IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Review Article
February 2024 Vol.:30, Issue:2

Methods of Extraction of *Punica granatum* L.: A Comprehensive Review

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ISSN 2349-7203
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Submitted: 25 January 2024
Accepted: 31 January 2024
Published: 29 February 2024

Keywords: Pomegranate, phytochemicals, bioactive compounds, ultrasound-assisted extraction, supercritical fluid extraction.

ABSTRACT

The pomegranate (Punica granatum L.) has recently garnered substantial attention due to its multitude of health benefits encompassing polyphenols, ellagitannins, and anthocyanins. Extracting bioactive compounds from pomegranates plays a pivotal role in realizing its potential for a variety of applications across the food, pharmaceutical, and cosmetic sectors. This comprehensive review presents an in-depth examination of the methods utilized for the extraction of compounds derived from pomegranates. It covers both traditional methods such as simple stirring, decoction, maceration, Soxhlet extraction, high voltage electric discharge, and pressurized liquid extraction. Additionally, this review delves into emerging trends in pomegranate extraction, encompassing environmentally conscious and sustainable practices aimed at mitigating ecological footprints. By amalgamating the existing knowledge, this review offers valuable insights for researchers, industries, and stakeholders keen on enhancing the extraction of bioactive compounds from pomegranates for diverse applications.

INTRODUCTION:

Pomegranate, scientifically known as *Punica granatum L.*, stands as one of the ancient consumable fruits, with a rich history in traditional medicine across various cultures. It is classified as a balausta fruit, characterized by a tough outer pericarp and a soft, juicy mesocarp housing the luscious arils.[1] Among the myriad bioactive components found in natural products, phenolic compounds have garnered significant attention due to their well-documented benefits for health and disease prevention. These compounds are abundantly present in various fruits, including apples, grapes and notably, pomegranate [2].

Fig. 01 Punica granatum L.

It's worth noting that pomegranate peels, constituting 38% of the fruit's total weight, are a compelling source of phenolic compounds. Surprisingly, they surpass pomegranate seeds and pulp in their phenolic content, thus offering great potential as a functional ingredient [3]. Pomegranate juice is a rich reservoir of diverse constituents, including polyphenols, tannins, and anthocyanins, as well as essential vitamins like vitamin C and vitamin E, alongside lipoic acid. Furthermore, it boasts the bioactive component punicalagin, which takes credit for over 50% of the antioxidant prowess found in pomegranate juice [4]. Previous research has established that pomegranate extract possesses the ability to effectively combat harmful hydroxyl (OH) and superoxide radicals [5]. Pomegranate fruits are a treasure trove of constituents dispersed across their various parts, including seeds, peels, and arils.

These components play a crucial role in managing health by influencing a variety of biological activities. The peels, which make up approximately 60% of the fruit, house a spectrum of ingredients, such as flavonoids, ellagitannins, proanthocyanidin compounds, and

an assortment of vital minerals like calcium, magnesium, phosphorus, potassium, and sodium.[6]

Pomegranate exhibits its potent antioxidant properties thanks to the rich presence of various compounds like flavonoids, flavones, anthocyanins, and catechins distributed throughout different parts of the fruit, including the fruits, seeds, and peels.[7] Moreover, studies have revealed the anti-inflammatory and analgesic potential of pomegranate in various forms, such as fruit rind, flowers, and leaves. Pretreatment with dried extracts from these sources displayed significant and dose-dependent reductions in edema compared to control groups. [8] In the realm of hepatoprotective effects, investigations with rats demonstrated that diets supplemented with pomegranate peel powder, whey powder, or a combination of both exhibited promising results in safeguarding the liver against injury when compared to the control group.[9]

