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Polyphenolic Composition, Antioxidant and Antiproliferative Activity of Edible and Inedible Parts of Cultivated and Wild Growing Pomegranate (*Punica granatum* L.)

Running head: Bioactivity of Cultivated and Wild Pomegranate

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SUMMARY

Research background. The aim of this study was to determine and compare the antioxidant and antiproliferative activities of juices and extracts of the peel, aril and membrane of the cultivated and wild growing pomegranate fruits.

Experimental approach. The contents of total phenols, total flavonoids, total flavonols, total flavan-3-ols, and total anthocyanins were determined spectrophotometrically. Individual phenolics were quantified by HPLC. Antioxidant activity was detected by DPPH and ABTS test, and neutralization of hydroxyl radical, while *in vitro* antiproliferative activity by sulforhodamine B (SRB) assay.

Results and conclusions. Total phenols were statistically the highest in wild pomegranate peel

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extract (WPPE) and amounted 340.92 mg GAE/g ($p<0.05$), while the content of total flavonoids was the highest in cultivated pomegranate peel extract (CPPE) and amounted 31.84 mg QE/g ($p<0.05$). The WPPE sample showed the highest antioxidant activity with respect to free DPPH and ABTS radicals. The CPPE and CPME (cultivated pomegranate peel and membrane extracts, respectively) samples showed almost identical and the strongest effect on hydroxyl-radical inhibition (41.24 μg/mL and 41.23 μg/mL, respectively). The WPPE sample showed the strongest effect on the growth inhibition of all tested tumor cell lines compared to all other samples.

Novelty and scientific contribution. In this study, bioactivity of different parts of cultivated and wild pomegranates were determined and compared. In the available literature, the individual antioxidant and antiproliferative activity of only some parts of pomegranate fruit were studied. In this work, all parts of the pomegranate fruit were investigated, including the membrane of the pomegranate, which was barely analyzed in other works. Also, wild pomegranate is less investigated in previous studies. Future research should be focused on *in vivo* studies of obtained pomegranate samples.

Keywords: cultivated and wild pomegranate; phenolics; antioxidant activity; antiproliferative activity

INTRODUCTION

Pomegranate (*Punica granatum* L.) fruit is considered as one of the oldest edible fruits which belongs to the family *Punicaceae*. This plant species originates from Asia, in the region from Iran to north India. Pomegranates that grow in nature are called wild pomegranates. The fruit of the wild pomegranate is smaller, while the fruit of the cultivated pomegranate is larger and heavier and has larger bright red grains (arils) of 8–12 mm in size. About 50 % of the total mass of pomegranate fruit corresponds to the pericarp, which contains many bioactive compounds such as flavonoids, ellagitannins, and proanthocyanidins. Arils, which are the edible part of the pomegranate, make up the remaining 50 % of the fruit weight. They are made up of an outer fleshy red part (78 %), and an inner seed (22 %) (*1*). Pomegranate fruit is divided into several cells (carpels) by membranous partitions (carpellary membranes), which are full of rounded succulent arils (*2*). It contains large number of different phytochemicals. A total of around 50 polyphenols were identified in different parts of the fruit. Pomegranate fruit contains hydrolyzing tannins (punicalagin and punicalin), condensed tannins, anthocyanins, phenolic and organic acids. The concentration of bioactive compounds is the highest in the pomegranate bark (*3*). Pomegranate peel has been found to possess exceptional phytochemicals of

medical and nutritional importance (*4*). Reactive oxygen species (ROS), formed in normal cellular metabolic processes or formed by exposure to ionizing radiation or xenobiotic substances, are considered as an important factor in the development of a large number of chronic diseases. The toxicity of ROS can be attributed to its ability to damage essential biological substrates, such as DNA, RNA, proteins, and membrane lipids. Reactive oxygen species include: superoxide radical, lipoperoxide oxides, hydrogen peroxide, and hydroxyl radical. It is known that diet has a key role in the prevention of many diseases. Due to the high content of polyphenolic components, pomegranate fruits are recognized as one of the foods whose antioxidants have numerous positive effects on human health. Phenolic compounds have the ability to 'scavenge' free radicals and chelate metal cations.The aim and novelty of this study was to determine and compare the polyphenolic composition, antioxidant and antiproliferative activity of juices and extracts of all parts (peel, aril and carpellary membrane) of cultivated and barely investigated wild growing pomegranate fruits.

