Emirates Journal of Food and Agriculture. 2021. 33(4): 320-327
doi: 10.9755/ejfa.2021.v33.i4.2683
http://www.ejfa.me/

RESEARCH ARTICLE

Cytotoxic effect of multifruit polyphenol preparation on human breast cancer cell lines

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ABSTRACT

Natural compounds are extensively used in the treatment of various diseases. Regular consumption of polyphenols plays an important role in the protection of health by reducing the risk of degenerative diseases, including cancer. The evaluation of the cytotoxic effect of the newly obtained multifruit polyphenolic preparation (composed of seven fruit) on T47D and MCF-7 breast cancer cells and MCF-12A normal cells. The PP was produced on the basis of combined ultrafiltrates obtained from chokeberry, raspberry, wild strawberry, apricot, peach, bilberry, and cranberry. The experiments were performed using human mammary gland cancer cell lines T47D (ductal cancer) and MCF-7 (adenocarcinoma) and normal breast cell line MCF-12A. Chromatographic techniques confirmed the highest contribution of cyanidin 3-*O*-glucoside, *p*-coumaroyl glucoside and chlorogenic acid in the PP. The PP exhibited dose-dependent cytotoxic effects towards MCF-7 and T47D cancer cell lines (IC_{50} = 1.2 μg. cm^{-3}) and MCF-12A cells (IC_{50} = 0.6 μg. cm^{-3}). The MTT cytotoxicity assay and microscopic observations confirmed the cytopathic effect of the PP on cell lines. It is supposed that berry polyphenols interfered with estrogen receptors leading to changes in the production of paracrine growth factors and therefore, PP was less cytotoxic towards the MCF-7 and T47D cell lines than against the MCF-12A cell line.

Keywords: Breast cancer; Coloured fruits; Flavonoid; Phenolic acid; Polyphenols

INTRODUCTION

Nowadays, cancer is among the main causes of death worldwide, and breast cancer is the leading cause of cancer-related deaths among women in developed countries (DeSantis et al., 2019). In most cases, research concerning the pathology on the molecular level has been focused on invasive breast cancer (IBC), and less attention has been given to the preinvasive nonobligate precursor, ductal carcinoma in situ (DCIS) which is present in a vast majority of IBC cases. Women diagnosed with DCIS face an increased risk of subsequently developing IBC (Seijen et al., 2019). DCIS is a heterogeneous group of lesions reflecting the proliferation of malignant cells within the breast ducts without invasion through the basement membrane (Fu et al., 2018).

It has been well documented that regular consumption of polyphenols plays an important role in the protection of long-term health by reducing the risk of degenerative diseases, including cancer, as reported in numerous reviews, e.g. the excellent work by Farvid et al. (2019) or Ahmeda et al. (2020). This is facilitated by the presence of phytochemicals, particularly polyphenols, carotenoids, alkaloids, nitrogen and sulphur compounds in the diet. According to numerous authors, e.g. Kumar et al. (2016), more than 30 plant-based foods such as garlic, turmeric, cruciferous vegetables and grape seed extracts display effective anticancer properties. One significant factor is the conformational flexibility of polyphenols that provides a broad range of biophysicochemical properties exhibited by phenol functional groups. For this reason, polyphenols are a unique and important group of natural compounds deserving research scrutiny (Basu and Maier, 2018). The anticarcinogenic potential of plant polyphenols may stem from the cancer-blocking or cancer-suppressing capacity, change in enzymatic activity at various stages of the disease (Ahmed et al., 2019), scavenging of free radicals, induction of enzymes

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Received: 29 December 2020; **Accepted:** 19 March 2021

involved in secondary metabolism, regulation of gene expression, alteration of cellular signalling pathways, enhancement of DNA repair, control of cell proliferation, apoptosis and invasion (Ullah et al., 2016).

It is known that estrogen receptors are strongly involved in the carcinogenesis of breast cancer and that plant polyphenols are structurally similar to the female hormone estrogen. Thus, polyphenols can act as estrogen receptor modulators (Basu and Maier, 2018). Nowadays, a great problem is the growing chemoresistance of breast cancer cells to commonly used drugs such as paclitaxel or doxorubicin. Therefore, phytoestrogens may be very useful in the therapy as alternative means of sensitising tumours during chemotherapy or radiation (Sinha et al., 2016). The aim of the study was to evaluate the cytotoxic effect of the newly obtained multifruit polyphenolic preparation (composed of seven fruit) on T47D and MCF-7 breast cancer cells and MCF-12A normal cells.