The administration of Punica granatum husk in its crude powder form led to a decrease in glucose concentration, triglycerides, cholesterol, and LDL cholesterol, while simultaneously raising the levels of HDL cholesterol and hemoglobin content in the blood. This effect was observed in both the normal group and the alloxan diabetic group of treated rats.[10] Punica granatum exhibits impressive antimicrobial properties, effectively inhibiting the growth of microorganisms.[11] The tannin found in the pericarp of the fruit demonstrates potent antiviral activity against the genital herpes virus (HSV-2), effectively eliminating the virus and preventing its absorption by host cells.[12] Extracts from pomegranate have shown significant efficacy in safeguarding human skin fibroblasts from cell death following exposure to UV radiation. Pretreatment with pomegranate-derived products has also been demonstrated to inhibit the formation of UVB-induced cyclobutene pyrimidine dimers.[13]

Furthermore, pomegranate juice has proven to be effective in combating dental plaque microorganisms, and reducing colony-forming units (CFU). Rinsing with pomegranate juice resulted in a substantial reduction in dental plaque microorganisms.[14] The impact of a hydroalcoholic extract from pomegranate fruits on dental plaque microorganisms was investigated, and the results demonstrated the effectiveness of the extract in combatting dental plaque microorganisms. In a separate study, young adults were examined to assess the effects of mouth rinsing with the pomegranate extract PomElla®. This treatment led to changes in salivary measures related to oral health, including improvements in conditions such as gingivitis. [15]

METHODS OF EXTRACTION OF POMEGRANATE:

1) SIMPLE STIRRING:

Presently, there exists a multitude of extraction techniques based on various physicochemical principles.[16] Among these, simple stirring stands out as one of the most commonly utilized and uncomplicated extraction methods. The efficiency of extracting compounds from plant tissues is influenced by several factors, including the chosen extraction method,[17] the composition and variations in the solvent mixture employed [18], and the selection of different materials.[19]

The extraction of antioxidant compounds from fruits and their by-products constitutes the initial and relatively straightforward step toward potential commercial-scale utilization. However, there has been insufficient exploration into the utilization of by-products such as pomegranate peel (PP) within the pomegranate juice industry. Consequently, the development of efficient extraction methods for antioxidant compounds like flavonoids, phenolics, proanthocyanidins, and their kinetic parameters contained in PP is essential to facilitate the selection of the most suitable extraction technique. Research has revealed that the antioxidant activity in fruits, particularly pomegranates, is typically higher in commercial juices extracted from whole pomegranates due to the presence of seeds and peels during the squeezing process, as opposed to juices obtained solely from the arils. In particular, the peel has been reported to exhibit relatively higher antioxidant activity compared to the seeds and pulp, making it a valuable source of natural antioxidants [20-21].

Typically, solvents like methanol, ethanol, acetone, and water are used for extracting pomegranate antioxidant compounds. However, these solvents often lead to significant co-extraction of unwanted substances and a reduction in the yield of target antioxidants [22]. Among these solvents, ethyl acetate may offer notable selectivity, while methanol and water may result in a higher total extract yield. For instance, in a study conducted by Pan et al. PP (1 g) was extracted using a magnetic stirrer with a stirring speed of 1200 rpm at 25°C and 50 mL of water for 60 minutes, yielding an extraction efficiency of 11.9% [23]. In another study, using the same method at 40°C, methanol as the solvent resulted in an 8.26% yield, whereas water as the solvent yielded 5.90%. Additionally, solvents such as ethanol, acetone, and ethyl acetate produced yields of 1.55%, 0.37%, and 0.18%, respectively. In a separate research study, Qu et al. [24] demonstrated that water served as an efficient "green" solvent for

extracting antioxidants from pomegranate marc, achieving high phenolic content (229 mg TAE/g) and DPPH scavenging activity (6.2 g/g) within just 2 minutes of extraction time.