MATERIALS AND METHODS

Plant material

Ripe wild growing pomegranate fruits were harvested in November 2019 in the Bosnia and Herzegovina, municipality of Stolac (43° 05′ N, 17° 58′ E). Samples of cultivated pomegranate fruits, originating from Turkey, were purchased in November 2019 at a local market in Banja Luka, Bosnia and Herzegovina.

Sample preparation

The peel, aril and membrane of cultivated and wild growing pomegranate fruits were hand-separated from the fruit. Parts of the obtained amount of arils were used to get pomegranate juice. After pressing the arils, the resulting juice was filtered and frozen at -18 °C. The remaining amount of arils was used to obtain aril extracts. Before extraction, arils were treated in a mortar and pestle (Haldenwanger, Berlin, Germany). The peel and membrane were air-dried at room temperature for 20 days, and then were grounded in a laboratory mill (E 1350 Blender, Ema, Turkey).

Determination of polyphenolic components

Powdered peel, aril, and membrane (200 grams) were extracted with 250 mL of solvent in a *Soxhlet's* extractor (Intos, Pula, Croatia). Extraction solvent was a mixture of 80 % ethanol (*V/V*) (Zorka

Pharma, Sabac, Serbia) and acetone (Lach-Ner s.r.o., Neratovice, Czech Republic) in a 1:1 ratio (*V/V*). The extraction time was 6 h. After the extraction was completed, the extracts were evaporated to dryness in a rotary vacuum evaporator (Elektormedicina, Ljubljana, Slovenia) at a 50 °C. The extracts were left for 6 days in a vacuum desiccator in a dark place for further drying. The obtained dry extracts were kept at +4 °C until the moment of analysis. Samples were marked as: CPJ - cultivated pomegranate juice, WPJ - wild pomegranate juice, CPPE - cultivated pomegranate peel extract, WPPE - wild pomegranate peel extract, CPAE - cultivated pomegranate arils extract, WPAE - wild pomegranate arils extract, CPME - cultivated pomegranate membrane extract, and WPME - wild pomegranate membrane extract.

Total polyphenolics content was determined according to Kırca and Arslan (*5*) with certain modifications. Briefly, 0.2 mL of a diluted extract (juice) was mixed with 1 mL of 7.5 % NaHCO3 (Sigma-Aldrich, St. Louis, MO, USA) and 1.5 mL of 0.2 N Folin reagent (Sigma-Aldrich, St. Louis, MO, USA). After 30 min in a dark place the absorbances were measured at 765 nm. The results were expressed as mg gallic acid equivalent per 1 gram of extract (mg GAE/g dm) and also as mg gallic acid equivalent per 1 gram of uice (mg GAE/ g fm).

Total flavonoids content was determined according to Mohammed *et al*. (*6*) by mixing 2 mL of the diluted sample with 2 mL of 2 % AlCl3 (Sigma-Aldrich, St. Louis, MO, USA) in 96 % ethanol. After 1 h at room temperature absorbances were read at 420 nm. The results were expressed as mg quercetin equivalent per 1 gram of extract (mg QE/g dm) and also as mg quercetin equivalent per 1 gram of juice (mg QE/g fm).

Total flavonols content was determined as previously described by Formagio *et al.* (*7*). The results were expressed as mg quercetin equivalent per 1 gram of extract (mg QE/g dm) and also as mg quercetin equivalent per 1 gram of juice (mg QE/g fm).

Total flavan-3-ols content was determined as previously described by authors Toro-Uribe *et al.* (*8*) with some modifications. Briefly, 2.5 mL of 10 % H2SO4 (Lach-Ner s.r.o., Neratovice, Czech Republic) in methanol (Lach-Ner s.r.o., Neratovice, Czech Republic) was mixed with 1 mL of diluted sample and 2.5 mL of 1 % vanillin (Sigma-Aldrich, St. Louis, MO, USA) in methanol. After 20 min at room temperature absorbances were read at 500 nm.The results were expressed as mg catechin equivalent per 1 gram of extract (mg CATE/g dm) and also as mg catechin equivalent per 1 gram of juice (mg CATE/g fm).