MATERIALS AND METHODS

Fruit. Chokeberries (*Aronia melanocarpa*), raspberries (*Rubus idaeus*) and wild strawberries (*Fragaria vesca*) were harvested in Bełżyce, Poland. Apricots *(Prunus armeniaca)* and peaches *(Prunus persica)* were harvested from a small orchard in Lublin, Poland. Bilberries (*Vaccinium myrtillus*) and cranberries (*Vaccinium macrocarpon*) were obtained from the Partnership Wholesale Market S.A. located in Ciecierzyn, Poland.

Preparation of the PP. Each fruit (1000 g) was processed in order to obtain ultrafiltrate as described in our previous work (Szwajgier et al., 2020) using repeated chromatography (typically 25 – 35 times) BioLogic DuoFlow system (BioLogic pumps, a QuadTec UV-VIS detector, and a BioFrac fraction collector; Bio-Rad, USA). Various eluent gradients were tested (for details, see Suppl. data) and fractions (2 cm^3) were collected. The preparation obtained from individual fruit was standardized with deionized water to obtain 250 mg of total phenolic compounds in 1 cm^3. To obtain the final PP equal volumes of the preparations were mixed together.

Preliminary analytical HPLC. Ultrafiltrates as well as the final PP were using HPLC as described in detail earlier (Szwajgier et al., 2020). Detection was performed using a UV-VIS detector (280 – 520 nm; Linear, California, USA) and a Merck-Hitachi F-1050 fluorescence spectrophotometer coupled with an IF2 interface (Knauer). Prior to the analysis, hydrolysis of glycosides was performed (Häkkinen et al., 1999). The procedure was duplicated. Compounds present in the chromatogram were identified using retention times of approx. 65 authentic HPLC grade standard polyphenolic compounds (Sigma-Aldrich, Fluka, USA; Extrasynthese, France; Roth, Germany; Apin Chemicals, England).

Qualitative and quantitative HPLC-ESI-Q-TOF-MS and HRMS/MS analysis.

For the determination of the PP composition, a similar analytical platform and the detection conditions as previously published (Szwajgier et al., 2019) were applied with modifications.

The PP (diluted to 9.5 mg total polyphenols/mL) was filtered through a nylon syringe filter (0.45 μm) and injected into the HPLC-MS system. The following linear gradient of 0.1% of formic acid with 10 mM of ammonium acetate (solvent A) and 95% acetonitrile with 0.1% formic acid and 10 mM ammonium acetate (solvent B) was applied on a Zorbax Stable Bond RP-18 Column (150 mm x 2.1 mm, 3.5 μm) by Agilent Technologies: 0 min – 1% of B, 70 min – 55% of B, 77 min – 95% of B, 83-90 min – 95% of B. The flow rate was set at 0.2 mL/min and the injection volume at 10 μL. The following MS conditions were introduced: gas temperature: 350 ºC, sheath gas temperature: 400 ºC, gas and sheath gas flows: 12 L/min, the nebulizer: 35 psig, fragmentation, capillary, nozzle and skimmer voltages of: 130 V, 4000 V, 1000 V, and 65 V, respectively. All solvents were of spectroscopic grade and were purchased at J.T. Baker (USA).

For quantitative analysis the following reference compounds: quercetin, naringin, citric acid, chlorogenic acid, protocatechuic acid, *p*-coumaric acid, gentisic acid and rosmarinic acid at the purity above 95% (Sigma Aldrich, USA) were used. The peak area measurements were obtained from 5 separate injections of each reference compound at 5 different concentrations. Quercetin was used as a reference compound for the determination of quercetin derivatives, naringin – for anthocyanin and flavonoid glucosides, *p*-coumaric acid – for *p*-coumaroyl glucoside.

