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Effect of Solvent Extraction and Blanching Pre-Treatment on Phytochemical, Antioxidant Properties, Enzyme Inactivation and Antibacterial Activities of 'Wonderful' Pomegranate Peel Extracts

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Abstract: 'Wonderful' pomegranate (*Punica granatum* L.) peel is rich in phytochemicals which are responsible for its strong antioxidant and antimicrobial activities, but it has low economic value as it is mainly discarded, causing an environmental waste management problem. To examine the best processing regime for pomegranate peel wastes, different solvents (ethanol, methanol and acetone) at various concentrations (50%, 70% and 100%) and blanching at 60, 80 and 100 °C for 1, 3 and 5 min, for each temperature, were tested. Ethanol at 70% (*v/v*) provided the highest extract yield, total phenolic and total tannin content at 29.46%, 10.61 ± 0.15, and 0.76 ± 0.02 mg GAE/g DM, respectively. Antioxidant activity using the 2,2 diphenyl-1-picryl hydrazyl assay (DPPH), ferric-reducing antioxidant power assay (FRAP) and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid assay (ABTS) were reported at 243.97 ± 2.43, 478.04 ± 73.98 and 718.79 ± 2.42 µmol Trolox/g DM, respectively. A blanching temperature of 80 °C for 3 min led to the highest extract that had a total phenolic content of 12.22 ± 0.08 mg GAE/g DM and total tannin content of 1.06 ± 0.06 mg GAE/g DM. This extract also exhibited the best antioxidant activity for the DPPH, FRAP and ABTS assays. Two blanching temperatures, 80 or 100 °C, significantly reduced polyphenol oxidase and peroxidase activities (*p* < 0.05). Although blanched peel extracts showed a broad-spectrum activity against test bacteria, blanching at 80 °C for 3 or 5 min was most effective. Hot water blanching is thus a suitable environmentally friendly post-harvesting processing method for pomegranate peels that are intended for use as extracts in value-added products with good antioxidant and antibacterial effects.

Keywords: agriculture waste; antibacterial activity; antioxidant activity; blanching; peroxidase; polyphenol oxidase; polyphenols; pomegranate fruit peel; value-addition

1. Introduction

Food production is expected to increase by approximately 70% by 2050 to meet global food demand [1]. However, 1.3 billion tonnes (about a third) of the total produced food is lost or wasted each year [1], with large quantities of more than 0.5 billion tonnes of this food waste being generated from fruit waste from agricultural processes globally [2]. This fruit waste is usually disposed in landfills or incinerated, raising several environmental concerns and health risks. However, it is an excellent source of numerous value-added materials such as bioactive components which include vitamins, antioxidants that may be polyphenols, natural pigments, flavours and aromatic agents [3–5].

Pomegranate (*Punica granatum* L.; Lythraceae) has gained popularity and demand worldwide due to its multi-functionality and health-promoting effects in the human diet [3,6,7]. Over 300,000 hectares of pomegranate cultivation area has been reported around the world, with a yield reaching approximately 3 million metric tonnes [8], with the northern hemisphere contributing 90% of the pomegranate fruit, while the southern hemisphere contributes only 10% [5]. In 2019, South Africa was reported as one of the major pomegranate producers in the southern hemisphere, among countries such as Peru and Argentina at 14%, 84% and 2%, respectively [9]. South African pomegranate cultivation area is reported at 1032 hectares, with 70,324.32 tons of pomegranate fruit produced in packhouses alone in the year 2020 [9]. ‘Wonderful’ is the most popular pomegranate cultivar grown in South Africa, with a cultivar distribution of 76%, and during its processing, it contributes to the majority of the pomegranate waste in the form of seeds and peel at 1406.49 tonnes [9]. The pomegranate industry faces a serious problem with regards to postharvest fruit losses and waste [10,11]. One tonne of pomegranate fruit generates approximately 520 kg of pomegranate peel after processing, when it could be a potential source of value by-products [12]. Finding ways to utilise this nutritionally rich waste would be beneficial to the farmers that cultivate and sell pomegranate fruit in South Africa, thereby boosting the economy of the country, and would ultimately be environmentally friendly as well.

There are many value addition opportunities caused by the great demand for natural products from ‘Super foods’ waste, making them economically attractive. The best alternative for this peel waste is to utilise it in food, nutraceuticals, pharmaceuticals, textile, packaging, cosmetics, and other industries, after proper processing of the bioactive compounds in the fruit peel. In order for this to occur, novel processing methods may be beneficial for the recovery of phytochemicals in pomegranate peel waste to compete on a global commercial scale. New ways of disposing or using this nutrient-rich pomegranate peel waste for value addition are thus highly sought after [3,5,13]. The health-promoting properties of pomegranate peel have influenced the food industry to incorporate pomegranate peel waste in food preparations, which include phenolic-rich diets, food supplements and nutraceuticals [5], fabrication of nanofibers with antimicrobial activity [14], as preservatives for the food industry [3,6,7,12,15–17], cosmetic formulations [3,6,18–22] and use for the textile industry for the application of a variety of disposable items [23–25]. The utilisation of pomegranate peels in food supplements will likely gain increasing popularity as the peels have many health-beneficial effects that have recently been shown in vivo using mice as pharmacological models [26]. The health-promoting benefits of pomegranate peel have prompted the nutraceuticals industry to increase the focus on phenolic-enriched diets and food supplements [3,27,28], and pomegranate peels are thus ideal for this purpose.

The fruit peel has substantial amounts of potentially valuable components such as dietary fibre, pectin, nutrients, phenolic acids (ellagic acids, gallic acids, chlorogenic acids, caffeic acids, ferulic acids, p-coumaric acids, syringic acid, cinnamic acid and vanillic acids), flavonoid (anthocyanins such as delphinidin, cyanidin, pelargonidin 3-glucosides and 3,5-diglucosides) and ellagitannins (punicalagin, punicalin, granatins, pedunculagin, tellimagradin, galla-gyldilactone, corilagin and castalagin) [3,5,29,30]. These compounds, especially hydrolysable tannins, are mainly found in the pomegranate peel at higher concentrations than other pomegranate fruit parts [5,29,31]. The chemical features of these compounds control the strong biological activity, such as antioxidant, antimicrobial, anticancer and anti-inflammatory activities linked to pomegranates [3,5,29,30,32,33]. Ellagitannins, such as punicalagin, have multiple hydroxyl groups that are structurally important for radical scavenging [12,29,31]. However, during processing (such as drying and extraction) of pomegranate peel waste, phytochemicals can undergo unpredictable chemical changes, resulting in the fluctuation of these valuable bioactive compounds and their altered biochemical functions [5]. To find the best practice in extraction of phytochemicals from pomegranate, numerous extraction methods and solvents are explored by several authors and have been previously reviewed in detail by Magangana et al. [5].

For example, Wang et al. [34] reported the highest extract yield of phenolics at 8.26% per g DM in methanol extract and the lowest in ethyl acetate extract at 0.18% in 'Wonderful' pomegranate peel extracts, while Muhson et al. [35] observed the highest total phenolic content at 168.26 mg gallic acid/100 g dry weight, total flavonoid content at 87.21 mg quercetin equivalents per 100 g dry weight and antioxidant activity using DPPH at 88.46% and ferric-reducing antioxidant power assay (FRAP) at 142.21 mg Trolox equivalents per 100 g dry weight using 50% acetone. However, these organic solvents are believed to be toxic, and the extraction conditions are often regarded as harsh. Contrary to this, water, ethanol and their mixtures pose as suitable alternatives to methanol and acetone as non-toxic food-grade solvents for commercial extraction of pomegranate peel waste [5,31]. Malviya et al. [36] reported, using 50% ethanol and aqueous extract, highest yield and total phenolic content at 16.3% and 438.3 mg/g respectively, in 'Ganesh' pomegranate peel extracts.

Furthermore, conventional organic solvents extraction methods such as Soxhlet, maceration and hydrodistillation are time-consuming, require pure and expensive solvent and exert a negative impact on the environment by emission of volatile organic compounds [5,37,38]. These traditional extraction methods are less efficient towards extraction, especially in the context of liberating bound phenolics from cellulosic structures in the plant material [5,37]. On the other hand, ultrasound-assisted solvent extraction is an eco-friendly approach that provides higher yields compared to traditional methods, plus it can be used not only in the laboratories but on a large scale, making it an economically viable industrialised strategy [5,12]. To generate an aqueous pomegranate peel extract from 'Sishe Kape-Ferdos', Sharayei et al. [38] optimised an ultrasound-assisted extraction procedure with an ultrasonic exposure time of 6.2 min and ultrasonic amplitude of 60%, resulting in optimum extract yield and total phenolic content of 13.1% and 42.2 mg GAE/g, respectively. Given the influence of processing on the phytochemical profile of pomegranate peel extracts, fluctuations may also be a result of several factors, such as cultivar variation, environmental conditions, fruit maturity status and storage [3,5,6,39]. Thus, making it difficult to determine the best practice. Furthermore, it is impossible to develop a standard solvent and extraction parameter(s) that is suitable for all kinds of phytochemical compounds found in plants, including pomegranate peel waste. Therefore, a screening process is necessary to identify the best solvent and concentration for a specific extraction procedure for a particular sample. Ultrasound solvent-assisted extraction was used to facilitate this process and aid in efficient extraction of phenolics in 'Wonderful' pomegranate peel using various solvents and at varying concentrations in this experiment. We therefore hypothesised that more efficient extractability of phytochemicals in 'Wonderful' pomegranate peel extracts is dependent on the solvent type and concentration.

Furthermore, during processing (drying), the phytochemical profile of pomegranate peel may be altered, reducing heat-sensitive phytochemicals. Applying a pre-treatment technique such as blanching before drying may help to preserve and even improve the phytochemical profile of the peel by breaking down of the cell wall and releasing bound phenolics which can be recovered during extraction [5,40]. This is a practical step to produce high-quality value-added pomegranate peel products. To the best of our knowledge, no studies have been reported concerning the effects of blanching pre-treatment on pomegranate peel extracts. However, numerous studies have shown the potential of blanching pre-treatment to improve the quality attributes and the recovery of phytochemicals and nutrients of various fruit peel and vegetable peel waste for other species [41–43]. Blanching can reduce the drying time of peel waste during processing, thus reducing heat-sensitive bioactive compounds [5]. Hot water blanching is the most common blanching technique and involves immersing food material into hot or boiling water [41–43]. Blanching is an important intermediate thermal processing step to enhance preservation and quality of foods by inactivating enzymes such as peroxidase (POD) and polyphenol oxidase (PPO) that can cause losses in phytochemical and nutrient content, as well as flavour and colour to the final peel product [41].

