Chemical profile and biological activity of tart cherry twigs: possibilities of plant waste utilization

PAVLE MAŠKOVIĆ – MARIJA RADOJKOVIĆ – ALEKSANDRA CVETANOVIĆ – MILAN MITIĆ – ZORAN ZEKOVIĆ – SAŠA ĐUROVIĆ

Summary

Fruits of *Prunus cerasus* L. (tart cherry) represent a significant source of health beneficial compounds and have been widely used in food industry. Despite that, other plant parts, such as twigs, have not been investigated. Aim of this study was to investigate chemical profile of twig extracts prepared from plant material of three cultivars. Twig extracts were prepared using maceration and Soxhlet extraction approaches, and investigated regarding phenolics (5.85–14.80 $mg \cdot l^{-1}$) and saccharides (123.38–339.46 $g \cdot l^{-1}$) concentrations using high performance liquid chromatography (HPLC) analysis. Total phenolics (99.34–139.77 $g \cdot kg^{-1}$), flavonoids (14.18–42.88 $g \cdot kg^{-1}$), anthocyanins (76.71–84.97 $g \cdot kg^{-1}$), condensed tannins (52.65–79.76 $g \cdot kg^{-1}$) and gallotannins (19.03–37.23 $g \cdot kg^{-1}$) contents were determined using spectrophotometric analysis. Biological activity was investigated using four antioxidant assays (total antioxidant capacity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, lipid peroxidation assay and hydroxyl radical-scavenging activity). Cytotoxic activity was assessed on three different cell lines (Hep2c, RD and L2OB), while antimicrobial activity was established on 15 bacterial strains. Obtained results confirmed the presence of health beneficial compounds in twigs and indicated the possibility of utilization as a secondary source of those compounds.

Keywords

tart cherry; twig; phenolic profile; saccharide; biological activity

Prunus cerasus L. (sour cherry, tart cherry) represents cherry fruit, which is characterized by its colour, sweetness, sourness and firmness [1]. They are commercially important and known as table fruits [2]. It has been considered that sweetness originates from the presence of glucose and fructose, while sourness is mainly due to the presence of organic acids, in particular malic acid [3, 4]. Besides carbohydrates and organic acids, the fruits contain other active phytochemicals such as phenolic compounds, vitamins (C, B, A, E and K), carotenoids, as well as alkaloid melatonin [5–7]. Among the phenolic compounds, flavonoids and their subclass of anthocyanins are of wide interest regarding potential health benefits. The latter are common components of fruits. Phenolic compounds exhibit a wide range of biological activities [5, 6, 8–11], such as antioxidant, anticancer, anti-inflammatory or antidiabetic [12]. Also, phenolic compounds exhibit antioxidant activity due to their redox properties. Such properties allow them to act as donors of a hydrogen atom, reducing agents and singlet oxygen quenchers [13]. It should be mentioned that this class of compounds possesses chelation potential towards metal ions [14].

The chemical composition of tart cherry fruits makes them healthful edible stone fruits [15]. Previously conducted studies reported presence of different phenolic acids and flavonoids in them: neochlorogenic acid, *p*-coumaric acid, chlorogenic

Pavle Mašković, Department of Agronomy, Faculty of Agronomy, University of Kragujevac, Cara Dušana 34, 32000 Čačak, Serbia.
Marija Radojković, Aleksandra Cvetanović, Zoran Zeković, Department of Biotechnology and Pharmaceutical Engineering, Faculty of Technology, University of Novi Sad, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia.
Milan Mitić, Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia.
Saša Đurović, Institute of General and Physical Chemistry, Studentski trg 12/V, 11158 Belgrade, Serbia.

Correspondence author:
Milan Mitić, tel.: +381 18 53 30 14; fax: +381 18 53 30 14; mobile: +381 63 101 29 05; e-mail: milanmitic83@yahoo.com

acid, quercetin, kaempferol, isorhamnetin, apigenin, catechin and their derivatives [1, 2, 5, 12, 16]. Although much information is available regarding the tart cherry, they are all connected with the chemical composition as well as biological activity of fruits. To our best knowledge, no data are available on tart cherry twigs.

Regarding this fact, the aim of the present study was to investigate composition of tart cherry twigs in order to evaluate the possibility of their utilization in food and/or pharmaceutical industries. To accomplish this task, twigs of three different tart cherry cultivars were processed by extraction using maceration and Soxhlet extraction techniques. Obtained extracts were investigated to establish phenolic profile, contents of glucose and fructose, as well as total phenolics, flavonoids, condensed tannins, gallotannins and anthocyanins contents. Besides that, extracts were evaluated for their antioxidant, cytotoxic and antimicrobial activities.

