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Efficacy of Ultrasound-Assisted Extraction in the Isolation of Bioactive Compounds from Pomegranate Peel (*Punica Granatum* L.): A Comparative Study of Solvent Performance

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A B S T R A C T

The growing nutraceutical value of *Punica granatum* L., also known as pomegranate, has increased in its largely discarded peel due to the content of polyphenols, flavonoids, tannins, saponins, and alkaloids. In this research, it is focused on extracting bioactive compounds from thepomegranate peel using a non-destructive environmentally friendly UAE method with hydromethanol, ethanol, and aqueous solutions.

Pomegranate peels were obtained locally, dried, and powdered before extraction. Results revealed that hydromethanol was the best solvent as it produced the highest number of anthocyanins (90 mg/100g), total phenolics (2.87 GAE mg/ml), flavonoids (91.24 mg/100g), saponins (1.48%), tannins (46.29 mg/g), and alkaloids (2.87 mg/g). Ethanol extracts also exhibited promising results, especially for alkaloids (12.66 mg/g).

This research puts UAE in its rightful position as a superior extraction method, further emphasizing the use of pomegranate peels as an eco-friendly bioactive compound source for functional foods and nutraceuticals. It advocates the usage of agricultural by-products and zero waste to develop natural additives in food industry that may contribute to health and environmental sustainability.

Keywords: Pomegranate peel, flavonoids, bioactive compounds, ultrasound-assisted extraction, antioxidants, nutraceuticals, green extraction

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INTRODUCTION

Pomegranate or *Punica granatum* L. (Lythraceae) is a shrub native to Iran, China, and India with the extensive cultivation throughout the Mediterranean region, North and South Africa. The plant has a temperate temperature that can grow with ease in dry and semi-arid regions.It can withstand salt, water scarcity, and agronomic conditions that typically hinder the growth of traditional crops. Due to its remarkable adaptability to Mediterranean climates, pomegranates have spread throughout this region and given rise to new types(1).

In contrast, it has recently been referred to as a "super food" and listed as one of the top ten fruit having significant nutraceutical potential. The juice is a high source of polyphenols such as anthocyanins (glycosides of cyanidin, delphinidinand pelagonidin), tannins (ellagitannins) and flavonoids (proanthocyanidins and flavanols). Pomegranate peel (PP) which makes up upto half of the fruit's weight, are frequently thrown away as waste. Nonetheless, it has been noted that fruit peel possesses the highest quantities of bioactive substances compared to the juice with more potent biological activity (2). Pomegranate fruit and fruit extracts have demonstrated preventative and attenuating properties against a variety of chronic and life-threatening illnesses during the past ten years, including cancer, atherosclerosis,type 2 diabetes and cardiovascular disorders (3).

About 50% of the weight of a pomegranate is made up of its peel, which is rich in complex polysaccharides. It also includes high levels of minerals such as potassium, calcium, phosphorus, magnesiumand sodium. Bioactive compounds are also found in PP including phenolics, flavonoids, proanthocyanidin compounds and ellagitannin (ETs), along with decreasing concentrations of punicalin, gallagic acid, ellagic acid, and ellagic acid glycosides (4). PP, therefore, is a waste as it contains higher amount of potentially beneficial compounds than the edible part.Because of their antioxidant properties, these bioactive compounds can be used as food additives.

To produce extracts high in phenolic compounds, the physiologically active components of the plant matrix must often be separated using specific solvents. Different methods and extraction conditions can be used to carry out the extraction processes. Typically, traditional extraction methods (maceration, shaking, soxhlet, etc.) use a lot of solvents and are time-consuming and ineffective. The drawbacks of traditional extraction methods have prompted the creation of new methods that are less harmful to the environment and more effective and selective (5).

UAE techniques have gained much popularity in the last few decades and are applied to a vast variety of natural materials, including phenolicsfrom PP (6). It has received much attention due to its cost-effectiveness, simplicity of operation, and great efficiency (7). By increasing mass and momentum transfer through acoustic cavitation events, UAE can facilitate bioactive compounds extraction.This results in a faster disruption of plant tissues and breaking of a liquid's cohesiveness due to mechanical, physical, and chemical outbursts that cause bubble cavitation with an interparticle impact, macroturbulence, and cavity collapse.