Nurhuda et al. [42] studied the effects of blanching using boiling water at 100 °C (for 2.5 and 5 min) on total anthocyanin content as well as browning enzymes (peroxidase (POD) and polyphenol oxidase (PPO)) in 'Anak Sekolah' rambutan fruit peel. Duarte et al. [43] investigated the effects of water blanching (at 90 °C for 1 min) as a pre-treatment on the total phenolic and total fibre content of yellow passion fruit (*Passiflora edulis* var. *flavicarpa*) peel. Several studies on mango peels [44], citrus peels [45], persimmon peels [46] and banana peels [47], to name a few, reporting the inactivation of browning enzymes (PPO and POD) and potential recovery of phytochemicals after blanching are evident in the literature. Therefore, it was important to know whether hot water blanching could positively affect the extraction of phytochemicals in 'Wonderful' pomegranate peel extracts, and the influence of temperature and time to generate fruit peel extracts of a high quality needed to be evaluated. Therefore, we hypothesised that blanching would positively affect the extraction of phytochemicals in 'Wonderful' pomegranate peel extracts and secondly, the best blanching regime is dependent on temperature and time.

To the best of our knowledge, no comparative study investigating the effect of solvent extraction and hot water blanching of 'Wonderful' pomegranate fruit peel waste has been reported in the literature. Therefore, the objectives of this study were two-fold: (1) to determine best extraction solvent to obtain the best phytochemicals and antioxidant activity, and (2) to determine the best blanching temperature and time for high extraction of phytochemicals, antioxidant, enzyme inactivation and antibacterial activities in 'Wonderful' peel extracts.

2. Materials and Methods

2.1. Plant Materials

Commercially mature pomegranate fruits ('Wonderful') were harvested from Blydeverwacht farm, Wellington (33°48'0'' S, 19°53'0'' E), in the Western Cape Province, South Africa. Twenty fruit of the same size, shape and colour, without physical defects from ten randomly selected trees were collected on the 29 March 2019. The fruits were transported to the Postharvest Technology Research Laboratory at Stellenbosch University in an air-conditioned vehicle and stored at 7.5 ± 0.5 °C and $92\% \pm 3\%$ relative humidity (RH) before processing to preserve quality [13]. For each fruit, fresh pomegranate peel was cut in the dimensions of 20 ± 0.5 mm (length), 20 ± 0.5 mm (width) and 5 ± 0.5 mm (thickness) [6].

2.2. Sample Preparation and Phytochemical Extraction

Pomegranate peels were collected after juice extraction using a hand juice pressing machine throughout the study. Clean pomegranate fruit peel was dried at 60 ± 2 °C, at a relative humidity of 18.63% and 1.0 m/s air velocity for 18 h to a moisture percentage of 8% dry basis [34]. The experiment was performed in triplicate, with the averages reported. A moisture analyser (KERN, DBS60-3, Balingen, Germany) was used to measure the moisture content in pomegranate peel. Phytochemicals from the ground pomegranate peels were extracted using the method described by Wang et al. [34] with slight modification. The modifications included the particle size of the pomegranate peel (PP) of less than 1 mm, the use of an ultrasonic bath (Scientific, South Africa) equipped with a maximum power of 700 W, 40 kHz frequency and internal dimensions of $500 \times 300 \times 150$ mm and extraction of 0.01 g of dried pomegranate peel powder mixed with 10 mL solvent (methanol, ethanol, acetone) at varying concentration (50%, 70% and 100%). To determine the best solvent type and concentration, only unblanched (pomegranate peel not subjected to any blanching, but only oven-dried at 60 °C for 18 h and ground to less than 1 mm) were used in this experiment. Once the best extraction solution was determined, based on solvent type leading to the best extract yield, total phenolic content, total tannin content and antioxidant activity, this was then chosen and used in the blanching experiment. The ultrasonic extraction was performed in the following conditions of temperature (40 ± 2 °C), extraction

time (1 h) [48,49], solvent ratio (15:1 *w/w* dry weight; solvent/sample) and maximum power and frequency extraction of 700 W and 40 kHz, respectively.

For the blanching experiment, pomegranate peels were also collected after the production of juice. Clean pomegranate fruit peels were hot water-blanching in a water bath (Scientific, Maraisburg, South Africa) at pre-set temperature-time combinations of 60, 80 and 100 °C for 0, 1.0, 3.0 and 5.0 min. Blanching peels were shocked in iced water (30 s) to stop the continuous cooking process and were carefully drained before weighing and oven-dried at 60 ± 2 °C, at a relative humidity of 18.63% and 1.0 m/s air velocity for approximately 10 to 18 h depending on the treatment to reach a moisture content of 8% dry basis [34]. The unblanching pomegranate peel (peels not subjected to hot water blanching) were taken as the control and this experiment was performed in triplicate ($n = 3$). Moisture content of dried pomegranate fruit peels was evaluated using a moisture analyser (KERN, DBS60-3, Balingen, Germany). The dried peels were ground to a particle size less than 1 mm and stored at -20 °C until extraction. Only 70% ethanol was further used in the blanching experiment, as it recorded the highest extract yield, TPC, TTC and antioxidant activity in the initial solvent extraction experiment.

2.3. Determination of Phytochemicals and Antioxidant Activity

2.3.1. Extract Yield

For each treatment, ten grams of pomegranate peel powder was weighed in a digital balance (ML3002, Mettler Toledo, Switzerland) and placed in different conical flasks and was extracted with different solvents (methanol, ethanol and acetone) at varying concentrations (50%, 70% and 100%). Dilutions for the varying concentrations were accomplished using distilled water. The liquid extracts were sonicated and filtered using Whatman filter paper number 1 under a vacuum. Thereafter, the extracts were dried in a fume hood at room temperature. To further remove any solvent and concentrate the extracts, extracts were subjected to vacuum evaporation (G3 Heidolph, Schwabach, Germany) at 50 ± 2 °C. All experiments were performed in triplicate. The dried extracted peel samples were weighed to calculate extract yields, and further analyse phenolic content, antioxidant and antibacterial potential. The extract yield was calculated based on Equation (1) [34]:

$$\text{Total extract yield (\%)} = \frac{\text{g. dried extract}}{100 \text{ g peel powder}} \times 100 \quad (1)$$

The extract yield was expressed as % per g DM.

2.3.2. Total Phenolic Content (TPC)

Determination of total phenolic (TPC) concentration was measured using Folin-Ciocalteu reagent (Folin-C) with the method described by Fawole et al. [3], with slight modifications. Diluted peel extracts (50 µL) were mixed with 450 µL of 50% methanol followed by the addition of Folin-C (500 µL), and 2% sodium carbonate (2.5 mL) after 2 min. The mixed solution was vortexed for 30 s and stored for 40 min at room temperature in the dark and followed by recording the absorbance at 725 nm using a UV-visible spectrophotometer (Thermo Scientific Technologies, Madison, WI, USA). Gallic acid was used as a standard and the results were expressed as mg gallic acid equivalent per g peel extracts (mg GAE/g DM), using a gallic acid (0–0.014 mg/mL) standard curve.

2.3.3. Total Tannin Content (TTC)

The total tannin determination was conducted using a method reported by Makkar [50]. Polyvinylpyrrolidone (PVPP) was used to separate non-tannin compounds from those with tannin by adding 100 mg PVPP to 1.0 mL of distilled water and 1.0 mL peel extract in a test tube, making a mixture. Then, the mixture was vortexed for 30 s and stored for 15 min at 4 °C before it was centrifuged for 10 min at $4000 \times g$. Upon extraction, 50 µL of the supernatant was mixed with 450 µL of 50% methanol. After this, 500 µL of Folin-C was added followed by the addition of 2% sodium carbonate (2.5 mL), and after 2 min,

the reaction cocktail was further incubated for 40 min at room temperature. Thereafter, absorbance values were recorded at 725 nm using a UV-visible spectrophotometer (Thermo Scientific Technologies, Madison, WI, USA). The peel extracts that were not treated with PVPP were measured for TPC and TTC, and calculated based on Equation (2) [50]:

$$\text{TPC (in peel extract without PVPP)} - \text{TPC (in peel extract treated with PVPP)} \quad (2)$$

Results were expressed as mg gallic acid equivalent per g peel extracts (mg GAE/g DM).

2.3.4. Total Flavonoid Content (TFC)

The total flavonoid content (TFC) was determined using the method described by Yang et al. [51]. The samples were prepared by extracting 0.01 g of peel sample with 10 mL 50% methanol measured at 10 mL, which was vortexed for 30 s. Thereafter, the mixture was sonicated in an ultrasonic bath for 10 min and centrifuged at $4000 \times g$ for 12 min at 4 °C. The solution was prepared by taking 250 µL of the peel extract and 1.2 mL distilled water before 75 µL of 5% sodium nitrite was added. After 5 min, freshly prepared 10% aluminium chloride solution, measured at 150 µL, was added to the mixture. After 5 min, 500 µL of 1 mM sodium hydroxide was added to the mixture and 775 µL of distilled water was added to bring the final volume to 3 mL. The mixture was vortexed for 30 s and the absorbances were obtained soon after, using a spectrophotometer (Thermo Scientific Technologies, Madison, WI, USA) at 510 nm. Catechin (0.0–0.5 µg/mL) was used for the standard curve and the results were expressed as catechin equivalent per g peel extracts (mg CE/g DM).

2.3.5. Total Anthocyanin Content (TAC)

The pH differential method to quantify total anthocyanin content (TAC) and the protocol of Wrolstad [52] were used. The experiment was performed in triplicate with each extract measured at 1 mL and thereafter mixed with 9 mL of pH buffers 1.0 and 4.5 in different test tubes. The absorbance of the mixture was measured at 520 and 700 nm for pH buffers 1.0 and 4.5, respectively. To calculate the total absorbance, Equation (3) was used from below and the total anthocyanin content was calculated using Equation (4), also indicated below. The results were expressed as cyanidin 3-glucoside.

$$A = (A_{510} - A_{700})_{\text{pH}1.0} - (A_{510} - A_{700})_{\text{pH}4.5} \quad (3)$$

$$\text{Total anthocyanin (}\mu\text{g/mL)} = \frac{(A \times \text{MW} \times \text{DF})}{\epsilon \times L} \quad (4)$$

where A = absorbance, ϵ = *Cyd*-3-glucoside molar absorbance (26,900), MW = anthocyanin molecular weight of 449.2, DF = dilution factor and L = cell path-length (1.0 cm). The final results were expressed as *Cyd*-3-glucoside equivalent (C_3gE) per g dry matter (mg C_3gE /g DM).