MATERIALS AND METHODS

Chemicals and reagents

Folin-Ciocalteu reagent, aluminium chloride, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), rutin and potassium iodate were from Sigma Aldrich (St. Louis, Missouri, USA). All standards for high performance liquid chromatography (HPLC) analysis were of analytical grade and were purchased from Sigma Aldrich or Alfa Aesar (Ward Hill, Massachusetts, USA). Acetonitrile and phosphoric acid were of HPLC grade and were obtained from Tedia (Fairfield, Ohio, USA). Ethanol was of analytical grade and was purchased from Sigma-Aldrich. Cirsimarin, resazurin, amaricin, Sabouraud dextrose agar, Tween 80 and *cis*-diaminedichloroplatinum (*cis*-DDP) were purchased from Tedia. All other chemicals and reagents were of analytical reagent grade and were puchased from Sigma Aldrich.

Plant material

Twigs of Oblačinska, Mađarska and Šumadinka tart cherries were collected in Prijevor village, Čačak area, Serbia (43°54'08.8" N / 20°18'22.4" E) during the winter in 2014. Collected plant material was naturally dried in the shade on draft for one month. Dried plant material was grounded in the blender CombiMax 700 (Braun, Frankfurt, Germany) and kept in paper bags before its use.

Extraction procedures

Maceration was conducted using the following procedure: plant samples (10.0 g) were extracted using 96% ethanol (300 ml) as a solvent. The extraction process was carried out under laboratory conditions at a temperature of 22 °C in a sheltered, dry place for seven days, with occasional shaking every day for 5 min to improve the maceration process.

Soxhlet extraction was conducted in the following manner: plant material (75.0 g) was crushed and homogenized into small 3–5 mm pieces by a cylinder crusher and placed in the Soxhlet apparatus. Extraction process was carried out for 8 h using 96% ethanol as a solvent (600 ml).

Obtained extract was filtered through filter paper Whatman No. 1 (Whatman, Maidstone, United Kingdom) and the solvent was evaporated by a rotary evaporator Devarot (Elektromedicina, Ljubljana, Slovenia) under vacuum and dried at 60 °C to the constant weight. The dried extracts were stored in a dark glass bottle at 4 °C to prevent oxidative damage.

All extracts were designated as follows: O1 – macerate of Oblačinska tart cherries; O2 – Soxhlet extract of Oblačinska tart cherries; M1 – macerate of Mađarska tart cherries; M2 – Soxhlet extract of Mađarska tart cherries; S1 – macerate of Šumadinka tart cherries; S2 – Soxhlet extract of Šumadinka tart cherries

Total phenolics, flavonoids, condensed tannins, gallotannins and anthocyanins contents

Total phenolics content (*TPC*) in the extracts was determined by the Folin-Ciocalteu method described previously [17], which involves the reaction of the sample, the Folin-Ciocalteu reagent and saturated solution of sodium carbonate. The absorbance of the reaction mixture at 765 nm was measured by spectrophotometer Agilent 8453 (Agilent Technologies, Santa Clara, California, USA). Results were expressed as grams of gallic acid equivalents (GAE) per kilogram of dry extracts.

Total flavonoids content (*TFC*) was determined using a colorimetric method [18]. Shortly, extracts were diluted and mixed with 5% $NaNO_2$, 10% $AlCl_3 \cdot 6H_2O$ and 1 $mol \cdot l^{-1}$ NaOH solution. Results were expressed as grams of rutin equivalents (RU) per kilogram of dry extracts.

Condensed tannins (*CT*) and gallotannins (*GA*) were determined using the previously described potassium iodate assay [19]. Both results were expressed as grams of GAE per kilogram of dry extract.

Total anthocyanins content (*TAC*) was determined applying the previously described procedure [20, 21] using pH single and differential

methods. Absorbance was measured at 520 nm and 700 nm in pH 1.0 and pH 4.5 buffers. Results were expressed as grams of cyanidin-3-glucoside equivalents (CGE) per kilogram of dry extract.