2) MACERATION:

The maceration technique was utilized to extract polyphenols from powdered pomegranate, following the method Elfalleh et al. outlined with slight modifications [25]. Initial experiments were undertaken to ascertain the most suitable solvent concentration (25%, 50%, 75%, and 100%) and sample-to-solvent ratio (1:20, 1:15, and 1:10). Following these trials, three different solvents—acetone, methanol, and ethanol—were employed at two selected concentrations, specifically 50% and 75%. The sample-to-solvent ratio was fixed at 1:15, and the maceration process was conducted at a temperature of 40°C. Each conical flask contained three grams of the respective peels and 45 ml of solvent at the designated concentrations. A 'Shaking Water Bath' facilitated the procedure, with the flasks immersed for approximately 20 hours. Subsequently, all samples were filtered through Whatman filter paper 41, followed by centrifugation at 5,000 rpm for 10 minutes using a Beckman J2-21 centrifuge. The solvent was then evaporated using a Rotary Evaporator under a vacuum of 140 mbar and a temperature of 45°C until complete drying. The resulting extracts were collected in amber glass bottles and stored under refrigerated conditions at 4-6°C [26].

Fig. 2 Maceration

3) PRESSURIZED LIQUID EXTRACTION:

Pressurized liquid extraction (PLE) of pomegranate peels was conducted using a pressurized liquid extraction system. Deionized water served as the extraction solvent for all experiments. This deionized water was degassed for one hour using a continuous stream of nitrogen gas.

All extractions were carried out under the following standard conditions: 10 grams of dried pomegranate peels with a particle size ranging from 65 to 212 micrometers were combined with 40 grams of sea sand and placed within a 100 ml stainless steel extraction cell. To prevent suspended particles from entering the collection bottles, a circular cellulose filter (30 mm in size, Dionex) was positioned at the bottom of the extraction cell. The extraction process was then automated using an ASE 300 system. Once the oven temperature reached the designated set point of 40 degrees Celsius, the cell was preheated at this temperature for 5 minutes, followed by a 5-minute static extraction step. After this static extraction phase, the extracts were transferred into 250 ml collection vials. Fresh solvent, equivalent to 60% of the cell volume, was subsequently pumped into the extraction cell, followed by a nitrogen gas purge lasting for 100 seconds. The extracts collected in vials were then transferred into 250 ml volumetric flasks, and the total volume was adjusted to 250 ml with water. This single extraction cycle was employed to determine the effective extraction factors. Various independent factors, including particle size, temperature, static time, and flush volume, were investigated separately. The effects of these independent factors on the system's responses were determined by altering the level of each factor while keeping the others constant. Once the optimum levels of independent factors were determined, the number of optimal extraction cycles was established under these conditions.

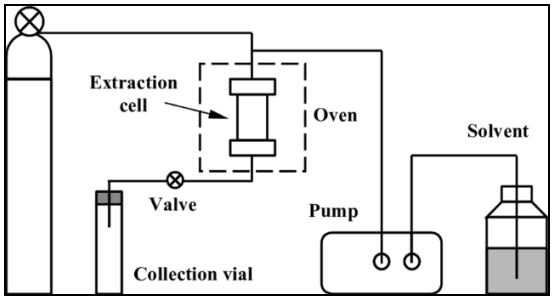

Fig. 3 Pressurized liquid extraction

In addition, pomegranate peels were subjected to extraction using different solvents, namely methanol, ethanol, ethyl acetate, acetone, and water. This was done using a magnetic stirrer to compare the results with those obtained via PWE. In this process, 10 grams of pomegranate peels were extracted with 100 ml of methanol at 40 degrees Celsius for 1 hour. The resulting extract was filtered through a Whatman No. 1 filter paper, and any remaining residue underwent a re-extraction with the same volume of the same solvent. The extracts were combined, and their volume was adjusted to 250 ml using the same solvent. This same

procedure was followed for the other solvents used. All extracts were filtered using a 0.45-micron filter before analysis [27].