Total anthocyanins content in samples was determined by the pH differential method by Giusti and Wrolstad (*9*). Samples were extracted with HCl (Lach-Ner s.r.o., Neratovice, Czech Republic)/ethanol (85:15 % V/V) for 24 h at 0 °C, and 0.5 ml of extract was mixed with 9.5 ml of KCl buffer (Sigma-Aldrich,

St. Louis, MO, USA), pH 1.0. Absorbances were measured at 510 nm and 700 nm after 15 min incubation at room temperature. The absorbance was calculated as:

$$A=(A_{510\ nm}-A_{700\ nm})_{pH=1.0} \quad /1/$$

The anthocyanin content (TAC/(mg/L)) of each sample was calculated from the following equation:

$$TAC=(A\cdot M\cdot DF\cdot 1000)/(\varepsilon\cdot l) \quad /2/$$

where A is absorbance, M is the molecular mass (449.2 g/mol), DF is the dilution factor (20), ε is the molar absorptivity of cyanidin-3-glucoside (26 900 L/mol cm), and l is light path length (1 cm). The anthocyanin content, mg/L, is then converted to mg per gram of sample. The results were expressed as mg cyanidin-3-glucoside equivalentper 1 g of extract (mg CyGE/g dm) and also as mg cyanidin-3-glucoside equivalent per 1 g of juice (mg CyGE/g fm).

Identification and quantification of phenolic acids and flavonoids by HPLC method

Samples were analyzed by a Shimadzu Prominence (Shimadzu, Kyoto, Japan) chromatographic system. Chromatograms were recorded using different wavelength for individual compounds: 280 nm and 320 nm for phenolic acids, and 360 nm for flavonoids. Separation was performed on a Luna C-18 RP column, 5 mm, 250 mm×4.6 mm with a C18 guard column, 4 mm×30 mm (both from Phenomenex, Torrance, CA, USA). Two mobile phases, A (acetonitrile) and B (1 % formic acid) were used at flow rates of 1 mL/min with the following gradient profile: 0–10 min from 10 to 25 % B, 10–20 min linear rise up to 60 % B, and from 20 min to 30 min linear rise up to 70 % B, followed by 10 min reverse to initial 10 % B with additional 5 min of equilibration time.

Antioxidant activity

The antioxidant activities of the samples regarding to 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic-acid) ABTS radical were determined according to the methods of Liyana-Pathiranan and Shahidi (*10*) and Re *et al.* (*11*), respectively. The antioxidant capacities of samples to inhibit DPPH (Sigma-Aldrich, St. Louis, MO, USA) and ABTS (Sigma-Aldrich, St. Louis, MO, USA) radicals were presented as IC_{50} values (μg/mL).

Total antioxidant capacity to neutralize hydroxyl radicals (•OH) was determined by spectrophotometric method described in the study by Xican (*12).* After keeping the samples at 50 °C for 20 minutes, 1 mL of 5 % TCA (Lach-Ner s.r.o., Neratovice, Czech Republic) and 1 % TBA (Sigma-Aldrich, St. Louis, MO, USA) were added. After mixing, all tubes were thermostated at 100 °C for 20 min. The

samples were cooled to room temperature. The measurements were performed at 530 nm. The antioxidant capacities of samples to inhibit hydroxyl radical were presented as IC_{50} values (μg/mL).

Antiproliferative effect

Human tumor cell lines: HeLa (cervix epitheloid carcinoma), MCF7 (breast adenocarcinoma), HT-29 (colon adenocarcinoma) and MRC-5 (normal fetal lung fibroblasts) were used for the estimation of cell growth activity.

Cell lines were harvested and plated into 96-well microtiter plates (Sarstedt, Newton, NC, USA) at seeding density of $4–8·10^3$ cells per well, in a volume of 199 or 180 μL, and pre-incubated in medium supplemented with 5 % FCS, at 37 ºC for 24 h. Serial dilutions of samples and solvents and control (1 or 20 μL per well) were added to the test and control wells, respectively. Microplates were incubated at 37 ºC for an additional 48 h. Cell growth was evaluated using colorimetric sulforhodamine B (SRB) assay according to Cetojevic-Simin *et al.* (*13*). Effects on cell growth were calculated as:

$$\text{Control}=((A_t/A_c)\cdot 100 \qquad /3/$$

where A_t is the absorbance of the test sample and A_c is the absorbance of the control.

All spectrophotometric analysis were determined by UV-VIS spectrophotometry (Jenway 6305, Cole-Parmer, UK).

Statistical analysis

The experiments were carried out in at least three repetitions. The results were expressed as mean±standard deviation (SD). All results were subjected to a one-factor analysis of variance (ANOVA). The Duncan's test was performed to determine a statistically significant difference between the arithemtic means at $p<0.05$. The obtained results were performed using the software programs: Excel (Microsoft Office 2010), Origin 5.0 (OriginLab, USA) and Statistica 12.0 (StatSoft, Inc.) (*14*).