2.3.6. Ascorbic Acid Concentration

To determine the ascorbic acid content, we followed the method of Mphahlele et al. [6]. Each pomegranate peel extract (1.0 g) was combined with 50 mL of 1% metaphosphoric acid before sonicating on ice for 4 min. Afterwards, the supernatant of 1.0 mL, recovered after centrifugation at $4000 \times g$ for 12 min, was pipetted into a tube and mixed with 9 mL of 2,6 dichlorophenolindophenol dye (0.0025%). The mixture was incubated in the dark for approximately 10 min before the absorbance measurement at 515 nm. An authentic L-ascorbic acid standard curve, ranging from 0.01 to 0.1 µg/mL, was used to calculate ascorbic acid concentration in treated samples. The results were expressed as ascorbic acid equivalents per g dry matter (µg of AAE/g DM).

2.3.7. Radical-Scavenging Ability (RSA)

The 2,2-diphenyl-1-picryl hydrazyl (DPPH) assay was performed according to Karioti et al. [53], with slight modifications as indicated by Fawole et al. [3]. Eppendorf tubes carrying each test peel extract (15 µL) were mixed with 735 µL methanol and a 0.1 mM solution of DPPH (750 µM) that was also dissolved in methanol. The mixture was incubated for 30 min in the dark at room temperature before measuring the absorbance at 517 nm using a UV-visible spectrophotometer (Thermo Scientific Technologies, Madison, WI, USA) in dim light. Trolox (0–1.0 mM) was used for the standard curve and results were expressed as µmol Trolox/g DM according to Equation (5), as indicated below:

$$\text{RSA \%} = [1 - (A_{\text{test}}/A_{\text{blank}}) \times 100] \quad (5)$$

where A_{test} stands for the absorbance of the reaction mixture which has the standard or extract, and A_{blank} stands for the absorbance of the blank test.

2.3.8. Ferric Ion-Reducing Antioxidant Power (FRAP)

The colorimetric ferric ion-reducing antioxidant power (FRAP) test followed the method of Benzie and Strain [54] but including few modifications reported by Fawole et al. [3]. Each time, a FRAP solution was freshly prepared by mixing 25 mL of acetate buffer (300 mM acetate buffer at pH 3.6), 2.5 mL (10 mM of 2,4,6-tri [2-pyridyl]-s-triazine (TPTZ) solution) and 2.5 mL (20 mM ferric chloride (FeCl_3) solution). Fifty percent aqueous methanol (10 mL) was added to 1.0 mL peel extract, sonicated for 10 min in cold water and centrifuged for 5 min (4000 (revolutions per minute) rpm) at 4 °C. The methanolic extract of 150 µL was added to 2850 µL of FRAP solution and vigorously shaken with vortex for 30 s, and was incubated in the dark at room temperature (25 ± 2 °C) for 30 min. The reduction of the Fe^{3+} -TPTZ complex to a coloured Fe^{2+} -complex by the extract was analysed using a UV-vis spectrophotometer (Thermo Scientific Technologies, Madison, WI, USA) at a wavelength of 593 nm. The control was prepared as above with no extract addition, and methanol was used as a blank. Results were expressed as µmol Trolox/g DM. The changes in the absorbance values of the test reaction mixtures from the initial blank reading were considered as FRAP activity.

2.3.9. 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic Acid) Assay (ABTS)

Determination of antioxidant activity was performed using the ABTS assay according to Chirinos et al. [55], with slight modifications. Freshly prepared potassium persulfate solution at a concentration of 2.6 mM was mixed with 7.4 mM ABTS solution to make a stock solution (1:1, v/v). The reaction was left in the dark for 12 h at room temperature (25 ± 2 °C) to obtain an ABTS radical working solution. When the absorbance value of the working solution was 0.70 ± 0.02 at 750 nm (by taking 1.5 mL stock solution and adding 60 mL methanol), the concentration was used for the ABTS method. Briefly, 15 µL peel extract was added to 200 µL of the ABTS working solution. The solution was allowed to react until a steady absorbance was reached, and this took approximately 6 min. The antioxidant activity was reported as Trolox equivalents per g sample (µmol Trolox/g DM).

2.4. Determination of Enzyme Activity

The enzyme extracts were prepared according to Gonzalez et al. [56], where 1 g of dried pomegranate peel extract was blended into a powder and mixed with 10 mL extraction solution, which consists of 0.1 M phosphate buffer at pH 7, 0.05 M/L EDTA and 60 g/L polyvinyl polypyrrolidone. This mixture was continuously stirred for 5 min and further kept at 4 °C in the dark for 2 h. Afterwards, the mixture was centrifuged at 4000 rpm at 4 °C for 25 min and the supernatant was collected into clean vials and stored at -80 °C for further use.

2.4.1. Polyphenol Oxidase (PPO) Assay

The assay was performed using spectrophotometric means according to Gonzalez et al. [56], measuring the initial rate of increase in absorbance at 420 nm. The assay was performed by adding 0.2 mL of the enzyme extract to 300 μ L (0.1 M) catechol and 2.5 mL of 0.1 M potassium phosphate buffer (pH 6). The absorbance using a UV-vis spectrophotometer (Thermo Fisher Scientific Model Helios Omega UV-Vis) was taken continuously over 3 min. A blank sample containing the extraction solution without the enzyme extract was used. The results were expressed as unit per g of fresh mass.

2.4.2. Peroxidase (POD) Assay

Peroxidase activity was assayed by spectrophotometric methods using a UV-vis spectrophotometer (Thermo Fisher Scientific Model Helios Omega UV-Vis) at 470 nm according to Meighani et al. [57], with slight modifications by Arendse et al. [58]. The reaction was initiated by the addition of guaiacol substrate made up to a concentration of 0.045 M in a reaction mixture containing 2.73 mL of a 0.1 M sodium phosphate buffer at pH 6, 0.1 mL of 0.045 M guaiacol, 0.15 mL of 0.225 M hydrogen peroxide (H_2O_2) and enzyme extract added at 0.02 mL. The activity was expressed as absorbance change at 470 nm over 2 min. The blank sample contained only the extraction solution. The results were expressed as unit per g fresh mass.

2.5. Antibacterial Activity Determination

The in vitro antibacterial activity of pomegranate peel extracts ('Wonderful') was tested by microdilution antibacterial assay for the minimum inhibitory concentration (MIC) values according to Fawole et al. [3], except in the present study, the initial concentration of 10,000 μ g/mL of peel sample was prepared by dissolving dried extracts in 70% (v/v) ethanol. Four bacterial strains, which included two Gram-positive bacteria, namely, *Bacillus subtilis* ATCC 6051 and *Staphylococcus aureus* ATCC 12,600, and two Gram-negative bacteria, *Escherichia coli* 11,775 and *Klebsiella pneumonia* ATCC 13,883, were used in this experiment. All four bacteria strains were cultured in sterile autoclaved Mueller Hinton broth. Firstly, under aseptic conditions, 100 μ L of sterile distilled water was added in 96-well microplates followed by 100 μ L for each peel extract. Extracts were serially diluted two-fold before 100 μ L of the bacterial culture was added to all wells and a 0.5 McFarland absorbance at 600 nm was used as a reference. Streptomycin at 100 μ g/mL was used as a positive antibiotic control against each bacterium. Bacteria-free broth, 70% (v/v) ethanol or sterile distilled water were included as negative controls. The final concentration of peel extracts ranged from 19.53125 to 2500 μ g/mL, whereas streptomycin concentrations ranged from 0.1953125 to 25 μ g/mL, in the respective wells. The plates were left for 18 h at 37 $^{\circ}$ C. After incubation, bacterial growth in the well plates was indicated by adding 40 μ L of *p*-iodonitrotetrazolium chloride (Sigma-Aldrich, Darmstadt, Germany) after incubation. Bacterial growth was indicated by pink colour, while clear wells indicated inhibition. The results were recorded in terms of the MIC, which is regarded as the lowest concentration of the extract without bacterial growth. Each time, the assay was conducted in triplicate.

2.6. Liquid Chromatography-Mass Spectrometry

To compare the general metabolite profiles of the blanched (treatment) and unblanched peel extracts, liquid chromatography-mass spectrometry (LC-MS) was carried out on a Waters Acquity ultra-performance liquid chromatograph (UPLC) coupled to an Acquity photo diode array (PDA) detector and Waters Synapt G2 quadrupole time-of-flight mass spectrometer. The protocol followed the method described by Mphahlele et al. [6], with a few modifications using a Waters HSS T3 column (2.1 $\times$ 150 mm, 1.8 μ m particle size) and an automated sample injection of 3 μ L, with a mobile phase flow rate of 0.25 mL/min (operated at 20 $^{\circ}$ C). The mobile phase contained solvent A (0.1% formic acid) and solvent B (0.1% acetonitrile), and chromatographic separations were initiated with 100% solvent A and 0% solvent B. A linear gradient elution was employed, increasing solvent B from 0% to

5% over the first 1.10 min, 25% over 17 min, and finally, 100% at 18 min. The column was subjected to 100% solvent B for an extra 3 min that preceded a 5 min re-equilibration, to yield a 5 total run time of 26 min.

The mass spectrometry analysis was performed as described by Mphahlele et al. [6] and pure reference standards (Sigma-Aldrich, Germany) were used to confirm presence or absence of catechin, ellagic acid, epicatechin, gallic acid, punicalagin α , punicalagin β and punicalin α and β . The extracted mass chromatogram of the molecular ion of the respective compound was recorded in negative mode using a scanning range of m/z 120 to 1500. This assisted with tentative identifications of the above-mentioned metabolites. Five replicates for each sample (blanched and unblanched peel) were used for method validation.

2.7. Statistical Analysis

All statistical analysis was performed using SAS Software (SAS Enterprise Guideline 7.1, Carrey, NC, USA). Factorial analysis of variance (ANOVA) at the 95% confidence interval was performed to check for interactions between solvent type and concentration, and, also for interactions between temperature and time during blanching. Values were expressed as means $\pm$ standard error (SE) of the triplicate ($n = 3$). Mean values were separated according to Duncan's multiple range test. Principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) were carried out using XLSTAT software version 2019.4.1.63305.