The chamber collapses, and this causes shocks that impart mechanical properties, increasing extraction efficiency through hot spots, particle size reduction, cell wall breakage, and intense mixing.Additionally, swelling and solvent penetration into the cells are caused by structural changes in plant tissue (8). Moreover, by producing a high-value green extract as a byproduct of renewable PP using an alternative solvent, UAE satisfies the six principles of green extraction. Economical, sturdy, and free of technical issues like equipment devaluation and tip erosion. UAE is therefore a "green and environment-friendly" extraction technique (9). In this study, we are extracting bioactive chemicals from PP, utilizing UAE approach,employing several solvents, including water, ethanol and methanol.

Materials and methods

Plant material

PPwere sourced from a local market in Banasthali Vidyapith, Rajasthan, India. After gently removing the fruit's outer skin, the peels were thoroughly cleaned under running water to remove any remaining dirt. The peels were dried for 18 to 20 hours at 80°C in an air-circulated oven until they were totally dry. After drying, PP was pulverized using an electric grinder into a coarse powder, which was then used to extract bioactive chemicals. The moisture loss method was used to compute the yield from fresh to dry, and the findings were expressed as dry weight (DW).

Extraction of bioactive compounds

Bioactive compounds extraction from PP was doneusing the method given by Mehmood (10). Distilled water, ethanol, and hydromethanol were used to create three extracts, which were designated aqueous extract, ethanol extract, and hydromethanol extract, respectively.

Aqueous extract (A.E.)

After dissolving 50g of dry material in 100ml of distilled water, the mixture was heated for two hours at 100°C in a water bath.

Ethanol extract (E.E.)

Fifty grams of the dried material were dissolved in one hundred milliliters of ethanol. It was then allowed to rest for forty-eight hours at room temperature (25-30°C).

Hydromethanol extract (H.M.E.)

After dissolving 50g of dried sample in 100ml of diluted methanol (1:1 v/v), the mixture was allowed to rest at room temperature (25–30°C) for 48 hours.After two hours of exposure to an ultrasonic water bath, the solvent was concentrated using a rotary evaporator set to 69 rpm and 45°C. To eliminate excessive moisture, the extracts were dried at 45–50°C in an air-circulated oven. The extracts were placed in an airtight container for additional examination.

Estimation of anthocyanin content

Using pH differential methodby two buffer systems-potassium chloride buffer (pH 1.0, 0.025 M) and sodium acetate buffer (pH 4.5, 0.4 M) wasused to estimate the total anthocyanin content.Methanolic extracts were combined with 3.6 ml of the appropriate buffers, and measured at 510 and 700 nm against water as a blank (11,12). With a molar extinction value of 29600, absorbance (A) was calculated from the formula:

$$A = [(A_{510} – A_{700})\ pH_{1.0} - (A_{510} – A_{700})\ pH_{4.5}]$$

The findings were reported in mg CGE/g DW, or cyanidin-3-glucoside equivalents per g DW.

Estimation of total phenol content

The total phenol content (TPC) was assessed using the Folin–Ciocalteu method as described by Singh (13). Under this procedure, 1 ml of 1 mg/m concentration extract was added to 5 ml of 10 % Folin–Ciocalteu reagent. Thereafter, 4 ml of 7.5 % sodium carbonate solution was also added to the mixture. The solution was kept at 27°C for 30 minutes, the absorbance was checked at 760 nm using UV–VIS spectrophotometer. The results were expressed as the milligrams of gallic acid equivalents (GAE) per 100 grams of the sample. A calibration curve was constructed making use of gallic acid as standard (14).