Cytotoxicity assay. The experiments were performed using human mammary gland cancer cell lines. The MCF-7 adenocarcinoma, cell line (cat. no. HTB-22) and MCF-12A normal, luminal, epithelial cell line (cat. no. CRL-10782) were obtained from the American Type Culture Collection (ATCC). The T47D ductal, epithelial cancer cell line (cat.no. 85102201) was obtained from the European Collection of Authenticated Cell Cultures (ECACC). The MCF-12A was used as a reference. The RPMI-1640 (Sigma-Aldrich) medium supplemented with 10% foetal bovine serum (Sigma-Aldrich), 100 U. cm^{-3} of penicillin and 100 μg. cm^{-3} of streptomycin (Genos) was used for T47D and MCF-7 cell lines. For the MCF-7 cell line, the

medium was additionally supplemented with 0.01 mg. cm^{-3} of human insulin (Sigma-Aldrich). The MCF-12A cell line was cultured in a mixture of Dulbecco's Modified Eagle's Medium and Ham's F12 medium (1:1) with the addition of 5% horse serum (Sigma-Aldrich), 20 ng. cm^{-3} of human epidermal growth factor, 100 ng. cm^{-3} of cholera toxin, 0.01 mg. cm^{-3} of human insulin, and 500 ng. cm^{-3} of hydrocortisone. A volume of 0.1 cm^3 of each cell line culture was then placed in 96-well polystyrene microplates (NUNC) at a cell density of $2x10^5$ cells per well. After the 24 h incubation (37°C, 5% CO_2, 90% humidity), the growth media were removed and the cells were treated with the PP diluted in the corresponding cell line medium with the addition of 2% of serum. The final concentration of the PP was 0.025 – 0.8 µg dry mass. cm^{-3} (MCF-12A cell line) and 0.1 – 1.4 µg d.m.. cm^{-3} (T47D and MCF-7 cell lines). The cell cultures were incubated for 24 h in standard conditions. The cytotoxicity of the PP was estimated using the MTT method as described earlier in (Ziaja-Sołtys, M. et al. 2020). All experiments were carried out in triplicate. The results were expressed as IC50 (concentration of the tested PP that reduced the absorbance of the studied samples by 50%), after the subtraction of cell control samples and the background originating from the PP.

Statistical analysis. The mean values and standard deviations were calculated for all quantitative experiments using Microsoft Excel software. The statistical processing of MTT data included the Student's t-test with the significance level of $p \leq 0.05$.

RESULTS

Preparative and analytical HPLC. Example chromatograms obtained from the preparative chromatography are presented in the Supplement (Fig. S:1,5,6,10,12,13,17,20). In the case of each ultrafiltrate, organic acids and reducing sugars were eluted at 0 – ~12 min (with DDI water). However, for the qualitative analysis the separation of compounds by the preparative HPLC was poor and analytical chromatography was employed as the next step in the analysis of all ultrafiltrates (Fig. S:2–4, 7–S9, 11, 14–16, 18–19, 21–23). An example chromatogram obtained after the separation of phenolic compounds from the final PP is shown in Fig. 1. The insightful analysis reveals the presence of a number of flavonoids and phenolic acids: kaempferol, (-)-gallocatechin, pelargonidin, quercetin, phloretin, myricetin, phloridzin, delphinidin, petunidin, cyanidin, cyanidin-3-*O*-glucoside, cinnamic acid, *p*-coumaric acid, ferulic acid, gentisic acid, 3-hydroxybenzoic acid, vanillic acid, chlorogenic acid, caffeic acid, gallic acid, protocatechuic acid, and 4-hydroxybenzoic acid.

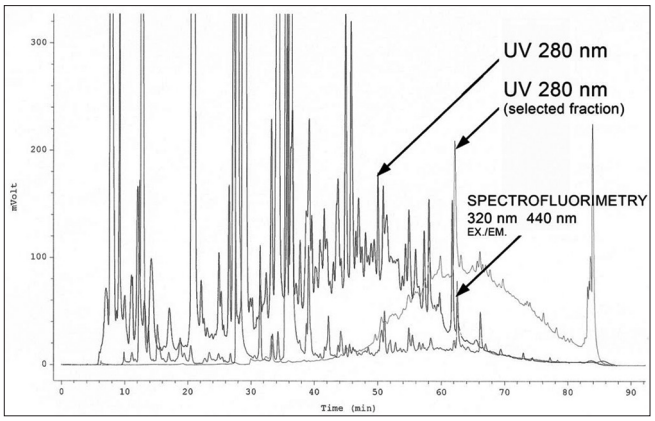

Fig 1. HPLC chromatogram after the separation of phenolic compounds present in PP (UV and spectrofluorimetric detection).