3. Results

3.1. Influence of Solvent Type and Concentration on Phytochemical Extraction

3.1.1. Phytochemical Analysis of 'Wonderful' Pomegranate Peel Extracts

A two-way ANOVA was conducted to compare the main effects of solvent and concentration as well as their interaction with the quality attributes. Statistical analysis showed that there were significant ($p < 0.05$) interactions between the main factors (solvent and concentration) for all the quality attributes measured, indicating that there was a combined effect for the main factors and all attributes evaluated. This combined effect makes it difficult to know where the influence is coming from. Table 1 shows that using 70% (v/v) ethanol recorded the highest extract yield, total phenolic content (TPC) and total tannin content (TTC) at $29.46\% \pm 0.75\%$ per g DM, 10.61 ± 0.15 mg GAE/g DM and 0.76 ± 0.02 mg GAE/g DM, respectively. While 100% acetone recorded the lowest extract yield at $14.45\% \pm 0.63\%$ per g DM, TPC at 5.45 ± 0.05 mg GAE/g DM and TTC at 0.14 ± 0.02 mg GAE/g DM.

Table 1. Extract yield, total phenolic content, total tannin content and antioxidant capacity (DPPH, FRAP and ABTS) of 'Wonderful' pomegranate peel extracts of three different solvents (methanol, ethanol and acetone) at three different concentrations (50%, 70% and 100%).

Solvent	* Concentration, % (v/v)	Extract Yield (%)	TPC	TTC	DPPH	FRAP	ABTS
Methanol	50	19.79 ± 1.54^c	8.76 ± 0.04^c	0.45 ± 0.04^{bc}	203.95 ± 4.85^c	354.34 ± 4.85^{bc}	562.94 ± 3.64^d
	70	22.94 ± 0.95^b	8.97 ± 0.02^b	0.54 ± 0.04^b	218.50 ± 2.43^b	394.36 ± 33.96^{ab}	708.48 ± 1.05^b
	100	17.11 ± 0.36^d	7.60 ± 0.04^d	0.31 ± 0.09^{de}	182.12 ± 0.61^d	302.79 ± 6.39^c	512.01 ± 1.05^f
Ethanol	50	23.52 ± 0.74^b	9.08 ± 0.05^b	0.50 ± 0.04^{bc}	219.71 ± 1.21^b	409.52 ± 4.25^{ab}	591.45 ± 3.03^c
	70	29.46 ± 0.75^a	10.61 ± 0.15^a	0.76 ± 0.02^a	243.97 ± 2.43^a	478.04 ± 73.98^a	718.79 ± 2.42^a
	100	18.19 ± 0.44^{cd}	8.76 ± 0.04^c	0.38 ± 0.03^{cd}	188.18 ± 5.46^d	339.18 ± 10.02^{bc}	541.72 ± 1.60^e
Acetone	50	16.32 ± 0.57^{de}	5.93 ± 0.04^f	0.31 ± 0.04^{de}	111.17 ± 1.21^f	216.08 ± 1.21^{de}	420.44 ± 1.21^h
	70	18.23 ± 0.62^{cd}	6.79 ± 0.04^e	0.22 ± 0.03^{ef}	127.55 ± 0.61^e	267.01 ± 2.43^{cd}	478.05 ± 1.60^g
	100	14.45 ± 0.63^e	5.45 ± 0.05^g	0.14 ± 0.02^f	95.41 ± 1.82^g	159.69 ± 0.61^e	385.87 ± 2.43^i
Solvent (A)		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Concentration (B)		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
A*B		0.0035	<0.0001	0.0047	0.0059	<0.0001	<0.0001

Factorial ANOVA was performed for Factor A (solvent) and Factor B (concentration). Values are means $\pm$ SE of triplicate ($n = 3$) determinations. Different letter(s) in each column indicate statistically significant ($p < 0.05$) differences according to Duncan's multiple range test. Extract yield = % per g DM, TPC = total phenolic content (mg GAE/g DM), TTC = total tannin content (mg GAE/g DM), DPPH = 2,2 diphenyl-1-picrylhydrazyl assay (μ mol Trolox/g DM), FRAP = ferric-reducing antioxidant power assay (μ mol Trolox/g DM), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assay (μ mol Trolox/g DM), * Concentration = dilution with distilled water.

Overall, results of extracted yield, TPC and TTC were higher in ethanol than in methanol and acetone at any tested concentration, while highest attributes were observed using a concentration of 70% (*v/v*) followed by 50% (*v/v*), regardless of solvent. In contrast, the use of 100% (*v/v*) concentration led to lowered amounts compared to 50% and 70% (*v/v*) concentration, irrespective of the solvent used. It was evident that ethanol was the best extractant for all measurements, with acetone-derived extracts having the lowest values. Out of those extracts produced with ethanol, those generated using a solution of 70% (*v/v*) had the highest phytochemical concentration. It was thus evident that the most abundant components of 'Wonderful' pomegranate peel extracts are phenolic compounds, which are most effectively extracted using 70% (*v/v*) ethanol, and for this reason, this method was then routinely used.

3.1.2. Antioxidant Activities of 'Wonderful' Pomegranate Peel Extracts

Antioxidant activity as shown in Table 1 followed the same trend as the phytochemical attributes. The results showed a significant ($p < 0.05$) effect of interaction between main factors (solvent and concentration) and antioxidant activity. Ethanol at 70% (*v/v*) provided the highest DPPH, FRAP and ABTS activities at 243.97 ± 2.43 , 478.04 ± 73.98 and 718.79 ± 2.42 $\mu\text{mol Trolox/g DM}$ respectively, while antioxidant activity using 100% acetone were almost three-fold lower at 95.41 ± 1.82 , 159.69 ± 0.61 and 385.87 ± 2.43 $\mu\text{mol Trolox/g DM}$ for DPPH, FRAP and ABTS, respectively. It was noted that pomegranate peels extracted using 70% (*v/v*) ethanol possessed the highest FRAP reducing power and DPPH and ABTS scavenging activities compared to methanol and acetone extracts, validating the decision to choose this particular type of extract for the rest of the experiments that focused on a blanching study.

3.2. Influence of Blanching Temperature and Time on Phytochemical Extraction

3.2.1. Phytochemical Analysis of Blanched 'Wonderful' Pomegranate Peel Extracts

Statistical analysis showed that there was a significant ($p < 0.05$) effect of interaction between temperature and time on all the phytochemical attributes evaluated. Table 2 shows that blanching at 80 °C for 3 min provided the highest extract yield at $31.28\% \pm 0.60\%$ per g DM, total phenolic content at 12.22 ± 0.08 mg GAE/g DM, total tannin content at 1.06 ± 0.06 mg GAE/g DM and vitamin C at 53.94 ± 0.46 μg of AAE/g DM, respectively. The highest total flavonoid content was at 0.08 ± 0.00 mg CE/g DM for treatments 60 °C for 1 min, 80 °C for 3 min and 80 °C for 5 min, and lowest for treatment 60 °C for 5 min and 100 °C for 5 min at 0.06 ± 0.00 mg CE/g DM. Total anthocyanin content decreased with increase in temperature and time, and was reported highest at 60 °C for 1 min at 1.84 ± 0.00 mg Cy3dE/g DM, followed by 80 °C for 3 min at 1.50 ± 0.00 mg Cy3dE/g DM and by 80 °C for 1 min at 1.39 ± 0.11 mg Cy3dE/g DM. Blanching at 100 °C for 5 min provided the lowest record for extract yield, total phenolic content, total tannin content, total flavonoid content, total anthocyanin content and vitamin C at $20.42\% \pm 0.85\%$ per g DM, 9.79 ± 0.05 mg GAE/g DM, 0.48 ± 0.03 mg GAE/g DM, 0.06 ± 0.00 mg CE/g DM, 0.22 ± 0.05 mg Cy3dE/g DM and 14.37 ± 0.69 μg of AAE/g DM, respectively.

The quality of the blanched products depends significantly on the temperature–time combination. Blanching at 80 °C for 3 and 5 min gave the best results, while blanching at low temperatures of 60 °C and high temperatures of 100 °C, regardless of time, significantly reduced phytochemicals. Furthermore, a strong positive correlation was observed between the extract yield with numerous components, such as TTC ($r = 0.681$) and TFC ($r = 0.758$) (Table 3). Similarly, strong correlations were reported between TTC and TFC ($r = 0.666$), TTC and TAC ($r = 0.732$), TTC and vit C ($r = 0.834$) and TAC and vit C ($r = 0.913$). The relationships show that when the extract yield rises, total tannin and total flavonoid content increase. Similar trends were observed with an elevation of total tannin content and the high extraction of total flavonoid content, total anthocyanin and vitamin C. Moreover, high total anthocyanin also resulted in high vitamin C content.

Table 2. Extraction yield, bioactive compounds, enzyme activity and antioxidant activity of ethanol (70% (*v/v*)) extracts from blanched (60, 80 and 100 °C for 1, 3 and 5 min) ‘Wonderful’ pomegranate peel.