Estimation of flavonoids

The flavonoid content was assessed using the aluminum chloride technique. After treating each pomegranate peel extract (PPE) with 4 ml of distilled water and 0.3 ml of 5% sodium nitrite, the mixture was left to incubate for five minutes. After adding 0.3 ml of 10% aluminum chloride, the samples were incubated for further six minutes. After that, 2 ml of sodium hydroxide (1 mol/l) was added, and distilled water was used to bring the solution's volume to 10 ml. The absorbance was measured at 510 nm after the premix was allowed to incubate for an additional fifteen minutes. Using catechin form, the total flavonoid content was calculated and expressed in µg/ml (15).

Estimation of alkaloids

5 grams of PPE were esterified by placing them in a mixture consisting of 200 ml, 10 %acetic acid in ethanol volume, covered and allowed to stay for four hours. The mixture was then filtered and the volume of the filtrates reduced to about one quarter its original volume by heating on a water bath. To achieve precipitation, concentrated NH_4OH was then systematically added to the filtrate. The mixture was allowed to stand overnight for the precipitate to settle. It was then washed with diluted NH_4OH solution and filtered. Alkaloids were recovered as a dry residue and weighed (16).

Estimation of total saponins

A conical flask containing 20 g of PP powder and 100 m of diluted ethanol (1:5 distilled water) was used to extract total saponin. It was then heated in a water bath for almost four hours while being constantly stirred. To gather the entire mixture, 200 ml of ethanol (1:5 distilled water) was used as a wash. A thermostatically controlled water bath at 90°C was used to evaporate the extract concentration to a volume of 40 ml.

The extract was concentrated in a separating funnel, then 20 m of diethyl ether was added, and everything was thoroughly mixed. Ether layer was thrown away, but the aqueous layer was retrieved. 60 ml of n-butanol was added after the purification procedure was repeated. 10 ml of 5% aqueous sodium chloride was used twice to wash the combined n-butanol extracts. After that residual solution was evaporated till concentrated in a water bath and further dried in an oven to a consistent weight, the saponin concentration was determined (16).

Estimation of tannins

Tannins were estimated using the Folin-Ciocalteu technique. 0.5 ml of the Folin reagent was combined with 0.1 ml of PPE that had been dissolved in 7.5 ml of distilled water. The solution was then diluted with 10 ml of distilled water and 1 ml of 35% sodium carbonate was added. The mixture was thoroughly shaken and then allowed to sit at room temperature for half an hour. Reference standard solutions containing 20, 40, 60, 80, and 100 µg/ml of gallic acid were made. The absorbance of the test and standard solutions was measured against the blank at 725 nm using a UV/Visible spectrophotometer. Gallic acid equivalents (GAE) in milligrams per gram of extract were used to calculate the quantity of tannin (17).

RESULTS

After 18 to 20 hours of oven drying, the fresh to dry yield of PPE was 38.3%. Subsequently, PPE's aqueous, ethanol, and hydro methanol extracts were examined to look for bioactive substances. Figure 1 shows an illustration of PP fresh and dry.

Figure 1: Pomegranate peel (fresh and dry)

Anthocyanin

As seen in Fig. 2, PPE was discovered to be a rich source of anthocyanins. shows that hydromethanol extract has the highest anthocyanin content (90 mg/100 g), followed by ethanol and aqueous extract (62.29 mg/100 g and 58.60 mg/100 g, respectively).