4) SOXHLET EXTRACTION:

The powdered pomegranate peel (PP) was mixed with a 60:40 (v/v) ethanol-water solvent at a ratio of 1 gram to 50 milliliters, following the method outlined by Živković et al. in 2018. This mixture underwent sonication at 400 watts and a frequency of 28 kHz for varying durations and temperatures using an ultrasonic bath. Subsequently, the samples were subjected to magnetic stirring (MR Hei-Tec) at different speeds for 24 hours at a temperature of 25°C. The treated samples were then centrifuged at 7000 rpm for 20 minutes, and the supernatant was filtered through a vacuum filtration setup using Whatman filter paper No. 1 to eliminate any large particles. Ethanol was separated from the extracts using a rotary evaporator at 50°C. The remaining PPE was then lyophilized at -30°C and under a pressure of 0.07–0.1 mbar for a period of 48 hours. Finally, the resulting powders were stored in plastic zip-lock bags at -18°C for future use [28].

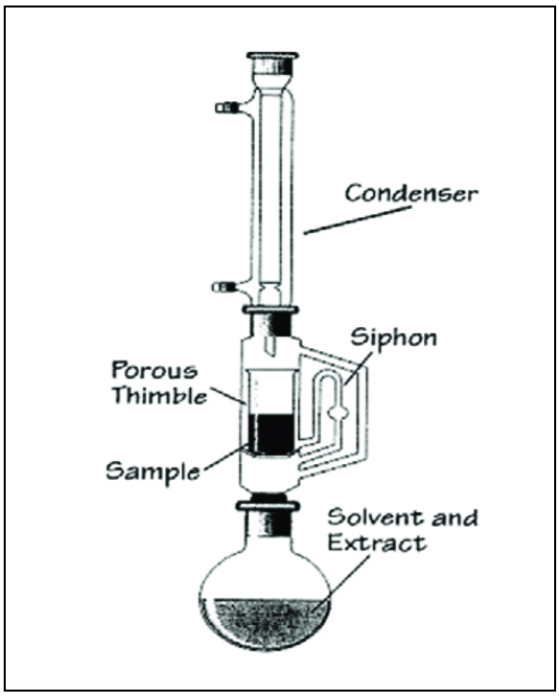

Fig. 4. Soxhlet Apparatus.

5) DECOCTION:

The traditional decoction method for extracting compounds from pomegranate peels involved combining the milled, dried materials with 40 mL of Milli-Q water in a beaker. This mixture was then heated and maintained at a boiling point for a duration of 10 minutes. Subsequently,

it underwent filtration using a Buchner funnel fitted with Whatman No. 1 filter paper, followed by centrifugation at a relative centrifugation force of 2016 for 10 minutes. The resulting extracts were stored at -20°C until further analysis [29].

6) HIGH VOLTAGE ELECTRICAL DISCHARGE:

The continuous High-Voltage Electric Discharge (HVED) extraction system consists of several key components, including a high-voltage pulse generator, a continuous treatment chamber, data acquisition systems, a fluid handling and cooling system, and voltage and current measuring devices. The high-voltage pulse generator (model TP3020) was sourced. This generator produced exponentially decaying bipolar triangle waveform pulses characterized by a 2-microsecond pulse duration and a frequency range of up to 1000 Hz. It was capable of delivering discharges ranging from 20 kV to 10 kA for a few microseconds [30].

The treatment chamber utilized in this system followed the "converged electric field type" design. This chamber was engineered to achieve higher electric field intensity within a confined volume without the need to escalate the voltage applied to the electrodes [31].

It featured a pair of parallel disc mesh electrodes constructed from stainless steel and an insulating plate with a small aperture positioned between the electrodes. This aperture created a limited treatment region where the electric field intensity was notably concentrated.

The electric discharge intensity within the central aperture of the insulating plate significantly exceeded that of other areas within the chamber. Consequently, this central hole in the insulator served as the focal point for High-Voltage Electric Discharge (HVED) processing.

One end of the stainless-steel electrode received high-voltage pulse power, while the other end was grounded. Liquid materials were continuously pumped and introduced into the chamber through the holes in the disc electrodes. However, only the materials located inside the hole of the insulating plate underwent HVED treatment.