Temp (°C)	Time	Extract Yield (%)	TPC	TTC	TFC	TAC	Vit C	DPPH	FRAP	ABTS	PPO	POD
Unblanched		29.46 ± 0.75	10.61 ± 0.15	0.76 ± 0.02	0.07 ± 0.00	1.17 ± 0.00	45.24 ± 0.06	243.97 ± 2.43	478.04 ± 73.98	718.79 ± 2.43	0.40 ± 0.00	3.00 ± 0.00
60	1	25.77 ± 1.01 ^b	10.66 ± 0.08 ^d	0.75 ± 0.06 ^{bcd}	0.08 ± 0.00 ^a	1.84 ± 0.00 ^a	48.95 ± 0.05 ^{bc}	266.41 ± 1.05 ^b	482.89 ± 6.55 ^{bc}	736.37 ± 1.60 ^c	0.70 ± 0.00 ^a	5.25 ± 0.00 ^a
	3	25.48 ± 1.25 ^b	9.911 ± 0.03 ^{ef}	0.73 ± 0.07 ^{bcd}	0.07 ± 0.00 ^b	1.22 ± 0.05 ^c	48.49 ± 0.26 ^c	246.40 ± 1.05 ^c	480.47 ± 1.21 ^c	723.03 ± 2.77 ^d	0.68 ± 0.03 ^{ab}	4.88 ± 0.38 ^{ab}
	5	21.73 ± 0.89 ^c	9.22 ± 0.05 ^g	0.71 ± 0.03 ^{cde}	0.06 ± 0.00 ^c	0.83 ± 0.00 ^d	34.50 ± 0.40 ^e	223.96 ± 1.60 ^d	445.30 ± 0.60 ^d	682.40 ± 2.18 ^e	0.60 ± 0.00 ^b	4.50 ± 0.00 ^b
80	1	21.77 ± 1.04 ^c	11.01 ± 0.08 ^{bc}	0.87 ± 0.02 ^b	0.07 ± 0.00 ^b	1.39 ± 0.11 ^b	49.82 ± 0.20 ^b	309.45 ± 11.56 ^a	503.50 ± 9.70 ^b	754.56 ± 2.42 ^b	0.28 ± 0.03 ^{cd}	2.25 ± 0.00 ^c
	3	31.28 ± 0.60^a	12.22 ± 0.08^a	1.06 ± 0.06^a	0.08 ± 0.00^a	1.50 ± 0.00^b	53.94 ± 0.46^a	319.16 ± 4.20^a	525.33 ± 15.76^a	778.82 ± 2.42^a	0.33 ± 0.03^c	1.50 ± 0.00^d
	5	30.67 ± 0.45 ^a	10.08 ± 0.05 ^e	0.81 ± 0.02 ^{bc}	0.08 ± 0.00 ^a	0.83 ± 0.00 ^d	43.32 ± 0.23 ^d	269.43 ± 4.85 ^b	495.63 ± 4.57 ^{bc}	725.46 ± 1.60 ^d	0.30 ± 0.00 ^{cd}	2.25 ± 0.00 ^c
100	1	24.81 ± 0.51 ^b	11.14 ± 0.05 ^b	0.66 ± 0.02 ^{de}	0.07 ± 0.00 ^b	0.50 ± 0.00 ^e	19.42 ± 0.55 ^f	180.30 ± 1.21 ^e	479.86 ± 3.03 ^c	663.00 ± 2.77 ^f	0.23 ± 0.03 ^{de}	1.50 ± 0.00 ^d
	3	21.45 ± 0.64 ^c	10.94 ± 0.06 ^c	0.61 ± 0.02 ^{ef}	0.07 ± 0.00 ^b	0.44 ± 0.05 ^e	16.17 ± 0.05 ^g	149.37 ± 0.60 ^f	403.45 ± 1.60 ^e	625.40 ± 4.37 ^g	0.15 ± 0.05 ^e	1.13 ± 0.38 ^d
	5	20.42 ± 0.85 ^c	9.79 ± 0.05 ^f	0.48 ± 0.03 ^f	0.06 ± 0.00 ^c	0.22 ± 0.05 ^f	14.37 ± 0.69 ^h	136.64 ± 2.64 ^f	348.88 ± 1.60 ^f	602.36 ± 1.21 ^h	0.18 ± 0.03 ^e	1.13 ± 0.38 ^d
Temp (A)		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Time (B)		0.0191	<0.0001	0.0043	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
A*B		<0.0001	<0.0001	0.0335	<0.0001	<0.0001	<0.0001	0.0021	<0.0001	<0.0001	<0.0001	0.0481

Factorial ANOVA was performed for Factor A (temperature) and Factor B (time). Values are means ± SE of triplicate (*n* = 3) determinations. Different letter(s) in each column indicate statistically significant (*p* < 0.05) differences according to Duncan's multiple range test. Extract yield = % per g DM, TPC = total phenolic content (mg GAE/g DM), TTC = total tannin content (mg GAE/g DM), TAC = Total anthocyanin content (mg Cy3dE/g DM), Vit C = Vitamin C (µg of AAE/g DM), DPPH = 2,2 diphenyl-1-picryl hydrazyl assay (µmol Trolox/g DM), FRAP = ferric-reducing antioxidant power assay (µmol Trolox/g DM), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assay (µmol Trolox/g DM), PPO = Polyphenol oxidase (U/g FW), POD = Peroxidase (U/g FW), GAE = gallic acid equivalent, CE = catechin equivalent, Cy3De = cyanidin-3-glucoside equivalent, AAE = ascorbic acid equivalent.

Table 3. Pearson correlation matrix between chemical indices measured from ethanol (70% (v/v)) extracts from blanched (60, 80 and 100 °C for 1, 3 and 5 min) ‘Wonderful’ pomegranate peel.

Variables	Extract Yield	TPC	TTC	TFC	TAC	Vit C	DPPH	FRAP	ABTS	PPO	POD	<i>E. coli</i>	<i>K. pneumonia</i>	<i>S. aureus</i>	<i>B. subtilis</i>
Extract yield	1														
TPC	0.412	1													
TTC	0.681	0.598	1												
TFC	0.758	0.601	0.666	1											
TAC	0.461	0.344	0.732	0.618	1										
Vit C	0.615	0.237	0.834	0.592	0.913	1									
DPPH	0.598	0.375	0.927	0.626	0.857	0.954	1								
FRAP	0.696	0.490	0.881	0.706	0.744	0.822	0.881	1							
ABTS	0.661	0.420	0.926	0.679	0.890	0.966	0.986	0.924	1						
PPO	0.143	−0.363	0.186	0.122	0.641	0.577	0.387	0.334	0.441	1					
POD	0.047	−0.462	0.085	0.060	0.590	0.521	0.327	0.274	0.371	0.980	1				
<i>E. coli</i>	−0.509	−0.050	−0.751	−0.369	−0.624	−0.802	−0.835	−0.857	−0.835	−0.524	−0.478	1			
<i>K. pneumonia</i>	−0.691	−0.081	−0.818	−0.558	−0.735	−0.917	−0.901	−0.748	−0.883	−0.498	−0.440	0.823	1		
<i>S. aureus</i>	−0.576	−0.121	−0.839	−0.513	−0.756	−0.931	−0.950	−0.768	−0.911	−0.425	−0.388	0.837	0.971	1	
<i>B. subtilis</i>	−0.463	0.070	−0.745	−0.344	−0.702	−0.895	−0.872	−0.699	−0.840	−0.583	−0.546	0.882	0.945	0.957	1

Values in bold are significant at $p = 0.05$.

3.2.2. Antioxidant Activities of Blanched ‘Wonderful’ Pomegranate Peel Extracts

The antioxidant activity using DPPH, ABTS and FRAP followed the same trend as the phytochemical potential of blanched pomegranate peel extracts. The results presented in Table 2 show a significant ($p < 0.05$) effect of interaction between temperature and time on antioxidant activity. The highest antioxidant activity using DPPH, ABTS and FRAP assays was reported on peel extracts blanched at 80 °C for 3 min at 319 ± 4.20 , 778.82 ± 2.42 and 525.33 ± 15.76 $\mu\text{mol Trolox/g DM}$, respectively. The lowest DPPH, ABTS and FRAP was reported on peel extracts blanched at 100 °C for 5 min at 136.64 ± 2.64 , 602.36 ± 1.21 and 348.88 ± 1.60 $\mu\text{mol Trolox/g DM}$, respectively. Strong positive correlations were reported between extract yield with FRAP at $r = 0.696$ and ABTS being $r = 0.661$. Strong positive correlations were also reported between TTC and DPPH ($r = 0.927$), TTC and FRAP ($r = 0.881$), TTC and ABTS ($r = 0.926$), TFC and ABTS ($r = 0.679$), TAC and DPPH ($r = 0.857$), TAC and FRAP ($r = 0.744$), TAC and ABTS ($r = 0.890$), vit C and FRAP ($r = 0.822$), vit C and ABTS ($r = 0.966$), DPPH and FRAP ($r = 0.881$), DPPH and ABTS ($r = 0.986$) and FRAP and ABTS ($r = 0.924$), suggesting higher accumulation of phytochemicals that possibly lead to high levels of antioxidant activity.

3.3. Determination of Enzyme Activity (PPO and POD)

Polyphenol oxidase (PPO) activity has been reported to be the primary enzyme responsible for the oxidation of phenolic compounds into quinone compounds, leading to the degradation of valuable phenolics and the browning of the peel [42]. As shown in Table 2, there was a significant ($p < 0.05$) effect of interaction between temperature and time on enzyme activity. For all blanching temperatures and times, increased PPO activity was reported with a decline in blanching temperature and time. For instance, for all pomegranate peel extracts blanched at 60 °C for 1, 3 and 5 min, PPO activity increased to 0.70 ± 0.00 , 0.68 ± 0.00 and 0.60 ± 0.00 U/g FW respectively, compared to the unblanched peel extracts (0.40 ± 0.00 U/g FW). Pomegranate peel extracts blanched at 100 °C for 3 min exhibited the lowest PPO activity at 0.15 ± 0.05 U/g FW, followed by 100 °C for 5 min at 0.18 ± 0.03 U/g FW and 60 °C for 5 min, and at 100 °C for 1 min reduced PPO activity by almost half (0.23 ± 0.03 U/g FW). A decline in PPO activity in all pomegranate peel extracts blanched at 80 °C for 1, 3 and 5 min was also noticeable. Peroxidase activity followed the same trend as PPO activity, with blanched pomegranate peel extracts at 100 °C for 1 min reduced by half and 100 °C for 3 and 5 min almost 3 times lower than the unblanched peel extracts (3.00 ± 0.00 U/g FW). Furthermore, strong positive correlations were observed between TAC and PPO ($r = 0.641$) and between PPO and POD ($r = 0.980$) (Table 3).

3.4. Antibacterial Activity

The antibacterial activity of blanched ‘Wonderful’ pomegranate peel extracts is presented in Table 4. All peel extracts showed a broad-spectrum activity against bacterial strains used. The minimum inhibitory activity values varied from 160 to 630 $\mu\text{g/mL}$. Moreover, peel extracts blanched at 80 °C for 3 min and 80 °C for 5 min provided the best results for all four bacterial strains at 160 $\mu\text{g/mL}$, while all blanched peel extracts at 100 °C for 1, 3 and 5 min exhibited the lowest minimum inhibitory concentration at 630 $\mu\text{g/mL}$. However, *E. coli* bacterial strains at 100 °C for 1 min were similar with untreated peel extracts at 310 $\mu\text{g/mL}$. Furthermore, strong negative correlations were observed between extract yield and *Klebsiella pneumonia* bacterial strains ($r = -0.691$), TTC and *Escherichia coli* ($r = -0.751$), TTC and *Klebsiella pneumonia* ($r = -0.818$), TTC and *Staphylococcus aureus* ($r = -0.839$) and TTC and *Bacillus subtilis* ($r = -0.745$). Similarly, TAC, vit C, DPPH, FRAP and ABTS had a strong negative correlation with the four bacteria strains tested (Table 3), indicating that decrease in phytochemical and antioxidant activity resulted in high bacterial activity. It was also noted that the bacteria strains, for instance, *Escherichia coli*, had strong positive correlation against the other bacterial strains tested (see Table 3).