A more detailed analysis (HPLC-MS) allowed the identification of 30 compounds (Table 1) with the highest contribution attributed to cyanidin 3-*O*-glucoside (8.18%), *p*-coumaroyl-glucoside (7.96%), chlorogenic acid (6.35%), citric acid (5.64%), neochlorogenic acid (4.85%) and isoquercetin (1.97%) (Table 2). For the major constituents of the PP clear MS/MS spectra were recorded (Fig. 2). The identification of the individual constituents in the mixture was supported by the analysis of scientific literature and fragmentation patterns of compounds listed in Table 2. Positive mode of operation was used for the determination of flavonoids, anthocyanins and catechins in the sample, and negative mode delivered compositional data on phenolic acids and simple organic acids present in the PP (Table 2).

Activity against cancer and normal breast cells

Our results showed that the PP exhibited dose-dependent cytotoxic effects towards all the tested cell lines. In the case of the reference MCF-12A cell line, the suppressive effect was observed for the PP concentrated at 0.025 – 0.8 µg. cm^{-3} (Fig. 3) and IC_{50} was 0.6 µg. cm^{-3}. The IC_{50} values estimated for the MCF-7 and T47D cell lines (Fig. 4) were higher and reached 1.2 µg. cm^{-3}.

A microscopic analysis of the changes in MCF-12, MCF-7 and T47D cells after incubation with the PP revealed a cytotoxic effect (Fig. 5 B, D, F). Morphological, degenerative changes including increased granularity of the cell surface were observed as a result of cell membrane destruction. The round shape of cells resulted in their lower ability to adhere. In the microscopic image, the reduction in the cell number of cancer T47D and MCF-7 cell lines was observed in comparison to the healthy reference cell line MCF-12A.

DISCUSSION

In the past, many studies have shown that polyphenolic compounds show selective cytotoxicity against breast