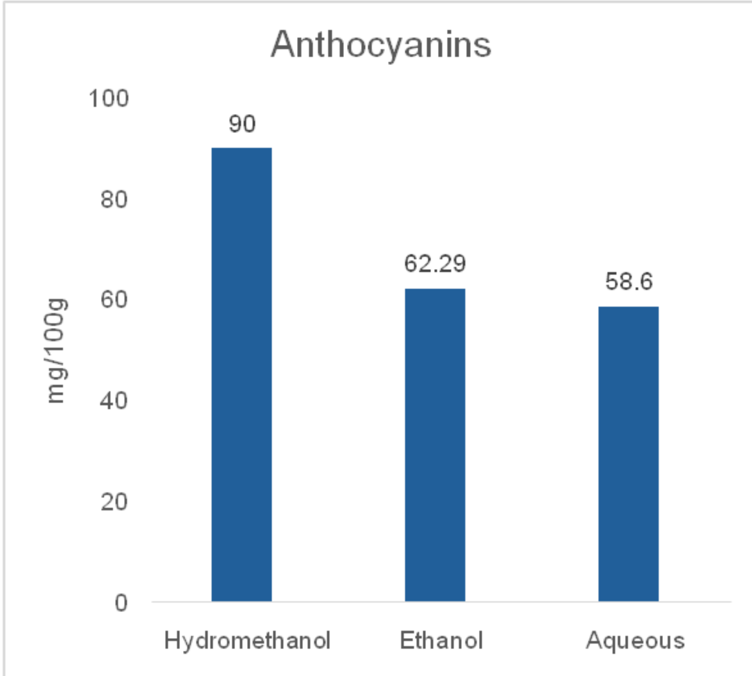

Figure 2: Anthocyanin content in hydromethanol, ethanol and aqueous extract of PPE

Total phenolic content

As shown in Fig. 3. the total phenolic content of PPE was found to be 2.87 GAE mg/ml in hydromethanol extract, 2.88 GAE mg/ml in ethanol and 2.45 GAE mg/ml in aqueous extract.

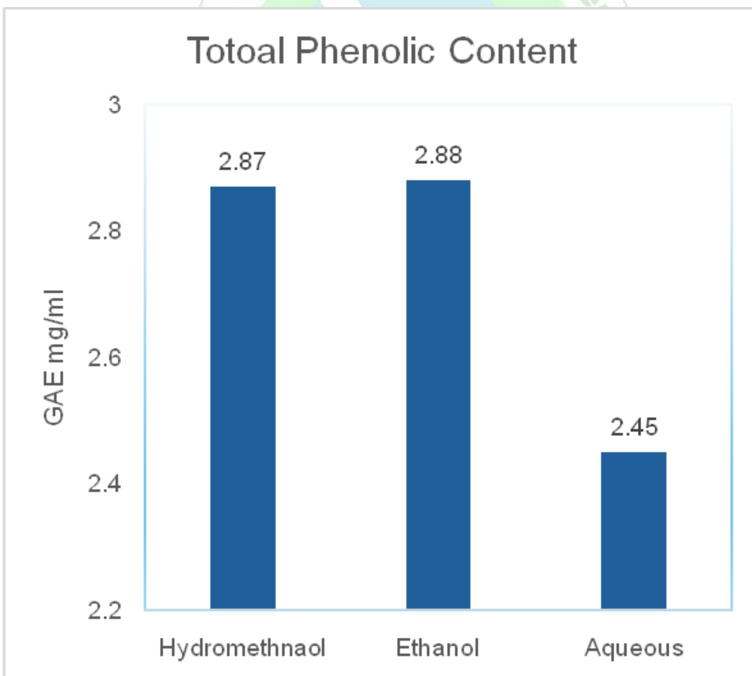

Figure 3: Total phenolic content in hydromethanol, ethanol and aqueous extract of PPE

Flavonoids

As shown in Fig. 4, flavonoids content was observed highest in hydromethanol extract at 91.24 mg/100gm while 84.18 mg/100gm in ethanol extract and 82.75 mg/100gm in aqueous extract.