Table 4. Antibacterial activity (MIC, µg/mL) of ethanol (70% (v/v)) extracts from blanched (60, 80 and 100 °C for 1, 3 and 5 min) ‘Wonderful’ pomegranate peel extracts.

Temperature (°C)	Time (min)	Gram-Negative		Gram-Positive	
		<i>Escherichia coli</i>	<i>Klebsiella pneumonia</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>
Unblanched		310	310	310	310
60	1	310	310	310	310
	3	160	310	310	160
	5	160	310	310	160
80	1	160	310	160	160
	3	160	160	160	160
	5	160	160	160	160
100	1	310	630	630	630
	3	630	630	630	630
	5	630	630	630	630
Streptomycin (µg/mL)		1.6	1.6	0.8	1.6
Solvent control (70% ethanol)		-	-	-	-

- Denotes not inhibited.

3.5. Principal Component Analysis (PCA) and Agglomerative Hierarchical Clustering Analysis (AHC)

To obtain an overview of the relationship between blanched ‘Wonderful’ pomegranate peel extracts, quality attributes and antimicrobial activity, a PCA and AHC were performed. In the PCA, the first two factors accounted for 84.92% of the total variation in the original dataset, as shown in Figure 1A. The first component (F1) explains the maximum variability at 67.13%, corresponding to an eigenvalue of 10.07, whilst factor 2 (F2) accounted for 17.79% variability with an eigenvalue of 2.67. Factor 1 is positively correlated with extract yield (0.689), TTC (0.901), TFC (0.668), TAC (0.875), vit C (0.974), DPPH (0.972), FRAP (0.905), ABTS (0.986) and PPO (0.525), whilst it was negatively correlated with *E. coli* (−0.867), *K. pneumonia* (−0.937), *S. aureus* (−0.938) and *B. subtilis* (−0.893). Treatment at 100 °C/5 min is associated with most of the observation observed in F1 at 35.45%. Factor 2 is positively correlated with TPC (0.853), which is negatively correlated with PPO (−0.748) and POD (−0.813), meaning TPC has the greatest negative effect on PPO and POD decrease, contributing 27.27% of the variation related to F2. Treatment at 60 °C/5 min is responsible for most of the contribution reported in F2 at 33.99%.

The AHC analysis of ‘Wonderful’ pomegranate peel extracts blanched at different temperatures (60, 80 and 100 °C) and at varying times (1, 3 and 5 min) are shown in Figure 1B. Treatments at 80 °C/1 min and 80 °C/3 min were clustered together based on the positive correlation with the extract yield, TFC, TTC, DPPH, FRAP and ABTS, while treatments at 60 °C for 1, 3 and 5 min and control were clustered together based on the positive correlation with TAC, vit C, PPO and POD. Furthermore, pomegranate peel extracts blanched at 100 °C for 1, 3 and 5 min were grouped based on their positive correlation with all four bacterial strains tested. This agreed with the PCA analysis. Treatments at 80 °C for 1 and 3 min influenced several valuable components compared to other treatments.

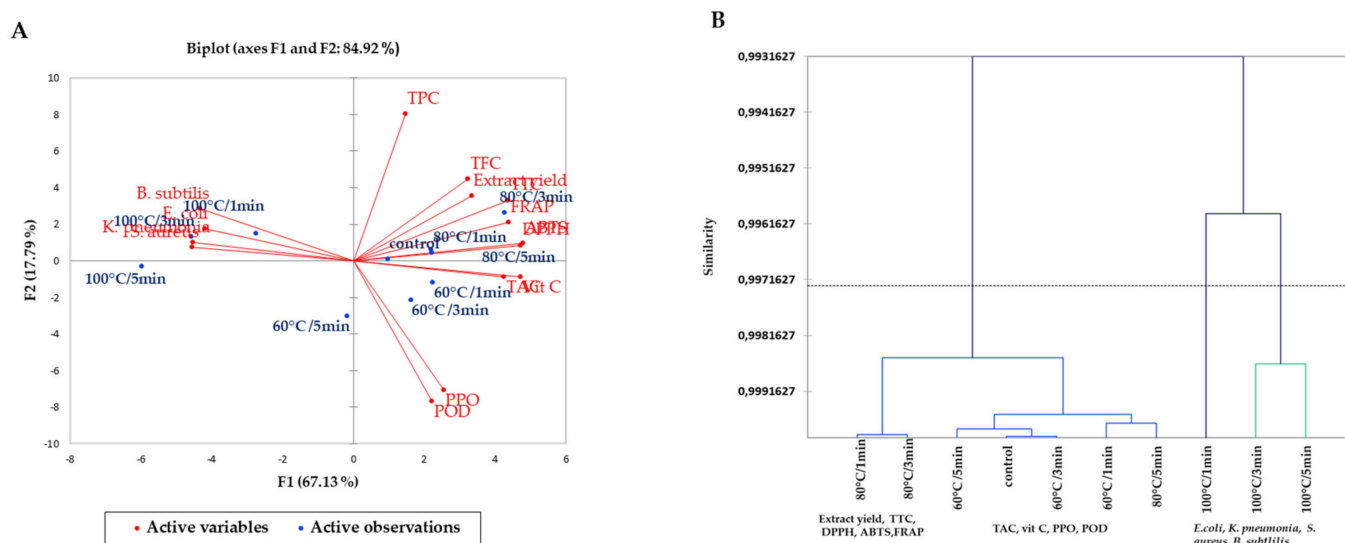


Figure 1. (A) Principal component analysis and (B) agglomerative hierarchical clustering (AHC) representing blanched ‘Wonderful’ pomegranate peel extracts blanched at 60, 80 and 100 °C for 1, 3 and 5 min, and the control shows unblanched peel extracts. TPC, TTC, TFC, TAC, Vit C, DPPH, FRAP, ABTS, PPO and POD represent total phenolic content, total tannin content, total flavonoid content, total anthocyanin content, vitamin C concentration, 2,2 diphenyl-1-picryl hydrazyl assay, ferric-reducing antioxidant power assay and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assay, polyphenol oxidase and peroxidase, respectively.

In general, the polyphenols appear to be higher in their relative percentage abundance in blanched ‘Wonderful’ pomegranate peel, for the 80 °C for 3 min group, as compared with unblanched peel extracts (Figure 2A). Punicalagin is the main phenolic compound present in pomegranate peel and is an important biomarker for the industry. Punicalin α and β (781.0671 m/z), gallagyl residues (600.9943 m/z) and ellagic acid (300.9987 m/z) are the main molecular ions that are important for detection of this phenolic compound (Figure 2B). Higher peak intensities were observed in blanched peel extracts and relative ion intensity increases were more prominent for hydrolysable tannins (α and β punicalagin) (Figure 2A). Furthermore, the absence of compounds such as galloyl-hexahydroxydiphenol-hexoside in unblanched peel extracts shows the effectiveness of blanching in loosening cellulosic structures within the peel for higher extraction of compounds and preserving the integrity of these compounds during the drying process. This work lends support to the higher antioxidant activities reported in Table 2.

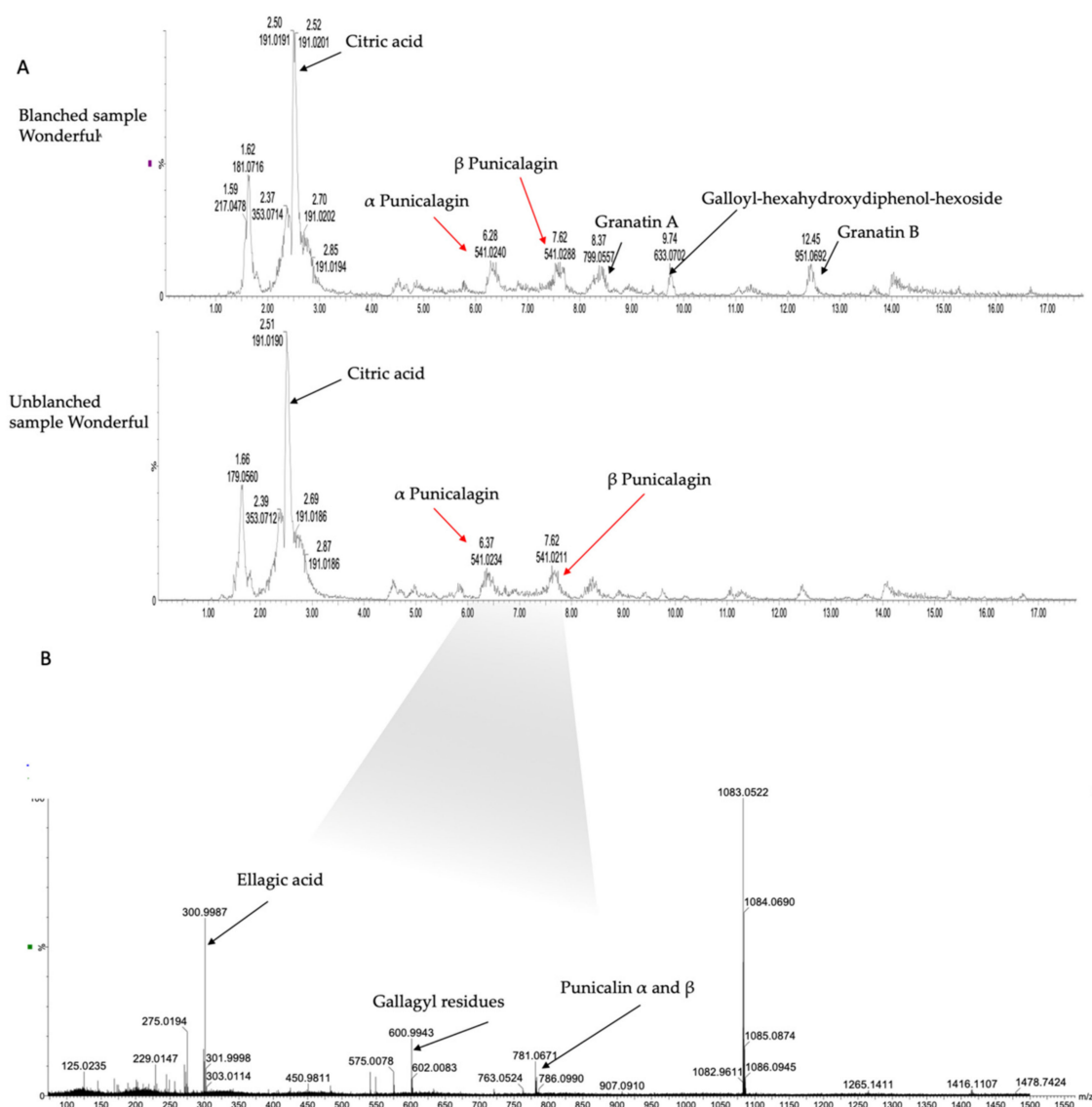


Figure 2. Metabolite profiling of peel extracts. (A) An example of a LC-MS chromatographic separation of blanching and unblanching pomegranate peels. (B) MS^E fragmentation patterns of studied pomegranate peels showing the main fragments observed in punicalagin compounds (punicalin α and β (781.0671 m/z), gallagyl residues (600.9943 m/z) and ellagic acid (300.9987 m/z)).