HPLC analysis of extracts

Phenolic compounds were quantified using a reversed phase HPLC method. The equipment used was an HPLC Agilent-1200 series (Agilent Technologies) with ultraviolet-visible diode array detector (UV-Vis DAD, Agilent Technologies) for multiple wavelength detection. After injecting 5 μl of sample, separation was performed in Eclipse XDB C-18 column (150 mm × 4.6·mm, particle size 5 μm; Agilent Technologies). The column was thermostated at 30 °C, flow was 0.8 ml·min^{-1}. Two solvents were used for the gradient elution: solvent A (deionized water with 5 % formic acid) and solvent B (80 % acetonitrile, 15 % deionized water and 5 % formic acid). The elution program used was as follows: from 0 min to 10 min 0 % B, from 10 min to 28 min gradual increase 0–25 % B, from 28 min to 30 min 25 % B, from 30 min to 35 min gradual increase 25–50 % B, from 35 min to 40 min gradual increase 50–80% B, and finally for the last 5 min gradual decrease 80–0 % of solvent B. All quantifications were carried out with external standards. Calibration curve, coefficient of correlation (R^2), limit of detection (*LOD*) and limit of quantification (*LOQ*) were previously described [22]. Contents of phenolic compounds were expressed as grams per kilogram of dry extract.

Determination of saccharides content was performed using Varian liquid chromatograph (Varian, Palo Alto, California, USA) coupled with refractive index (RI) detector. Column was Zorbax Carbohydrate (150 mm × 4.6 mm, particle size 5 μm; Agilent Technologies). Flow was 1.4 ml·min^{-1}, mobile phase was a mixture of acetonitrile and water (80 : 20, v/v), column temperature was 40 °C, injected volume was 5.0 μl. Results were presented in grams per kilogram of extract.

Assessment of biological activity

Antioxidant activity of previously prepared extracts was assessed using four different assays: total antioxidant capacity [23], lipid peroxidation assay [24], hydroxyl radical-scavenging activity [25] and DPPH radical-scavenging activity [26] with a slight modification [27]. Total antioxidant capacity (*TA*) was expressed as milligrams of ascorbic acid (AA) per kilogram of dry extract. In case of inhibition of lipid peroxidation assay, hydroxyl radical-scavenging activity test as well as DPPH test, the obtained results were expressed as a half maximal inhibitory concentration (ILP_{50}; OH_{50}; IC_{50}, respectively) in milligrams of dry matter per litre of extract.

Cytotoxic activity was determined according the elsewhere described test, using an earlier established 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay [28, 29]. The following cell lines were used: cell line derived from human rhabdomyosarcoma (RD), cell line derived from human cervix carcinoma – HeLa derivative (Hep2c) and cell line derived from murine fibroblast (L2OB), against which activity of obtained extracts were measured. Final results were presented as IC_{50} values, which was defined as the concentration of an agent inhibiting cell survival by 50 %, compared with a vehicle-treated control.

Antibacterial activity was estimated by measuring the minimum inhibitory concentrations (*MIC*). *MIC* values of the extracts and cirsimarin against the test bacteria were determined by microdilution method in 96-well microtitre plates according to the previously described method [30]. The obtained value was taken as the *MIC* for the tested sample and a standard drug.

Statistical analysis

Statistical analysis was carried out using trial version of OriginPro 2015 (OriginLab, Northampton, Massachusetts, USA). For correlation, 2-tailed test of significance was used. Also, statistical analysis was done by one-way analysis of variance (ANOVA), and significant differences between the results for total phenolics, *TPC*, *TFC*, *CT*, *GA* and *TAC* were determined by Duncan's multiple range test. All experiments were performed in triplicate, while results were expressed as mean values ± standard deviation. Levels of significance were as follows: $p < 0.1$; $p < 0.05$ and $p < 0.01$.

RESULTS AND DISCUSSION

Chemical profile of tart cherry twig extracts

Prepared macerate and Soxhlet extracts were analysed in order to establish their phenolics profile, while results are presented in Tab. 1. Presented results showed diversity of phenolic profiles of extracts. In most cases, highest yield of phenolic compounds was observed in macerates. The differences between same twigs might be explained by the application of different extraction techniques. In the case of Soxhlet extraction, fresh amount of solvent soaks the plant material after every exchange, while in the case of maceration, solvent is gradually transported from

Tab. 1. Chemical composition of tart cherry twig extracts.