RESULTS AND DISCUSSION

Obtained juice yields were: for cultivated pomegranate juice (CPJ) 80.51 % and for wild pomegranate juice (WPJ) 48.73 %. Higher yield of CPJ can be explained by the higher proportion of liquid phase in the arils of cultivated pomegranate fruit. The yield of cultivated pomegranate juice was in accordance with the studies of Zaouay *et al.* (*15*). The yields of CPPE, CPAE, and CPME were: 17.83, 11.01 and 22.27 %, respectively. Yields of WPPE, WPAE and WPME were: 14.05, 13.26 and 40.20 %,

respectively. Yield of CPPE in this study was similar to the results reported by Iqbal *et al.* (*16*) whose yield of ethanol extract of pomegranate peel was 21.14 %. Yield of cultivated pomegranate arils extract was lower than that obtained by Magangana *et al.* (*17*).

The content of total polyphenolics was statistically the highest in the WPPE sample ($p<0.05$). The CPPE sample had a slightly lower concentration of these components, with a statistically significant difference compared to the WPPE sample ($p<0.05$). The lowest content of these compounds was in the CPJ sample, which did not differ statistically significantly from the WPJ, CPAE, and WPAE samples (Table 1). Value for the CPPE sample (294.55 mg/g) was in accordance with the results reported by the authors Derakhshan *et al.* (*18*) for Natanz pomegranate peel (276 mgGAE/g). On the other hand, the authors Orak *et al.* (*19*) reported about two times the lower concentration of total phenols in ethanolic extracts of pomegranate peel of the Hicaznar variety. Our values for wild and cultivated pomegranate juices were in accordance with the values published by Gözlekçi *et al.* (*20*), which were in the range of 0.78–1.55 mg GAE/g, and slightly lower for CPJ sample compared to the study by Zaouay *et al.* (*15*). The content of total phenols in the CPAE sample was slightly higher than the literature values for ethanolic extracts of arils of different varieties of pomegranate (3.2−8.8 mg GAE/g) (*17*). The content of total phenols in the WPME sample was statistically higher compared to the CPME sample ($p<0.05$). The content of total flavonoids was the highest in the CPPE sample and was statistically significantly different from the WPPE sample ($p<0.05$). A similar trend was observed in the pomegranate carpellary membrane, where the content of these compounds in CPME was statistically higher compared to the WPME sample ($p<0.05$). The lowest concentration of these components was observed in the CPJ and WPJ samples (0.12 and 0.13 mg QE/g, respectively). The WPJ sample had a slightly higher concentration of these compounds, but without a statistically significant difference ($p>0.05$). Since the juice was obtained by pressing of arils and is an integral part of it, the sample WPAE was also richer in these compounds compared to CPAE and the difference was statistically significant ($p<0.05$). The concentration of flavonoids in CPJ was consistent with the results obtained by Li *et al.* (*21*), whose values, depending on the pomegranate variety, were in the range of 0.045–0.335 mg QE/mL. The content of total flavonoids in the CPPE sample was 2 to 3 times higher than the literature values (*19,22*). Taking the above data into account, we can conclude that flavonoids are mainly located in the peel and membrane of pomegranate fruits, while their content in the arils and pomegranate juice is significantly lower.

Table 1

The content of total flavonols was statistically the highest in the WPPE sample and was slightly

higher with a statistically significant difference ($p<0.05$) compared to CPPE. A similar results were observed in the WPME and WPAE samples, which had a statistically higher concentration compared to the CPME and WPAE samples ($p<0.05$). CPJ had the lowest concentration of flavonols, and their content was similar to the samples of WPJ and CPAE, without a statistically significant difference ($p>0.05$). The concentrations of total polyphenolic compounds and total flavonols were higher in all samples of wild pomegranate fruit compared to samples of cultivated pomegranate fruit. Also, from the presented results, it can be concluded that total phenols, flavonoids, and anthocyanins are mainly distributed in the outer part of the fruit (peel), and less in the interior, which is in line with the research of Saidani *et al.* (*23*).

The CPPE sample showed statistically the highest content of total flavan-3-ols, followed by the WPPE sample, which had about 2 times lower concentration of these compounds. The CPJ sample was the poorest in these substances and did not differ statistically significant from the CPAE, WPAE, and WPME samples ($p>0.05$). Also, CPJ had about two times the lower concentration of total flavan-3-ol compared to WPJ ($p>0.05$). The WPAE sample had a slightly higher concentration of these compounds compared to CPAE ($p<0.05$). The WPME sample also had a higher concentration of total flavan-3-ol and was statistically different from the CPME sample ($p<0.05$).