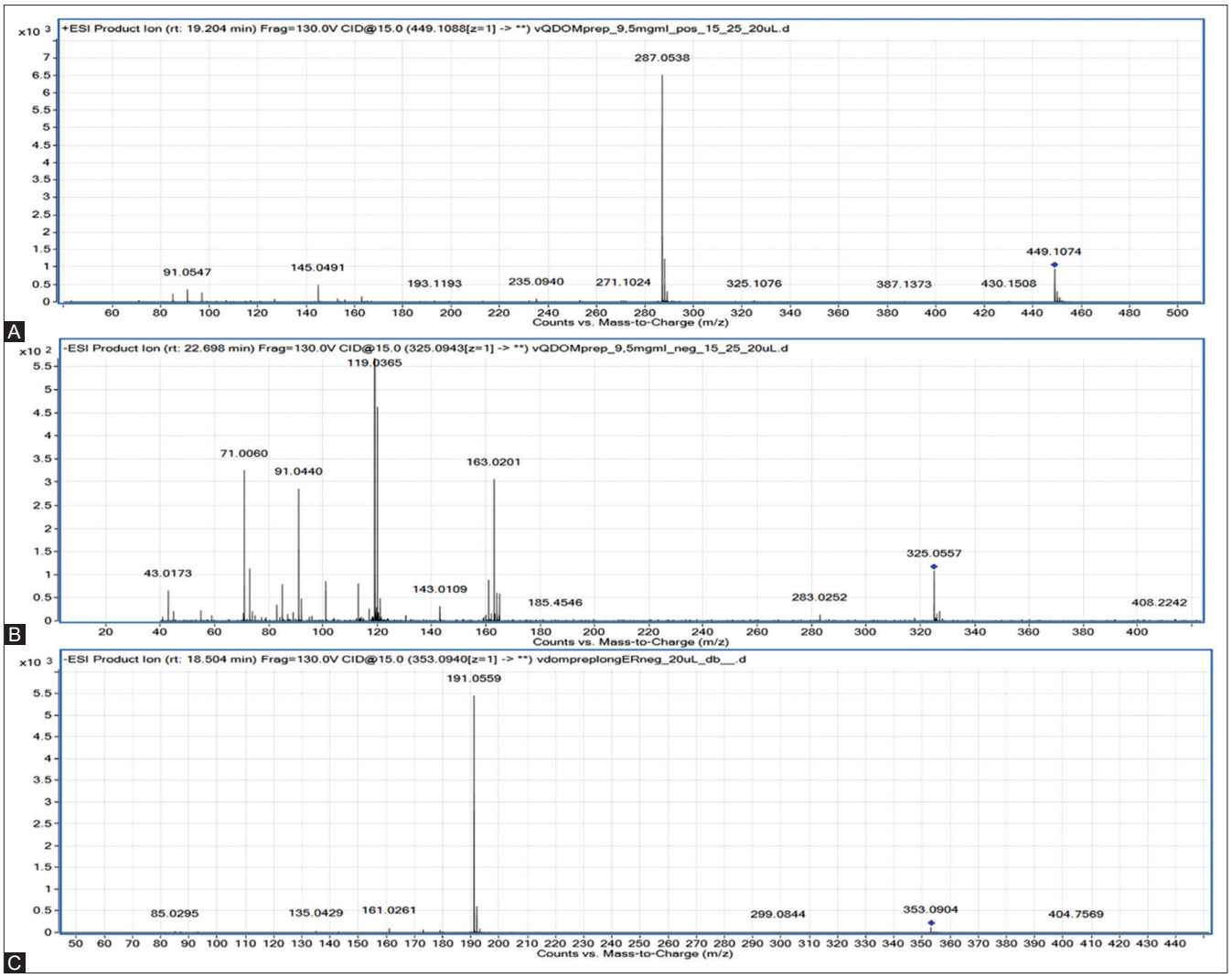

Fig 2. Selected MS/MS spectra of the major constituents present in the PP: (A) cyanidin 3-O-glucoside, (B) *p*-coumaroyl-glucoside, (C) chlorogenic acid.

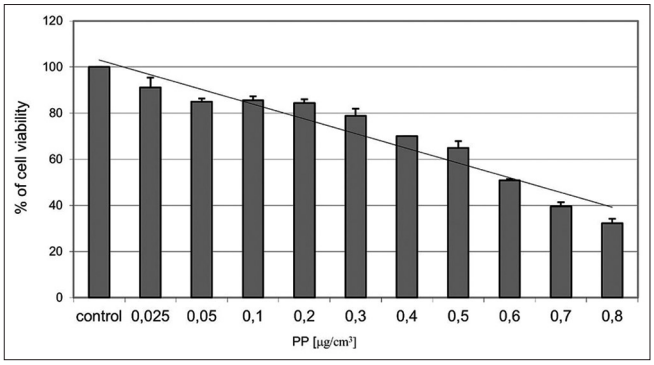

Fig 3. The PP cytotoxicity against MCF-12A cells using MTT assay.

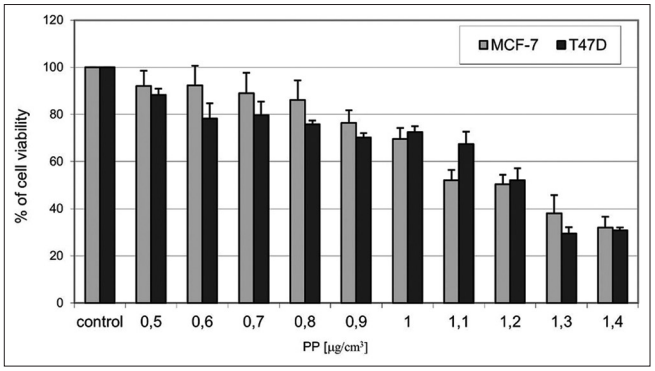

Fig 4. The PP cytotoxicity against MCF-7 and T47D breast cancer cells using MTT assay.

cancer cells. For example, Martel et al. (2016) found that selected polyphenols inhibited the glucose uptake by T47D and MCF-7 breast cancer cells, thus affecting the metabolism and survival of these cells. In addition, some polyphenols appeared to interfere with the availability of lactate in both *in vitro* models. Coumestrol isolated from soybean inhibited the proliferation and induced the apoptosis of MCF-7 cells through induction of the generation of reactive oxygen species and DNA fragmentation, up-regulation of p53/p21, cell cycle arrest at G1/S phase, mitochondrial membrane depolarization

Table 1: Qualitative HPLC-ESI-Q-TOF-MS study of PP (delta – the calculated molecular weight error, DBE – double bond equivalent)