	Content [g·kg^{-1}]					
	O1	O2	M1	M2	S1	S2
Phenolic compounds						
Protocatechuic acid	ND	ND	ND	ND	ND	ND
p-Hydroxybenzoic acid	0.038	0.049	0.040	0.064	0.056	0.079
Caffeic acid	0.083	ND	ND	ND	ND	ND
Vanillic acid	0.853	0.249	0.683	0.180	0.283	0.174
Chlorogenic acid	1.932	0.372	0.012	0.239	ND	0.134
Syringic acid	ND	ND	ND	0.137	ND	0.134
p-Coumaric acid	0.486	0.137	0.086	0.275	0.351	0.086
Ferulic acid	1.278	0.369	1.414	0.530	0.841	0.430
Sinapic acid	0.192	0.705	0.481	0.178	0.737	0.397
Rutin	1.825	0.903	0.431	0.696	1.145	1.321
Luteolin-glycoside	ND	ND	ND	0.033	ND	0.016
Apigenin-glycoside	0.316	0.103	ND	0.117	ND	0.296
Rosmarinic acid	1.501	0.629	0.757	1.192	0.866	0.350
Quercetin	1.391	0.760	0.872	1.099	1.119	0.671
Luteolin	0.387	0.214	ND	0.152	ND	0.011
Naringenin	3.561	2.607	1.401	0.289	1.845	1.474
Kaempferol	0.202	ND	0.644	0.265	0.287	0.141
Apigenin	0.753	0.645	0.865	0.403	0.776	0.504
Total phenolics	14.798^{a}	7.742^{c}	7.686^{c}	5.849^{d}	8.306^{b}	6.218^{d}
Saccharides						
Glucose	162.11	251.85	166.52	218.96	123.38	140.64
Fructose	62.61	87.61	62.77	–	–	66.53
Total saccharides	224.72^{bc}	339.46^{a}	229.29^{b}	218.96^{c}	123.38^{e}	207.17^{d}
Total phenolics content	99.34^{e}	126.37^{b}	107.87^{d}	139.19^{a}	113.40^{c}	139.77^{a}
Total flavonoids content	14.18^{f}	32.81^{c}	22.57^{e}	36.24^{b}	28.55^{d}	42.88^{a}
Condensed tannins	52.65^{d}	67.11^{c}	54.33^{d}	73.45^{b}	64.84^{c}	79.76^{a}
Gallotannins	19.03^{d}	33.44^{b}	25.14^{c}	34.87^{ba}	27.11^{c}	37.23^{a}
Total anthocyanins content	79.32^{cb}	81.39^{b}	83.42^{ab}	78.77^{cd}	76.71^{d}	84.97^{a}

Different letters in superscript in the same row represent significant differences between samples ($p < 0.05$).
Total phenolics content are expressed as grams of gallic acid equivalents, total flavonoids content are expressed as grams of rutin equivalents, condensed tannins are expressed as grams of gallic acid equivalents, gallotannins are expressed as grams of gallic acid equivalents, total anthocyanins content are expressed as grams of cyanidine-3-glucoside equivalents.
O1 – macerate of Oblačinska tart cherries; O2 – Soxhlet extract of Oblačinska tart cherries; M1 – macerate of Mađarska tart cherries; M2 – Soxhlet extract of Mađarska tart cherries; S1 – macerate of Šumadinka tart cherries; S2 – Soxhlet extract of Šumadinka tart cherries. ND – not detected.

the plant material to the solvent over a certain extent of time. The highest yield was noticed in the case of O1 sample (14.798 mg·l^{-1}) followed by S1 sample (8.306 mg·l^{-1}). Protocatechuic acid was not detected at all, while caffeic acid was noticed only in O1 sample. Chlorogenic acid was quantified in all samples with the exception of S1, while syringic acid was detected in M2 and S2 samples. The same case was with luteolin glycoside, while it was vice versa for luteolin and apigenin glycoside. Kaempferol was not detected in any sample with the oxception of O2 sample. The highest yield was noticed in the case of naringenin in O1 sample followed by the same compound in O2 and S1 samples.

Presence of quercetin, kaempferol and their derivatives, apigenin-glucoside, *p*-coumaric acid and chlorogenic acid in sweet cherry, sour cherry

and tart cherry were in accordance with previous studies [2, 5, 7, 12], although the contents were different (292.6 mg·kg^{-1} and 85.9 mg·kg^{-1} for quercetin and kaempferol, respectively [7]). Chlorogenic acid was previously detected in the range of 5.8–57.7 mg·kg^{-1} [12]. Another study reported a similar range for chlorogenic acid (2.5–57.4 mg·kg^{-1}) [2]. Analysis of 33 cultivars of tart cherries revealed presence of chlorogenic acid in contents of 662.4–5 224.5 mg·kg^{-1}, while apigenin-glucoside was detected in the range of 10.2–119.8 mg·kg^{-1} [5].