4. Discussion

This work screened for the best solvent type and concentration to assist with the best extraction of phytochemicals and antioxidant potential of 'Wonderful' pomegranate peel extracts. Solvent type and differing concentrations both influenced the extraction of phenolic compounds. The extract yield, total phenolic content, total tannin content, DPPH, FRAP and ABTS were dependent on solvent type ($p < 0.0001$) and concentration ($p < 0.0001$) (Table 1). Ethanol as a 70% solution gave the highest extract yield at 29.46% per g DM, total phenolic content at 10.61 mg GAE/g DM, total tannin content at 0.76 mg GAE/g DM and antioxidant potential (DPPH, ABTS and FRAP at 95.41, 385.87 and 159.69 μmol Trolox/g DM) respectively, compared to other solvent types and concentrations used. In addition to this, it was noted that ethanol, followed by methanol and lastly acetone gave the best phytochemical extraction and antioxidant potential. Similarly, the concentrations that had the highest yields were in the following order, 70% followed by 50% and lastly 100% concentration. The pure (100%) solvents all showed weak extraction potential while combinations of the pure solvents with water to reach 70% and 50% concentrations re-

spectively, resulted in high phytochemical yields and antioxidant activity. The greater extraction potential of aqueous solvents indicates that the mixing of less polar solvent with water may increase the polarity index of the solvents, therefore enhancing the (solubility) extraction potential of a particular solvent [35].

Malviya et al. [36] studied the antioxidant and antibacterial potential of 'Ganesh' pomegranate peel extracts using different solvents (ethanol, water and methanol) at different concentrations (50%, 70% and 100%). They reported that the 50% (v/v) ethanol solution gave the highest extract yield, at 16.3% per g DM, while 70% ethanol gave the highest antioxidant activity using inhibition activity for DPPH at 79.5% and ABTS at 94.6%. Tabaraki et al. [59] used ultrasonic-assisted extraction with optimal conditions (70% (v/v) ethanol, temperature 60 °C, extraction time of 30 min and maximum power and frequency extraction of 140 W and 35 kHz) and reported highest extract yield of 45.38%, TPC of 8677.83 mg GAE/100 g DM and FRAP of 63.37 mmol Fe²⁺/100 g in pomegranate peel extracts. Similarly, Živković et al. [60] optimised ultrasonic-assisted extraction of natural antioxidants from pomegranate peel extracts using the following parameters of 59% (v/v) ethanol, solid to solvent ratio of 1:44, extraction temperature of 80 °C and extraction time of 25 min. They reported highest TPC of 149.12 mg GAE/g DM, ellagic acid content of 11.65 mg/g DM, gallic acid content of 2.87 mg/g DM, punicalin of 61.93 mg/g DM and punicalagin of 18.05 mg/g DM. However, the frequency and power for ultrasonic extraction were not stated by the authors even though these two factors are known to improve the penetration rate of the solvent into the plant cellular material and subsequently lead to the disruption of the plant tissue, releasing bioactive compounds into the solvent [12,48,49]. There are several studies using ethanol combinations for the best phytochemical extraction in pomegranate peel extracts cited in the literature, regardless of extraction techniques [20,38,61–63]. Moreover, ethanol and its water combinations are food-grade solvents suitable for human consumption and for commercial extraction to add value to 'Wonderful' pomegranate peel waste. The high phenolic content and antioxidant activity when using an ultrasonic extraction with 70% ethanol, at temperature 40 ± 2 °C, extraction time 1 h, solvent ratio (15:1 w/w dry weight; solvent/sample) and maximum power and frequency extraction of 700 W and 40 kHz respectively, were subsequently used for the blanching experiment extraction. The preservation of these valuable compounds in the peel during extraction depends on the extraction technique, solvent type and mixture of different solvents used during extraction, amongst others [5,34,39].

Contrary to our findings, previous studies have suggested that methanolic and acetone extraction of pomegranate peel waste shows higher extract yields and antioxidant activity compared to other solvents [34,64,65]. Shibani et al. [66] reported highest extract yield, total phenolic content and total flavonoid content at 45.4% per g DM, 274 mg gallic acid equivalents (GAE)/g DM and 56.4 mg flavonoids RE/g DM respectively, in 'Yemeni varieties' pomegranate peel extracts using 80% methanol. Similarly, Wang et al. [34] investigated solid-solvent extraction of antioxidants from 'Wonderful' pomegranate marc peel and how different solvents (acetone, ethanol, ethyl acetate, methanol and water), temperature conditions and solvent-solid ratios affect the extraction of antioxidant compounds. They reported the highest extract yield of phenolics at 8.26% per g DM in methanol extract and the lowest in ethyl acetate extract at 0.18%. Similarly, Pradmaja and Prasad [28] investigated pomegranate peel extracts as potential sources of natural antioxidant using different solvents (acetone, chloroform, ethanol, ethyl acetate and methanol). Methanolic extract was shown to have the highest total phenolic content, while chloroform peel extracts had the lowest at 78.92 and 2.70 mg/gm gallic acid equivalents. Muhson et al. [35] studied the effect of solvent (acetone, ethanol, methanol and water) and concentration (50%, 80% and 100%) for extracting antioxidant compounds from pomegranate peel. They observed highest total phenolic content at 168.26 mg GAE/100 g DM, total flavonoid content at 87.21 mg quercetin equivalents per 100 g DM and antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl at 88.46% and ferric-reducing power at 142.21 mg Trolox equivalents per 100 g DM using 50% acetone. However, these organic solvents are believed to be toxic and the extraction

conditions are often regarded as harsh. It is thus clear that there is no congruency in terms of extraction solvent type that may lead to best recovery of phytochemicals with extracts that hold high antioxidant power. Water, ethanol and their mixtures pose as suitable alternatives to methanol and acetone as nontoxic food-grade solvents for commercial extraction of pomegranate peel waste [5,31]. The ability of acetone, ethanol, methanol, water and other solvents to extract phenolic compounds from pomegranate peel extracts may also be linked to the polarities of both the phenolics within the pomegranate peel material and solvents [28,34,60,67].

Our aim was also to determine the best blanching temperature (60, 80 and 100 °C) and time (1, 3 and 5 min) for efficient extraction of phytochemicals, antioxidant, enzyme inactivation and antibacterial activity in 'Wonderful' peel extracts. We report for the first time, to our knowledge, that blanching 'Wonderful' pomegranate peel improved the extraction of phytochemicals during blanching, to a degree. The results presented in Table 2 show a significant ($p < 0.05$) effect of interaction between temperature and time with all investigated attributes. 'Wonderful' pomegranate peel extracts subjected to hot water blanching at 80 °C for 3 and 5 min significantly increased all phenolic content and inhibiting PPO and POD enzymes responsible for phenolic compound degradation, and had the highest antioxidant activities compared to other treatments. This could be a result of optimum thermal blanching conditions that cause structural changes in the tissue by breaking down the cellulosic membranes, or loosening them and altering cell wall porosity, thus improving the extraction of phytochemicals within the cell structures [41]. In addition, increased extractability of phytochemicals may also result in enhanced bioaccessibility, which leads to increased bioavailability [5,34,42]. Higher temperatures of 100 °C led to significant reductions in all measured phenolic components and antioxidant activity, worsening over the blanching time. Over-blanching is associated with reductions in nutrient and sensory qualities in many foods as a result of the Maillard reaction [42,68]. Exposure of food materials to high temperatures usually has a negative effect on the quality attributes and also a decline in content and bioavailability of some nutrients [42,68]. Thus, it is important to find a blanching temperature and time that adequately inactivates these enzymes and at the same time has minimal effects on the valuable phytochemicals [41,42,68]. The loss of all quality attributes at high temperatures of 100 °C blanched for 5 min could be attributed to some low molecular weight components present in the cell, such as minerals, vitamins, sugars and heat-sensitive phytochemical compounds that leach from the cell structure to the water medium during blanching [41,42,69]. Furthermore, pomegranate peel extracts blanched at 100 °C for 3 min exhibited the lowest PPO activity at 0.15 ± 0.05 U/g FW, followed by 100 °C for 5 min at 0.18 ± 0.03 U/g FW and 100 °C for 1 min, reducing PPO activity by almost half (0.23 ± 0.03 U/g FW), suggesting that loss of phytochemical content at high temperatures for 'Wonderful' pomegranate peel extracts may not necessarily be a result of enzymatic activity. These results agreed with the PCA and AHC used to obtain an overview of the relationship between blanched 'Wonderful' pomegranate peel extracts, quality attributes and antimicrobial activity (Figure 1). The results showed that factor 2 in the PCA is positively correlated with TPC (0.853), and at the same time, TPC is negatively correlated with PPO (−0.748) and POD (−0.813). This suggests that when total phenolic compounds increase, PPO and POD activity decrease. Polyphenol oxidase (PPO) activity has been reported to be the primary enzyme responsible for the oxidation of phenolic compounds into quinone compounds, leading to the degradation of valuable phenolics and to the browning of the peel [42]. Another enzyme, peroxidase (POD), also uses polyphenol substances by oxidation. However, this is performed in the presence of H_2O_2 [58]. The two enzymes are known to have a synergistic effect [58], which explains the strong positive correlation between PPO and POD ($r = 0.980$) that we observed in our results (Table 3). Contrary to our findings, Nurhuda et al. [42] studied the effects of blanching using boiling water at 100 °C (for 2.5 and 5 min) and steam at 100 °C (for 5, 10 and 15 min) in total anthocyanin content (TAC), as well as browning enzymes, POD and PPO, in 'Anak Sekolah' rambutan fruit peel. They reported that total anthocyanin