No	*Ion (+/-)*	*Rt (min)*	*Molecular formula*	*m/z experimental*	*m/z calculated*	*Delta (mmu)*	*DBE*	*MS/MS fragments*	*Proposed compound*
1	-	4.8	$C_7H_6O_5$	169.0153	169.0142	-6.19	5.0	-	**Gallic acid**
2	-	5.1	$C_6H_8O_7$	191.0210	191.0197	-6.63	3.0	111, 87, 57	**Citric acid**
3	-	10.8	$C_7H_6O_4$	153.0200	153.0193	-4.34	5.0	109, 91, 65	**Gentisic acid**
4	-	11.1	$C_7H_6O_4$	153.0188	153.0193	3.46	5.0	109, 91, 65, 53	**Protocatechuic acid**
5	-	14.0	$C_{16}H_{18}O_9$	353.0877	353.0878	0.3	8.0	191, 179, 135	**Neochlorogenic acid**
6	-	15.5	$C_7H_6O_3$	137.0245	137.0244	-0.6	5.0	108, 94, 91	***m*-Hydroxybenzoic acid**
7	-	16.5	$C_7H_6O_3$	137.0245	137.0244	-0.6	5.0	120, 107, 93, 65	***p*-Hydroxybenzoic acid**
8	-	18.4	$C_{16}H_{18}O_9$	353.0895	353.0878	-4.78	8.0	191, 161, 135	**Chlorogenic acid**
9	+	18.9	$C_{27}H_{30}O_{16}$	611.1613	611.1607	-1.05	13.0	565, 433, 287, 227, 116	**Kaempferol-3-sophoroside derivative**
10	+	19.2	$C_{21}H_{21}O_{11}$	449.1074	449.1078	0.98	12.0	287, 235, 145	**Cyanidin 3- glucoside**
11	-	19.4	$C_8H_8O_4$	167.0367	167.0350	-10.2	5.0	-	**Vanillic acid**
12	-	20.3	$C_9H_8O_4$	179.0360	179.0350	-5.65	6.0	135, 117, 89	**Caffeic acid**
13	-	23.3	$C_{17}H_{20}O_9$	367.1042	367.1035	-2.02	8.0	249, 191, 173, 93	**4-*O*-feruloylquinic acid**
14	-	23.7	$C_{15}H_{18}O_8$	325.0943	325.0956	-4.32	7.0	163, 119, 91, 71	***p*-Coumaroyl-glucoside**
15	-	24.5	$C_8H_8O_4$	167.0349	167.0350	0.49	5.0	-	**Isovanillic acid**
16	-	24.9	$C_9H_8O_3$	163.0420	163.0401	-11.78	6.0	119, 104, 93	**Coumaric acid**
17	+	25.1	$C_{21}H_{21}O_{10}$	433.1147	433.1129	-4.11	12.0	365, 301, 229, 133	**A quercetin derivative**
18	-	25.5	$C_{18}H_{16}O_8$	359.0737	359.0772	9.83	11.0	197, 153, 135, 89	**Rosmarinic acid**
19	-	26.7	$C_{10}H_{10}O_4$	193.0499	193.0506	3.77	6.0	-	**Ferulic acid**
20	+	27.4	$C_{21}H_{24}O_{10}$	437.1427	437.1442	3.49	10.0	-	**Phloretin 2′-β-D-glucoside** (Phloridzin)
21	-	28.4	$C_7H_6O_3$	137.0241	137.0244	2.3	5.0	108, 93, 75, 65	***o*-Hydroxybenzoic acid derivative**
22	-	28.7	$C_9H_8O_2$	147.0439	147.0452	8.46	6.0	-	**Cinnamic acid**
23	+	30.7	$C_{15}H_{10}O_7$	303.0508	303.0499	-2.88	11.0	285, 257, 229, 165, 153, 137	**Quercetin**
24	+	31.4	$C_{15}H_{11}O_7$	303.0489	303.0499	3.41	11.0	-	**Delphinidin**
25	-	31.7	$C_{11}H_{12}O_5$	223.0633	223.0612	2.58	6.0	-	**Sinapinic acid**
26	+	31.8	$C_{15}H_{10}O_8$	319.0456	319.0448	-2.38	11.0	-	**Myricetin**
27	+	35.5	$C_{21}H_{20}O_{12}$	465.1039	465.1028	-2.47	12.0	333, 303, 257, 165, 127	**Quercetin 3-glucoside (isoquercetin)**
28	+	36.9	$C_{27}H_{30}O_{15}$	595.1613	595.1626	7.48	13.0	435, 398, 223, 287, 147	**Kaempferol 3-rutinoside**
29	+	38.2	$C_{22}H_{23}O_{12}$	479.1175	479.1190	1.89	12.0	-	**Petunidin glucoside**
30	+	43.4	$C_{30}H_{26}O_{12}$	597.1532	579.1497	-6.05	18.0	-	**Procyanidin B2**