The content of total anthocyanins was statistically the highest in the WPJ sample and the lowest in the WPME sample. Compared with the CPJ sample, WPJ and WPAE samples had a statistically higher concentration of these compounds. The CPJ sample had a higher concentration of total anthocyanins compared to the results of Li *et al.* (*21*) (0.026–0.160 mg CyGE/mL). The content of total anthocyanins in the CPAE and CPPE samples was significantly lower compared to the results of Osama *et al.* (*24*) (11.04 and 15.24 mg CyGE/g, respectively). The content of total anthocyanins was statistically higher in CPPE compared to WPPE, as well as in the CPME sample compared to the WPME sample, with a statistically significant difference ($p<0.05$). Results in this study with respect to the CPPE sample were lower than those reported by Fawole *et al.* (*25*), whose values ranged from 0.058–0.32 mg CyGE/g, depending on the variety of pomegranate fruit. Based on the above data, it can be concluded that in pomegranate fruit, these compounds are slightly more stored in the outer part of the fruit, in contrast to the wild pomegranate fruit, in which anthocyanins are more located inside the fruit.

The contents of individual phenolic compounds were shown in Table 2. The highest total content of phenolic compounds detected by HPLC method was in WPPE sample (46.61 mg/g), closely followed by CPPE (42.40 mg/g) and WPME (40.92 mg/g). The highest contents of epicatechin (9.53 mg/g), *p*-hydroxybenzoic (9.24 mg/g), syringic (4.38 mg/g), ellagic (1.23 mg/g) and ferulic (1.97 mg/g) acids were

in WPPE sample. The same sample showed the best results in antioxidant (DPPH, ABTS and OH radicals) tests (Table 3). Epicatechingallate was found in peel and membrane (CPPE, WPPE, CPME and WPME) samples. Gallic acid was found in the greatest amount in CPPE (3.58 mg/g), as well as coumaric (3.65 mg/g) and chlorogenic (6.15 mg/g) acids. CPPE sample closely followed WPPE sample in good antioxidant results. Protocatechuic acid was the highest in CPME sample (4.98 mg/g), which is in accordance with bit worse results in bioactive tests results in Table 3, comparing to peel samples. Vanillic acid was detected only in WPPE and WPME samples.

Table 2

Table 3

The WPPE sample showed the highest antioxidant activity against free DPPH and ABTS radicals, followed by the CPPE, WPME, and CPME samples, with no statistically significant difference ($p>0.05$) (Table 3). On the other hand, CPJ showed the lowest antioxidant activity against DPPH and ABTS free radicals, while WPJ had about 2.6 times higher acitivity compared to CPJ sample. In all samples, it was stated that the components of wild growing pomegranate had a higher antioxidant capacity compared to cultivated pomegranate fruit. Authors Sharayei *et al.* (*26*) reported significantly lower antioxidant activity of pomegranate peel extract obtained by aqueous extraction assisted by ultrasonic waves (UAE), whose IC_{50}^{DPPH} values ranged from 0.2−1.2 mg/mL. On the other hand, the authors Okonogi *et al.* (*27*) in their study with pomegranate peel extract in 95 % ethanol, reported an IC_{50}^{DPPH} in amount of 3 μg/mL. A similar result was reported by Kanatt *et al.* (*28*) (IC_{50}^{DPPH}=4.9 μg/mL). Our results were consistent with the values obtained by Mansour *et al*. (*29*) for aqueous extract of pomegranate peel, where IC_{50}^{DPPH} valueswere in the range of 10.2−13.1 μg/mL. Authors Robert *et al.* (*30*) reported an IC_{50}^{DPPH} value of 2.12 mg/mL for pomegranate juice, which means that this sample showed about two times better antioxidant activity compared to our CPJ sample, but also slightly worse activity compared to our sample WPJ. The CPAE and WPAE samples showed significantly better antioxidant activity compared to the literature data (IC_{50}^{DPPH}=1.73 mg/mL for ethanol extract) (*30*).