content significantly increased in rambutan peel extracts in both water-blanching peel at 1.39 mg/100 g in 2.5 min and steam-blanching peel at 1.64 mg/100 g in 5 min of blanching, compared to unblanching peel at 1.00 mg/100 g. Notable reductions were observed for both blanching techniques after 5 min of blanching. Furthermore, significant reductions in PPO and POD for both water and steam blanching with increase in time were reported. Similarly, Duarte et al. [43] investigated the effects of water blanching (at 90 °C for 1 min) as a pre-treatment on the total phenolic and total fibre content of yellow passion fruit (*Passiflora edulis* var. *flavicarpa*) peel and significant increases in total fibre content during blanching from 63.3% (unblanching peel) to 69.2% (blanching peel). However, TPC reduced significantly when exposed to blanching for all measured parameters. Chung et al. [70] studied the effects of the hot water blanching process at 85 °C for 30 s on the total phenolic content and antioxidant activity of yam (*Dioscorea alata*) peel, but significant declines in total phenolic content of blanching peel extracts up to 40% were noted. Consequently, this led to lowered antioxidant activity of the yam peel extracts. In this particular study, we also observed that lower temperatures of 60 °C at 1, 3 and 5 min significantly increased ($p < 0.05$) both PPO and POD activity, and further led to the reduction in all phytochemical and antioxidant activity in 'Wonderful' pomegranate peel extracts (Table 2). This could be a result of under-blanching, which speeds up the activity of enzymes, for example PPO and POD, but this may be worse than not blanching [41].

Numerous studies have reported the inactivation of PPO and POD, and some examples of potential recovery of phytochemicals after blanching are illustrated in mango peels [44], citrus peels [45], persimmon peels [46] and banana peels [47], to name a few. These authors suggest that the temperature and timing of blanching are crucial and should be appropriately adjusted for size and type of fruit peel waste based on varying susceptibility to degradation to thermal processing, as under-blanching stimulates enzymatic actions and increases the degradation rate, whereas over-blanching causes loss of phytochemicals, nutrients, texture, colour and flavour qualities of the peel through leaching [41,42,70]. Phytochemicals can undergo chemical changes during blanching, based on several factors, including the blanching method, thermal stability of different phytochemicals (based on temperature and time used during blanching), enzyme activity and location of phytochemicals in the plant structure, and even the plant material type [41,42,70].

The antibacterial activity of blanching 'Wonderful' pomegranate peel extracts showed a broad-spectrum activity against bacterial strains tested, namely Gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus*, and Gram-negative bacteria, *Escherichia coli* and *Klebsiella pneumonia* (Table 4). MIC ranged from 160 to 630 µg/mL. The best MIC was reported using peel extracts blanching at 80 °C for 3 min and 80 °C for 5 min at 160 µg/mL for all tested bacteria strains, while at high temperatures of 100 °C, for 1, 3 and 5 min, we obtained weak antioxidant activity at 630 µg/mL compared to other treatments (Tables 3 and 4). This is in agreement with our PCA and AHC (Figure 1) results, which showed that Factor 1 is positively correlated with the extract yield (0.689), TTC (0.901), TFC (0.668), TAC (0.875), vit C (0.974), DPPH (0.972), FRAP (0.905), ABTS (0.986) and PPO (0.525), whilst it was negatively correlated with *E.coli* (−0.867), *K. pneumonia* (−0.937), *S. aureus* (−0.938) and *B. subtilis* (−0.893). We also observed that high temperature of 100 °C, for 1, 3 and 5 min, favoured increases in bacterial activity by reducing heat-sensitive phytochemicals and their antioxidant activity, and thus caused weak antimicrobial activity of cultivar 'Wonderful' peel extracts subjected at this high temperature.

Similar to our findings, these results are comparable with Mphahlele et al. [6], who tested the antibacterial activity of 'Wonderful' peel extracts dried at various temperatures. They reported a MIC ranging between 100 and 390 µg/mL for the same bacteria strains, especially *S. aureus* with the highest MIC at 100 µg/mL. Similarly, Fawole et al. [3] studied the antibacterial activity of methanolic pomegranate peel extracts of seven different cultivars (Arakta, Bhagwa, Ganesh, Herskowitz, Molla de Elche, Ruby and Wonderful) against both Gram-negative (*Escherichia coli* and *Klebsiella pneumonia*) and Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) bacteria using the microdilution technique. The authors

reported a minimum inhibitory concentration (MIC) ranging from 200 to 780 µg/mL against the tested bacteria strains. The strong antibacterial activity in pomegranate peel extracts were also reported by Rosas-Burgos et al. [71], who investigated both anti-bacterial (Gram-negative: *Escherichia coli*, *Shigella sonnei*, *Salmonella enterica*, and Gram-positive: *Bacillus subtilis*, *Enterococcus faecalis* and *Staphylococcus aureus*) properties of six pomegranate ('Mollar de Elche', 'Valenciana de Albatera', 'Piñón Tierno de Ojós', 'Hicaznar', 'Borde de Albatera' and 'Borde de Beniel') peel extracts. They observed that pomegranate peel extracts from cultivar Piñón Tierno de Ojós had the best results, with a radial inhibitory growth for all fungi species ranging from 39.2% to 70% for *A. flavus* and *F. verticillioides*, respectively. Furthermore, pomegranate peel extracts from Piñón Tierno de Ojós cultivar were reported with the lowest minimum inhibitory concentrations of 50% (MIC₅₀) and 90% (MIC₉₀) ranging from 1.5 (*B. subtilis*) to 171 µg/mL (*E. coli*), and 4.4 (*B. subtilis*) to 222 µg/mL (*S. sonnei*), respectively.

The pomegranate peel is known to have hydrolysable tannins higher in the peel matrix, compared to the rest of the other fruit parts [5,29,31], and during blanching at optimum temperatures of 80 °C and time of 3 and 5 min, release of these compounds may likely occur, thus influencing bacterial activity of 'Wonderful' peel extracts. The chemical features of these compounds control its high antioxidant and antimicrobial activity. For instance, ellagitannins such as punicalagin have multiple hydroxyl groups, and the presence of hydroxyl residues allows for radical scavenging, therefore quenching these harmful species [12,29,31]. The antimicrobial activity of tannins (punicalagin isomers) has also been suggested to be a result of their ability to precipitate proteins, therefore breaking down or destroying the cell membrane of the microorganism and ultimately causing cell death [3,71]. Tannins also have adjacent hydroxyl groups such as catechol -OHs that grant the strong attraction for metal ions during chelation, thus preventing the production of free radicals generated through the Fenton reaction [3,7,31,71]. It has also been suggested that transport of substrates into the cell of the microorganism may be affected by the membrane instability caused by the interaction of the hydrophilic region of the tannin chemical structure with the polar region of the microorganism, while the hydrophobic section is immersed in a non-polar section of the bacterial membrane [71].

Ellagitannins such as α-punicalagin, β-punicalagin and granatin A and B (Figure 2A) are regarded as potential compounds showing considerable free radical scavenging potential [3,6]. The high antioxidant and antibacterial activity in blanched peel extracts at 80 °C for 3 min may be a result of additive and synergistic interactions of a complex mixture of phytochemicals such as hydrolysable tannins and many other bioactives present in the pomegranate peel extracts [3,32]. Punicalagin is the main phenolic compound present in pomegranate peel test samples and contains a gallagic acid, ellagic acid and glucose [3,32]. In our study, α-punicalagin and β-punicalagin were detected (in both blanched and unblanched treatments) as a doubly charged ion displaying an $[M - 2H]^{2-}$ ion at m/z 541 and eluted at 6.46 and 7.69 min, respectively (Figure 2A). The UV max for both α-punicalagin and β-punicalagin was reported at 258 and 378. Moreover, the main fragments observed in punicalagin compounds were the presence of punicalin α and β (with $[M - H]^-$ at m/z 781.0671, molecular formula of C₃₄H₂₁O₂₂ and UV max at 259 and 270), ellagic acid (with $[M - H]^-$ at m/z 300.9987, molecular formula of C₁₄H₅O₈ and UV max at 254 and 364) and gallagyl residues (600.9943 m/z) (Figure 2B). Punicalagin derivatives were tentatively identified in blanched peel extracts as granatin A (with $[M - H]^-$ at m/z 799.0454, molecular formula of C₃₄H₂₃O₂₃) and granatin B (with $[M - H]^-$ at m/z 951.071, and molecular formula of C₅₂H₂₃O₁₉), respectively (Figure 2A) [32,72–74]. Galloyl-hexahydroxydiphenol-hexoside was also tentatively identified in blanched peel extracts and eluted at 9.74 min, with a molecular formula of C₂₇H₂₂O₁₈, precursor ion at m/z 633.0702 and UV max at 260 and 360 (Figure 2A). Organic acid, known as citric acid, was tentatively identified in both treatments (blanched and unblanched peel extracts) and eluted at 2.50 min, with $[M - H]^-$ at m/z 191.0221, and molecular formula of C₅H₇O₇

(Figure 2A). The current study thus forms a platform to quantify this metabolite and others in future work.

5. Conclusions

In this study, solvent and hot water blanching processes were combined to extract polyphenols from pomegranate peels, as no singular post-harvesting extraction method is currently available for use to produce an elite peel-based phytochemical extract. Out of the solvents tested, 70% ethanol was the best at extraction of phenolic compounds from 'Wonderful' pomegranate peels when combined with a blanching treatment lasting 3 min at 80 °C. Higher phytochemical content, antioxidant and enzyme inactivation and antibacterial activities were displayed with this particular method. It appears that blanching also leads to higher accumulation of hydrolysable tannins and other biomarker metabolites that have pharmacological effects. A more comprehensive profiling of phenolic acids, flavonoids (anthocyanins) and ellagitannins that influence strong antioxidant and antimicrobial potential of pomegranate peels is thus needed in order to provide insights into the biochemical changes that occur as a result of various extraction procedures. Novel processing innovations may help with the preservation, recovery and isolation of these health-promoting compounds to formulate new or fortify existing value-added and shelf-stable products. This will possibly encourage the pomegranate peel industry to explore alternative ways of making greater income whilst reducing raw pomegranate fruit peel waste. Added to this, such industrial activities may reduce the negative environmental effects associated with disposing of fruit waste in landfills or by incineration.

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