99% methanol, 50% v/v water in methanol, 10% v/v water in methanol), in 250 mL flasks and kept for sonication for 10 minutes at 60 °C , After extraction the content of the flasks were filtered through filter paper (Whatman No.1, UK), the filtrate partitioning between ethyl acetate and 2% aqueous acetic acid four times (400mL each) , then collect the ethyl acetate fractions and drying by air drier to obtain extract powder (table 1)

Table (1) Different extraction methods of pomegranate peel powder by using different solvent.

Extract #	Pomegranate peel powder + solvent	Method of extraction	Time	Temperature
1	(20 g) pomegranate peel powder + 80 mL dDW	Stirring extraction	60 min	60 °C
2	(20 g) pomegranate peel powder + 80 mL 99% methanol	Stirring extraction	60 min	60 °C
3	(20 g) pomegranate peel powder + 80 mL 50% v/v water in methanol	Stirring extraction	60 min	60 °C
4	(20 g) pomegranate peel powder + 80 mL 10% v/v water in methanol	Stirring extraction	60 min	60 °C
5	(20 g) pomegranate peel powder + 80 mL dDW	Ultrasonic extraction	60 min	60 °C
6	(20 g) pomegranate peel powder + 80 mL 99% methanol	Ultrasonic extraction	60 min	60 °C
7	(20 g) pomegranate peel powder + 80 mL 50% v/v water in methanol	Ultrasonic extraction	60 min	60 °C
8	(20 g) pomegranate peel powder + 80 mL 10% v/v water in methanol	Ultrasonic extraction	60 min	60 °C
9	(20 g) pomegranate peel powder + 80 mL 10% v/v water in methanol	Reflex extraction	60 min	60 °C

Reflex extraction method

For reflex extraction (20 g) of pomegranate peel powder was put into 250 mL round bottom flask containing (80 mL) of 10% v/v water in methanol, the extraction temperature was controlled at 60 C for 1 hour. After extraction the content of the flasks were filtered through filter paper (Whatman No.1, UK), the filtrate partitioning between ethyl acetate and 2% aqueous acetic acid four times (400mL each) , then collect the ethyl acetate fractions and drying by air drier to obtain extract powder (table 1)

Preparation of standard ellagic acid stock solution

The standard stock solutions were prepared by dissolving accurately weighed amount of ellagic acid in 99% methanol (table 2).

Table (2) Different standard ellagic stock solution.

Solution #	Concentration (mg/mL)
1	0.004
2	0.012
3	0.024
4	0.032
5	0.036

Determination of absorption maxima (λ max) of ellagic acid standard by UV-Vis. Spectrophotometer

Ultra violet scan for standard Ellagic acid stock solution no.1 was taken using a quartz cuvette with 1 cm path length. The solution was scanned using double beam UV-vis spectrophotometer (SHIMADZU, Japan) from 200 to 1100 nm at fast speed. An absorption maximum (λ max) of Ellagic acid standard was 259 nm (figure 3).

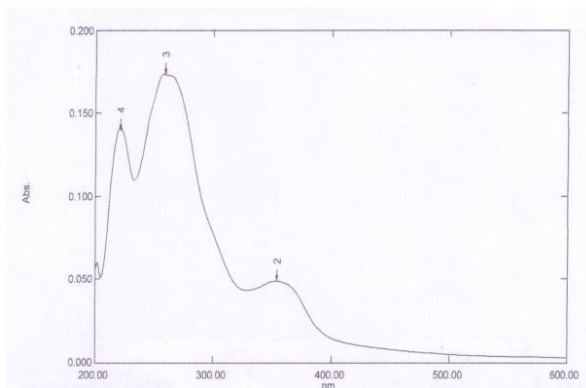


Figure 3. Absorption maxima (λ max) of Ellagic acid standard.

Drawing the calibration curve of different concentration of standard ellagic acid solutions

Five standard ellagic acid solutions were prepared using 99% methanol (table 2). UV scans were taken using a quartz cuvette with 1 cm path length. The solutions were scanned using double beam UV-Vis spectrophotometer at wave length 259 nm at fast speed, from absorbance of different standard ellagic acid solution. The standard curve was obtained, (figure 4).

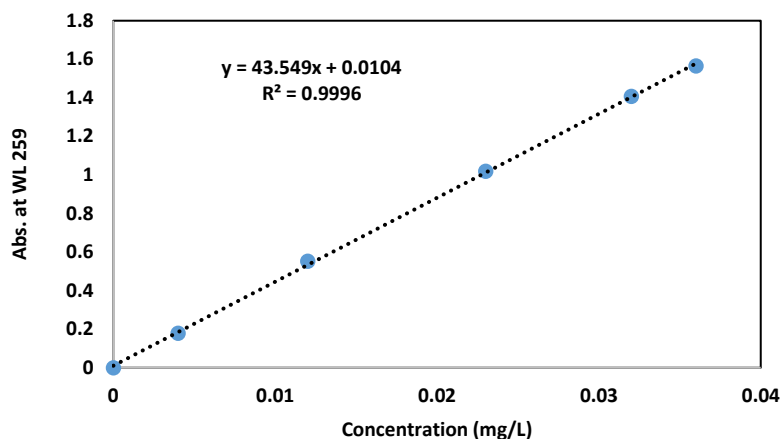


Figure 4. The calibration curve of different concentration of standard Ellagic acid solution.