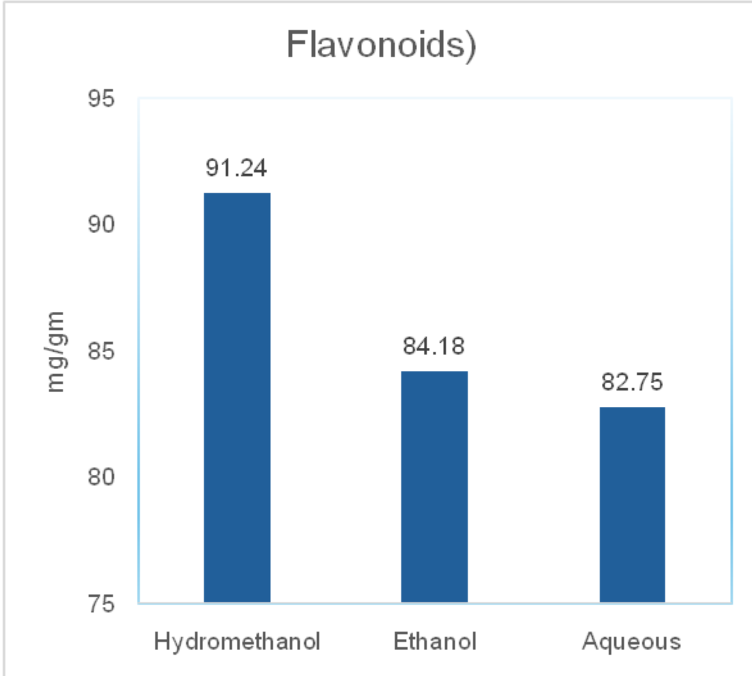

Figure 4: Flavonoids content in hydromethanol, ethanol and aqueous extract of PPE

Saponins

As shown in Fig. 5, saponin content in PPE is highest in hydromethanol at 1.48%, followed by ethanol at 1.45%, and lowest in aqueous at 1.42%. This indicates hydromethanol is slightly more effective for saponin extraction.

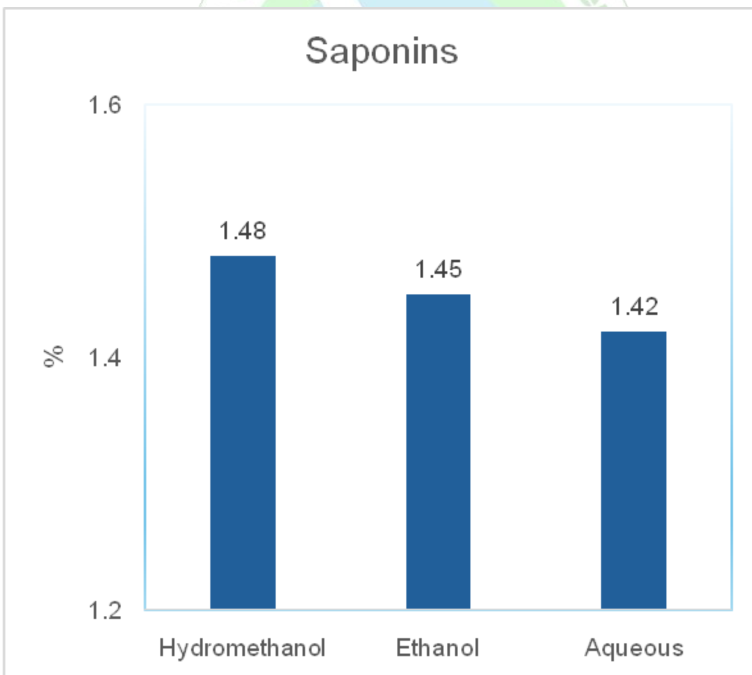

Figure 5: Saponin content in hydromethanol, ethanol and aqueous extract of PPE

Tannins

As shown in Fig. 6, tannin content in PPE is highest in hydromethanol at 46.29 mg/g, followed by ethanol at 18.29 mg/g, and lowest in aqueous at 16.56 mg/g. This indicates hydromethanol is the most effective solvent for tannin extraction.