Regarding to the ABTS radical, the CPJ sample was in line with the results found in the literature (IC_{50}^{ABTS}=525−3760 μg/mL) (*31*). Also, ethanolic arils extract obtained by Singh *et al.* (*32*) showed better antioxidant activity compared to our CPAE sample, but worse compared to the WPAE sample (IC_{50}^{ABTS}=81.31 μg/mL). The same authors confirmed the significant influence of solvent choice during extraction on the antioxidant activity of the extracts. The CPPE sample showed a significantly better effect compared to the Al-Hindi *et al*. (*33*) (IC_{50}^{ABTS}=54.63 μg/mL, aqueous extract). Likewise, our peel extracts

showed better activity compared to the results reported by Laosirisathian *et al*. (*34*) for extracts of pomegranate peel at different concentrations of ethanol as solvent.

Similary to the DPPH and ABTS tests, all samples of wild growing pomegranate fruit showed better antioxidant activity against hydroxyl radicals. The CPPE and CPME samples showed almost identical and the strongest effect on hydroxyl radical inhibition, as did the WPPE and WPME samples. The means of these samples did not differ statistically significantly ($p>0.05$). The CPJ sample had the weakest antioxidant activity compared to other samples, and was statistically significantly different from all other samples ($p<0.05$). The WPAE sample showed stronger antioxidant activity with respect to the OH radical compared to the CPAE sample, but with no statistically significant difference ($p>0.05$). Our results for pomegranate peel extracts were slightly better compared to the results of Arun *et al*. (*35*), whose IC_{50}^{OH} value for pomegranate peel extract in 70 % methanol was (54.9±0.43) μg/mL, while according to the same authors, the IC_{50}^{OH} value for peel extract obtained with pure methanol was (13.6±0.33) μg/mL. Our peel extracts showed better activity compared to the research of Rummun *et al.* (*36*), where IC_{50}^{OH} of methanolic peel extract was 0.111 mg/mL.

To-date, several studies have been conducted to evaluate the antiproliferative activity of pomegranate fruit against different types of tumor cells, such as colon cancer, breast cancer, prostate cancer, lung cancer, and cervical cancer (*37*). The published study (*38*) showed that ellagic acid and its by-products can contribute to the prevention of colon cancer by regulating the expression of multiple genes involved in key processes associated with cancer development. A study of 46 patients with experimental prostate cancer showed, in 16 of them, a significant reduction in PSA (prostate-specific antigen) during treatment with pomegranate juice (*39*).

Table 4 shows that CPJ and WPJ samples did not show antiproliferative activity on the tested tumor cells. In general, the WPPE sample showed the strongest effect on the growth inhibition of all tested tumor cell lines compared to all other samples. Based on research by Abdel Motaal and Shaker (*39*), our WPPE sample showed significantly lower antiproliferative activity with respect to MCF7 cells (IC_{50}=(7.7±0.01) μg/mL). Also, the Keta *et al.* (*40*), reported about 2.5 times better antiproliferative activity compared to our WPPE sample with respect to MCF7 cells (IC_{50}=(31.29±1.63) μg/mL). With the exception of CPJ and WPJ samples, the worst antiproliferative effect on the tested tumor cell lines was found in CPFE and WPFE samples. The CPME sample showed, with respect to the inhibition of HeLa and MCF7 cells, a better antiproliferative effect compared to the CPPE and WPME samples. The CPPE sample showed better antiproliferative activity compared to WPME. Authors Peršurić *et al*. (*41*) reported IC_{50}

values for the antiproliferative activity of pomegranate extracts with respect to HeLa cells in the range of 0.141–0.212 mg/mL. Comparing obtained IC_{50} values for antiproliferative activities with respect to HT-29 cells, the CPPE sample showed a better inhibitory effect compared to the WPME and CPME samples. With respect to HT-29 tumor cell line, the WPME sample showed a better effect compared to the CPME sample. With respect to MRC-5 tumor cell line, the CPPE sample showed slightly better antiproliferative activity compared to CPME samples and WPME samples, while CPME in WPME samples showed a similar effect in inhibiting the growth of these tumor cell lines. WPPE sample showed slightly better antiproliferative activity with respect to MRC-5 tumor cell line compared to studies reported by Keta *et al.* (*40*) who reported IC_{50}=(189.15±0.05) μg/mL.

Table 4

CONCLUSIONS

Extracts of wild pomegranate peel contained the highest amounts of total phenols and flavonols, while the highest concentration of flavonoids, flavan-3-ol, and total anthocyanins was detected in the extract of cultivated pomegranate peel. Also, extract of wild pomegranate peel showed the highest antioxidant activity against DPPH, ABTS, and hydroxyl radicals compared to other tested pomegranate samples. The same sample showed the greatest antiproliferative effect on the tested tumor cell lines. As it could be observed, total phenols, flavonoids, and flavonols showed a very strong effect on the antiproliferative activity of pomegranate samples against MCF7, HT-29, and MRC-5 tumor cell lines. In this study, all parts of the pomegranate fruits, cultivated and wild, including the membrane of the pomegranate were analysed, and used solvent for extraction was ethanol, which is a green solvent. Future studies of obtained pomegranate samples should be *in vivo* studies.