Determination of ellagic acid content in different extract solution

Accurately weighed amount of each pomegranate peel extract in table (1) was dissolve in 99% methanol to prepare solution with concentration (0.04 mg/mL) of extract. The absorbance of each extract solution was measure to determine the content of Ellagic acid in each sample according to the following equation (table 3):

$$\% \text{ of Ellagic acid in sample} = \frac{\text{wt of ellagic acid in sample}}{\text{wt of sample}} \times 100$$

Table 3. The absorbance of extract solution (2nd column) and Ellagic acid content in different extract solution (3rd column).

Extract #	Abs at WL 259	% of ellagic acid in sample
1	1.416	81.38%
2	0.827	47.25%
3	0.859	49.00%
4	0.525	29.78%
5	0.986	56.50%
6	0.635	36.25%
7	0.817	46.75%
8	0.497	28.25%
9	0.248	13.95%

RESULTS

The preliminary experiment was performed in order to establish the suitable extraction solvent to obtain Ellagic acid rich extract by using stirring extraction technique. Solvent include dDW and three different concentration of methanol (99% methanol, 50% v/v water in methanol, 10% v/v water in methanol) were studied.

Figure (5) shows the effect of using these solvent on percentage extraction of Ellagic acid with Application stirring extraction technique. The present results revealed that the highest percentage of extraction was obtained when the pomegranate peel extracted with dDW at 60C and achieved 81.38% extraction of Ellagic acid.

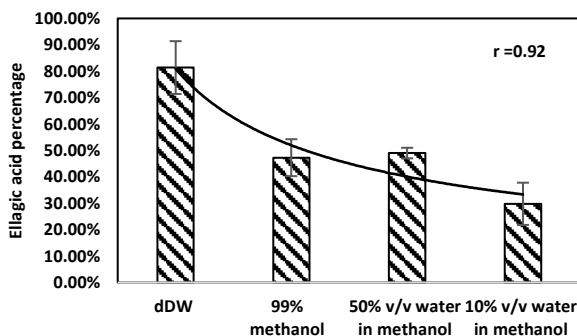


Figure 5: The effect of using different solvent on percentage extraction of Ellagic acid with Application stirring extraction technique.

By using ultrasonic extraction technique. Solvent include dDW and three different concentration of methanol (99% methanol, 50% v/v water in methanol, 10% v/v water in methanol) were studied. Figure 6. Shows the effect of using these solvent on percentage extraction of Ellagic acid with Application ultrasonic extraction technique. The present results revealed that the highest percentage extraction was obtained when the pomegranate peel extracted with dDW at 60Cand achieved 56.50% extraction of Ellagic acid.

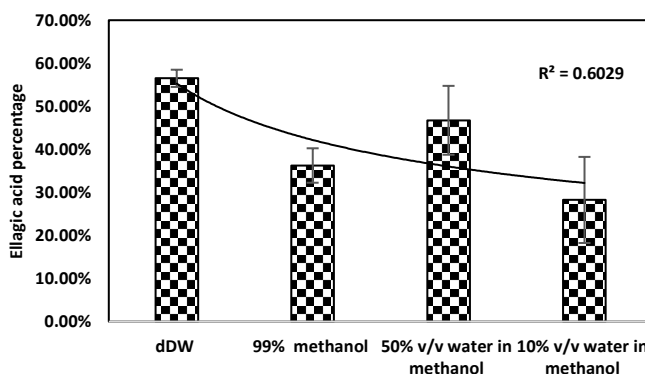


Figure 6: The effect of using different solvent on percentage extraction of Ellagic acid with Application ultrasonic extraction technique.

The percentage extraction of Ellagic acid when Application soxhlet extraction technique was 13.95%.

DISCUSSION

By comparing various extraction methods for pomegranate peel, the stirring extraction method by using dDW was more efficient the ultrasonic extraction reduce the extraction time and required only 10 minute to extract adequate amount of Ellagic acid, so the ultrasonic extraction method become ideal when extraction time be important factor (8).

In conclusion the result of the current study shows that the stirring extraction of pomegranate peel with dDW at 60 °C give extract contain significant concentration of Ellagic acid . so use of this extraction method is potentially a useful source of Ellagic acid for applications in the food or pharmaceutical industry and in scientific research. So we suggesting to used that methods to recycling of pomegranate peel and may other food waste

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