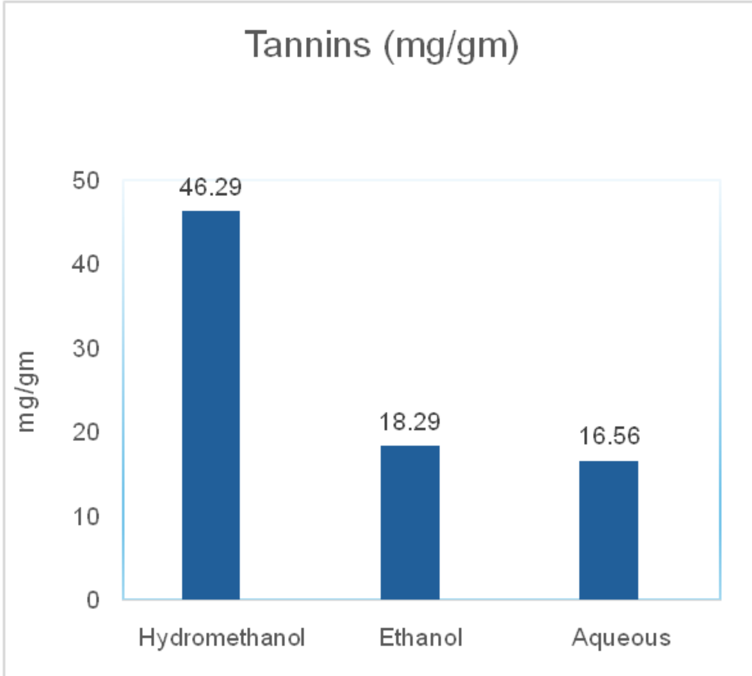

Figure 6: Tannins content in hydromethanol, ethanol and aqueous extract of PPE

Alkaloids

As shown in Fig. 7, alkaloid content in PPE is highest in ethanol at 12.66 mg/g, followed by aqueous at 11.28 mg/g, and lowest in hydromethanol at 2.87 mg/g. This shows that ethanol is the most effective solvent for alkaloid extraction.

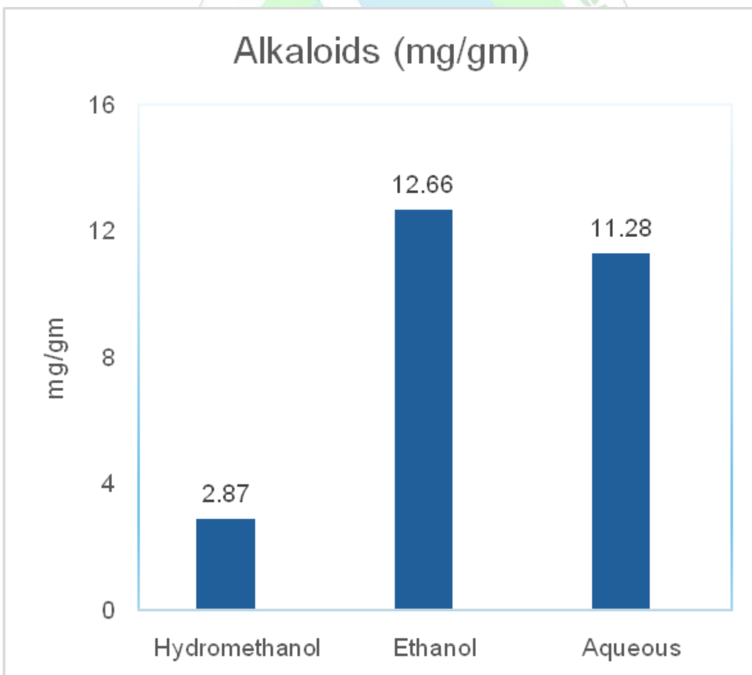

Figure 7: Alkaloids content in hydromethanol, ethanol and aqueous extract of PP

CONCLUSION

To sum up, *Punica granatum* L. certainly has bioactive value but attention is rarely paid to probably the most neglected PP. This peeling suggests that the bioactive ingredients such as antioxidants, polyphenols, saponins, and tannins that are often considered to be present in only some parts of the plant are actually in abundance where no one assumes it's present. This ensures supply of such by-products which is certainly worth the hype, especially since, as the population gets tired of synthetic fungicides and agents, natural barriers automatically come up.

The most interesting part about the extraction process of this study was how it didn't harm the environment and at the same time was, ultrasound assist extraction, efficient than any other alternate method. Hydromethanol, Ethanol, and Water were a few of the solvents that helped enhance extraction efficacy and most importantly, the selection profile of the active compounds.

Data derived emphasized that the UAE treatment with hydromethanol specifically enhanced the extraction of tannins, flavonoids and anthocyanins from thePP. Since the active components present in various extracts had been shown to differ, different extraction methods could be used to obtain desired health-enhancing components.

This study not only highlights the bioactive properties of PP, but also technology that contributes to environmental preservation – 'Green Extraction' techniques.

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