Table 2: Quantitative analysis of 12 selected major components of the PP and relevant reference compounds selected for the calculation of their content (SD – standard deviation of the results)

A reference compound	Ion (+/-)	Calibration equation	R^2 value	Compound identified	% content in the sample	SD
Quercetin	+	y = 53,760,657x + 8,171,143	0.9949	A quercetin derivative	0.85	0.064
				Isoquercetin	1.97	0.11
Naringin	+	y=52,263,581x + 637,745	0.9984	Cyanidin 3-*O*-glucoside	8.18	0.38
				Kaempferol rutinoside	0.014	0.001
Citric acid	-	y = 2,465,685x - 67,321	0.9993	Citric acid	5.64	0.48
Chlorogenic acid	-	y = 47,887,804x + 240,274	0.9960	Chlorogenic acid	6.35	0.23
				Neochlorogenic acid	4.85	0.31
Protocatechuic acid	-	y = 31,018,319x + 1,007,975	0.9987	Protocatechuic acid	0.53	0.02
p-Coumaric acid	-	y = 25,723,840x - 872,820	0.9977	*p*-Coumaric acid	0.24	0.007
				p-Coumaroyl-glucoside	7.96	0.51
Gentisic acid	-	y = 16,868,073x - 941,479	0.9996	Gentisic acid	1.053	0.048
Rosmarinic acid	-	y = 336,094,991x + 420,952	0.9993	Rosmarinic acid	1.1	0.08

and activation of caspases 9/3. All these activities led to the promotion of caspase-dependent apoptosis of MCF-7 cells (Zafar et al., 2017). Breast cancer is highly heterogeneous and its molecular subtypes differ greatly in terms of intracellular pathways responsible for cell growth and metastasis. Consequently, there is probably a wide variety of potential molecular targets for polyphenols (Braakhuis et al., 2016). Therefore, it should be expected that the response mechanism of normal and neoplastic breast cells will be different, which can also explain the higher sensitivity of normal MCF-12A cells compared to T47D and MCF-7 neoplastic cells, as observed in our study.

It is known that complex fruit preparations or fruit juices exert significantly higher antiproliferative effect on various types of cancer cells than their respective components applied individually. For example, a polyphenolic extract from cranberries significantly inhibited the proliferation of human breast cancer MCF-7 cells when dosed at 5 – 30 mg. cm^{-3} (Sun and Liu, 2006). Raspberry juice significantly suppressed the growth of human tumour cells: colon HCT-116, breast MCF-7, lung NCI-H460, and gastric AGS (by 50, 24, 54 and 37%, respectively), probably due to the very high anthocyanin content (Bowen-Forbes et al., 2010). Weaver et al. (2009) reported that a 50% reduction in the number of surviving cells was obtained after the application of 4 and 6 μg/ml of the strawberry extract to tumour and normal breast cells, respectively. In the case of MCF-7 cell line tested in the cited study, the IC_{50} value reached 55 μg. cm^{-3}. As follows from our results, the normal MCF-12A cell line turned out to be more sensitive (IC_{50}= 0.6 μg. cm^{-3}) to the tested PP than the T47D and MCF-7 cell lines (IC_{50} = 1.2 μg. cm^{-3}). In the work of Georgiev et al. (2019), MCF-7 and MDA-MB-231 cells turned out to be more sensitive to the extract from *Lycium barbarum* (IC_{50} 60.51 μg. cm^{-3} and 79.93 μg. cm^{-3},