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CONFLICT OF INTERESTS

The authors declare no conflict of interests.

AUTHORS' CONTRIBUTION

Mirjana Milošević was involved in designing/performing experiments and processing/interpreting

data. Jelena Vulić was writing/revising manuscript. Zoran Kukrić was also writing/revising manuscript. Biljana Lazić participated in designing/performing experiments. Dragana Četojević-Simin, was designing/performing experiments. Jasna Čanadanović-Brunet prepared the manuscript. All authors have read and agreed to the published version of the manuscript.

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Table 1. Content of total polyphenolics, total flavonoids, total flavonols, total flavan-3-ols and total anthocyanins in cultivated and wild pomegranate juices and extracts

Samples	*w*(total phenols as GAE/(mg/g)	*w*(total flavonoids as QE/(mg/g)	*w*(total flavonols as QE/(mg/g)	*w*(total flavonols as CATE/(mg/g)	*w*(total anthocyanins as CyGE/(mg/g)
CPJ	(0.85±0.03)^{a}	(0.12±0.00)^{a}	(1.56±0.01)^{a}	(6.93±0.68)^{a,b}	(0.45±0.01)^{c}
WPJ	(1.84±0.02)^{a}	(0.13±0.00)^{a}	(1.61±0.04)^{a}	(14.57±0.85)^{b}	(0.53±0.01)^{d}
CPAE	(6.61±0.20)^{a}	(0.52±0.006)^{b}	(6.94±0.05)^{a}	(10.47±0.68)^{a,b}	(0.24±0.006)^{a}
WPAE	(16.18±1.37)^{a}	(1.38±0.02)^{c}	(18.12±0.52)^{b}	(11.12±0.85)^{a,b}	(0.42±0.00)^{c}
CPPE	(294.55±19.94)^{b}	(31.84±0.16)^{d}	(258.79±7.08)^{c}	(76.10±11.36)^{c}	(1.68±0.01)^{e}
WPPE	(340.92±26.22)^{c}	(29.84±0.21)^{e}	(287.36±0.63)^{d}	(39.95±1.70)^{d}	(0.85±0.05)^{f}
CPME	(155.44±5.02)^{d}	(21.59±0.43)^{f}	(144.08±7.76)^{e}	(6.45±0.17)^{a}	(0.58±0.006)^{g}
WPME	(201.37±11.37)^{e}	(18.36±0.04)^{g}	(154.13±0.43)^{f}	(8.97±0.00)^{a,b}	(0.37±0.02)^{b}

The results are presented as mean (*N*=3)±standard deviation. Mean values with different superscript letters in the same column are statistically diferent ($p<0.05$). CPJ - cultivated pomegranate juice, WPJ - wild pomegranate juice, CPPE - cultivated pomegranate peel extract, WPPE - wild pomegranate peel extract, CPAE - cultivated pomegranate arils extract, WPAE - wild pomegranate arils extract, CPME - cultivated pomegranate membrane extract, and WPME - wild pomegranate membrane extract, GAE - gallic acid equivalent, QE - quercetin equivalent, CATE - catechin equivalent, CyGE - cyanidin-3-glucoside equivalent

Table 2. Content of phenolic compounds in samples of cultivated and wild pomegranate fruits

Phenolic compound	CPPE	WPPE	CPME	WPME	CPJ	WPJ	CPFE	WPFE
	w(phenolic compound)/(mg/g)							
Gallic acid	(3.58±0.04)	(1.39±0.03)	(1.54±0.03)	(2.65±0.02)	(0.11±0.03)	(0.20±0.02)	(0.15±0.01)	(0.44±0.02)
Protoca-	(4.25±0.02)	(1.97±0.02)	(4.98±0.02)	(2.47±0.03)	(0.12±0.03)	(0.05±0.02)	(0.22±0.02)	(0.61±0.02)