A photograph captured the UV light emanating from the treatment region, where an electric field intensity of 20 kV/cm was applied. The diameters of both the electrodes and the treatment region were 20 mm and 1 mm, respectively.

The mesh opening diameter measured 2 mm, while the distance between the electrodes (denoted as "d") varied between 1 and 10 mm. Specifically, tests were conducted at distances

of 2, 3, 4, and 5 mm, with a peak pulse voltage of 9 kV applied. The electric field intensity (E, in kV/cm) was calculated using the formula:

$$E = V / d$$

Where, - V represents the peak pulse voltage (in kV).

- d represents the gap distance between the electrodes (in cm) [32].

For the continuous High-Voltage Electric Discharge (HVED) extraction process, it's well-documented that water is an environmentally friendly and efficient solvent for extracting phenolic compounds from plant materials [33-34].

Hence, water was chosen as the extraction solvent in this study. To operate HVED effectively, the flow rate of materials (ranging from 8 to 14 mL/min), the distance between electrodes (2 to 5 mm, corresponding to electric field intensities ranging from 18 to 45 kV/cm), and the liquid-to-solid ratio (ranging from 20 to 50 mL/g) must be meticulously controlled for the continuous HVED system. The process involved finely blending 10 grams of dried pomegranate peel samples with an appropriate volume of distilled water, utilizing a stirrer. The resulting suspension was pumped through a cooling coil immersed in a water bath before being introduced into the HVED treatment chamber via a peristaltic pump at the specified flow rate. Subsequently, the suspension underwent electric discharge treatment at various distances between the electrodes while maintaining a constant peak pulse voltage of 9 kV and a frequency of 100 Hz. The treated suspension exiting the chamber and passing through the cooling coil was promptly collected. Following the extraction process, the mixture underwent filtration using quantitative filter paper with a diameter ranging from 8 to 10 mm. Subsequently, the extracting solution was subjected to centrifugation at 4000 rpm for a duration of 10 minutes, and the resulting supernatant was carefully collected through membrane filtration utilizing a 0.45 µm filter. The filtrate was then gathered and stored at 4°C in a refrigerator for subsequent analysis. As a control experiment, warm water maceration was conducted. Based on preliminary optimization, the optimal conditions for warm water maceration were as follows: a temperature of 70°C, a time duration of 60 minutes, and a liquid-to-solid ratio of 35 mL/g [35-36].

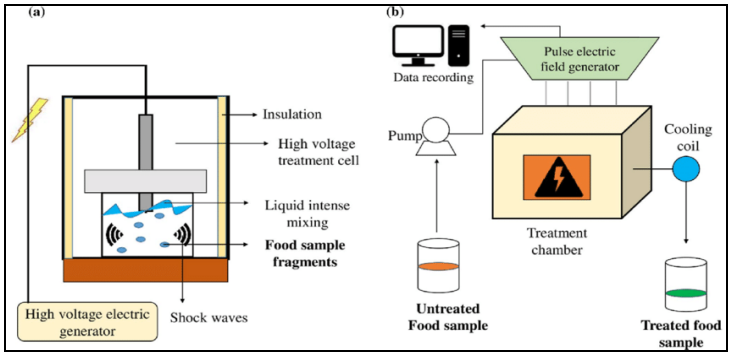

Fig.5 High Voltage Electrical Discharge

CONCLUSION:

The comprehensive review of the methods of extraction of Punica granatum L. reveals a diverse range of techniques employed to extract bioactive compounds from this plant. These methods include conventional techniques like maceration and Soxhlet extraction, as well as modern approaches such as ultrasound-assisted extraction and supercritical fluid extraction. Each method has its advantages and limitations, impacting the yield and quality of extracted compounds. Researchers must carefully select an extraction method based on the desired bioactive components and the specific properties of Punica granatum L. to enhance the efficiency and sustainability of the extraction process.

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