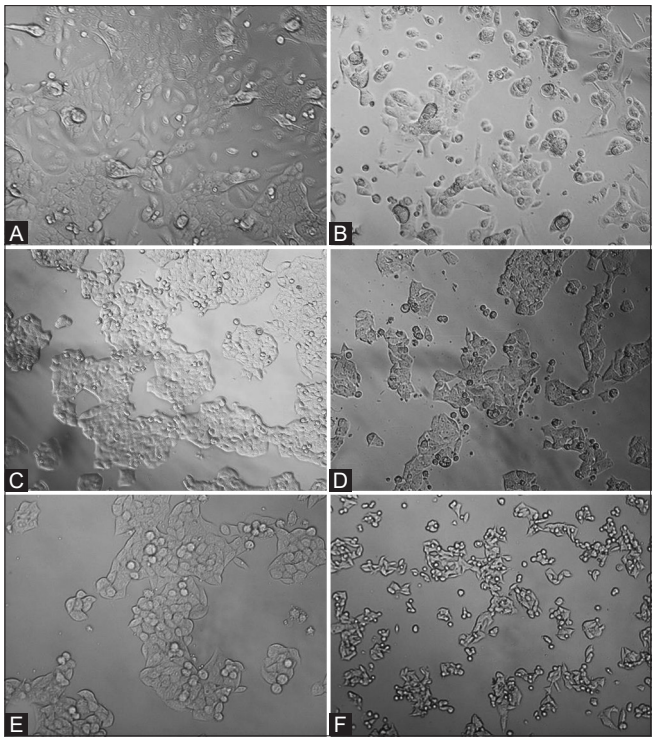

Fig 5. The effect of the PP (at IC_{50}) on cancer and reference cell lines (magnification 200x).A: MCF-12A (control without the PP), B: MCF-12A + PP at 0.6 µg ml-1, C: MCF-7 (control without the PP), D: MCF-7 + PP at 1.1 µg ml-1, E: T47D (control without the PP), F: T47D + PP at 1.2 µg ml-1.

respectively) than the normal MCF-10A cell line (IC_{50} 108.3 µg. cm^{-3}). Polyphenolic extracts from raspberries effectively inhibited the growth of colorectal cancer cell lines HT-29 and SW48 (Flis et al. 2012). Cranberry polyphenolic juice and extract (containing predominantly water-soluble phenolic compounds, apolar phenolic compounds and anthocyanins) effectively inhibited the growth of colon cancer cells HT-29 and LS-513 with IC_{50} in the range of 3.8 – 179.2 µg gallic acid equivalent. cm^{-3} (Dang et al., 2012).

It is well documented that berry phytochemicals can interfere with signalling in which estrogen receptors (ER) are involved. ER play a crucial role in the development of normal mammary glands but also in the development of breast cancer. In a normal mammary gland, ER– positive cells are in the minority and do not proliferate but produce paracrine growth factors which cause signal proliferation in adjacent cells. Many proliferating cells of primary neoplasia are ER–positive and may change growth signalling from paracrine to autocrine. Both tumorigenic and normal cell lines that we used in the study were ER positive. Based on this knowledge, we believe that the paracrine growth factor signalling in MCF-7 and T47D cancer cells was changed into autocrine growth factor signalling. For this reason, the studied preparation was less cytotoxic to MCF-7 and T47D cell lines than to MCF-12A cell line. More than 70% of diagnosed incidents of breast cancer are ER–positive and the ER status is a key criterion determining the choice of the therapeutic method. Moreover, many tumors retain their ER expression after the therapy but alter ER signalling pathway during the recurrence. For this reason, some authors suggested focusing on this pathway during the application of berry polyphenols. These authors claim that this class of compounds can influence the development of primary as well as secondary cancers (Aiyer et al., 2012).

CONCLUSION

Experiments aimed at further analysing the PP are currently still ongoing. Determining the chemical composition ("polyphenolic fingerprinting") of the active fractions (not observed in the case of separated preparations obtained from each fruit) is our present research priority. It is our belief that a major role in this context may be played by synergistic effects between individual compounds originating from different fruits.

Funding

This scientific work was supported by the Ministry of Science and Higher Education of the Republic of Poland (scientific project 2339/B/P01/2010/38) and co-funded from resources of the scientific project POIG 01.01.02-00-061/09.

Ethical approval

The procedures used to carry out the experiments do not require the approval of the Ethics Committee as they did not use human tissue, only commercially available cell lines.

DECLARATIONS OF INTEREST

None

AUTHOR CONTRIBUTIONS

Marta Ziaja-Sołtys: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing - Original Draft, Writing - Review and Editing, Visualization, Supervision

Dominik Szwajgier: Conceptualization, Methodology, Investigation, Resources

Wirginia Kukuła- Koch: Methodology, Investigation, Writing - Review and Editing

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