techinic acid								
Epicatechin	(3.77±0.03)	(9.53±0.03)	(7.24±0.03)	(6.93±0.02)	(0.24±0.03)	(0.28±0.02)	(0.21±0.01)	0
Catechin	(6.18±0.02)	(1.65±0.02)	(6.22±0.02)	(8.93±0.03)	0	0	(0.74±0.01)	(3.27±0.01)
Ferulic acid	(1.15±0.02)	(1.97±0.02)	(0.81±0.01)	(1.11±0.02)	(0.01±0.01)	(0.01±0.01)	(0.04±0.01)	(0.09±0.02)
Syringic acid	(3.46±0.02)	(4.38±0.01)	(1.13±0.01)	(2.84±0.01)	0	0	0	0
Ellagic acid	(0.42±0.01)	(1.23±0.01)	(0.14±0.02)	(0.17±0.02)	0	0	0	0
Coumaric acid	(3.65±0.02)	(2.60±0.02)	(1.63±0.02)	(2.98±0.02)	0	0	0	0
Chlorogenic acid	(6.15±0.01)	(4.48±0.02)	(2.80±0.02)	(4.36±0.02)	(0.01±0.00)	(0.01±0.00)	(0.04±0.01)	(0.83±0.01)
p-hydroxybenzoic acid	(5.04±0.01)	(9.24±0.02)	(3.12±0.02)	(3.96±0.02)	(0.24±0.02)	(0.46±0.02)	0	0
Vanillic acid	0	(3.23±0.02)	0	(1.15±0.02)	0	0	0	0
Epicatechingallate	(4.76±0.01)	(4.94±0.02)	(1.60±0.02)	(3.37±0.02)	0	0	0	0
Total	42.40	46.61	31.22	40.92	0.73	1.01	1.40	5.24

The results are presented as mean (*N*=3)±standard deviation

Table 3. Antioxidative activity of cultivated and wild pomegranate juices and extracts

Sample	DPPH test	ABTS test	Hydroxyl radical
	IC_{50}/(μg/mL)		

CPJ	(5,087.61±417.89)^{d}	(1,251.87±67.70)^{b}	(9,313.041±1,611.98)^{a}
WPJ	(1,955.64±177.74^{c}	(478.21±28.06)^{c}	(4,121.08±645.44)^{b}
CPAE	(598.84±18.72)^{b}	(148.72±5.60)^{d}	(659.42±108.62)^{c}
WPAE	(216.73±9.48)^{a}	(68.95±1.41)^{e}	(445.08±59.80)^{c}
CPPE	(14.37±0.70)^{a}	(3.53±0.19)^{a}	(41.24±4.61)^{c}
WPPE	(12.23±0.71)^{a}	(3.21±0.10)^{a}	(36.54±4.90)^{c}
CPME	(21.02±1.05)^{a}	(5.76±0.26)^{a}	(41.23±2.48)^{c}
WPME	(16.90±0.69)^{a}	(4.73±0.33)^{a}	(37.90±2.80)^{c}
Trolox	(12.56±0.72)	(2.97±0.19)	(4.46±0.41)

The results are presented as mean (N=3)±standard deviation. Mean values with different superscript letters in the same column are statistically diferent ($p<0.05$)

Table 4. Antiproliferative effects of cultivated and wild pomegranate juices and extracts (HeLa (cervix epitheloid carcinoma), MCF7 (breast adenocarcinoma), HT-29 (colon adenocarcinoma) and MRC-5 (normal fetal lung fibroblasts))

Sample	HeLa	MCF7	HT-29	MRC-5
	IC_{50}/(μg/mL)			
CPJ	n.a.	n.a.	n.a.	n.a.
WPJ	n.a.	n.a.	n.a.	n.a.
CPAE	>5000[a]	>5000[a]	>5000[a]	>5000[a]
WPAE	(4119.48±162.76)[b]	(3354.65±146.40)[b]	(4745.27±93.86)[a]	(4387.34±155.60)[a]
CPPE	(273.20±54.97)[c]	(139.61±7.85)[c]	(549.78±94.72)[b]	(209.62±10.35)[b]
WPPE	(74.55±23.31)[d]	(85.76±25.77)[d]	<312.5[c]	(145.16±11.03)[c]
CPME	(157.28±54.28)[e]	(124.17±23.67)[c]	(2060.20±111.83)[d]	(297.09±12.25)[b]
WPME	(437.83±77.20)[f]	(153.46±5.70)[c]	(1444.73±82.88)[e]	(300.15±25.34)[b]

The values represent means (*N*=4)±standard deviation. n.a.-no activity. Mean values with different superscript letters in the same column are statistically diferent ($p<0.05$)