Contents of saccharides are presented in Tab. 1. Results showed that glucose was present and dominated in all extracts, while fructose was not detected in M2 and S1 samples. The highest contents of glucose and fructose were determined in O2 sample, where their contents were 251.85 g·kg^{-1} and 87.61 g·kg^{-1}, respectively. The highest total yield of saccharides was in the same sample (339.64 g·kg^{-1}), while the lowest was achieved in S1 sample (123.38 g·kg^{-1}). Presence or absence of different compounds in the plant are known to be influenced by different environmental factors, thus the determined differences might be explained by a combinatorial influence of many factors on plant development.

Results for *TPC, TFC, CT, GA* and *TAC* are presented in Tab. 1. The highest contents were observed in S2 sample. In all cases (except *TAC* for M1), higher contents were observed in Soxhlet extracts. Such results are in contradiction with the results obtained by HPLC-DAD analysis. Results indicated that there were high contents of phytochemicals in twigs, which may be further explored. Comparing our results with the report on *TPC* and *TAC* in 34 cultivars of tart cherry, where *TPC* ranged from 740 mg·kg^{-1} to 7 540 mg·kg^{-1} (expressed as GAE) and *TAC* from 210 mg·kg^{-1} to 2 850 mg·kg^{-1} (expressed as milligrams of malvidin-3-glucoside equivalent) [6], it might be concluded that twigs indeed may represent a significant source of these compounds.

Another research group investigated *TPC* and *TAC* of three different cultivars of tart cherries. They reported *TPC* in a range of 1 621.0–3 120.0 mg·kg^{-1} (expressed as GAE) and *TAC* from 450.0–1 090.0 mg·kg^{-1} (expressed as CGE) of fresh cherry [12]. Different results regarding *TPC* and *TAC* were reported in the literature. Thus, *TPC* was in the range of 2 541.0–4 070.0 mg·kg^{-1} (expressed as GAE) of fresh cherry, while *TAC* ranged from 22.0 mg·kg^{-1} to 1 281.0 mg·kg^{-1} (expressed as CGE) of fresh

Tab. 2. Antioxidant and cytotoxic activity of tart cherry twigs.

Antioxidant activity	*TA* [mg·kg^{-1}]	ILP_{50} [mg·l^{-1}]	OH_{50} [mg·l^{-1}]	IC_{50} [mg·l^{-1}]
O1	114.55 ± 0.75	33.26 ± 0.22	37.55 ± 0.63	52.64 ± 0.72
O2	135.26 ± 0.39	21.54 ± 0.35	22.55 ± 0.18	30.29 ± 0.04
M1	115.32 ± 0.36	30.28 ± 0.06	33.22 ± 0.88	47.38 ± 0.33
M2	141.34 ± 0.67	17.11 ± 0.49	20.09 ± 0.48	26.42 ± 0.22
S1	124.54 ± 0.17	24.70 ± 0.89	27.67 ± 0.70	34.17 ± 0.87
S2	154.40 ± 0.34	14.19 ± 0.55	16.64 ± 0.51	23.30 ± 0.86

Cytotoxic activity	IC_{50} [mg·l^{-1}]		
	Hep2c cells	RD cells	L2OB cells
O1	33.28 ± 0.23	30.40 ± 0.27	32.11 ± 0.98
O2	24.33 ± 0.32	17.59 ± 0.72	22.50 ± 0.33
M1	33.17 ± 0.26	31.83 ± 0.18	29.19 ± 0.14
M2	19.54 ± 0.44	14.09 ± 0.57	17.19 ± 0.52
S1	30.32 ± 0.75	25.77 ± 0.43	21.34 ± 0.40
S2	15.12 ± 0.19	13.36 ± 0.24	13.03 ± 0.16
cis-DDP	0.94 ± 0.55	1.40 ± 0.97	0.72 ± 0.64

O1 – macerate of Oblačinska tart cherries; O2 – Soxhlet extract of Oblačinska tart cherries; M1 – macerate of Mađarska tart cherries; M2 – Soxhlet extract of Mađarska tart cherries; S1 – macerate of Šumadinka tart cherries; S2 – Soxhlet extract of Šumadinka tart cherries.

TA – total antioxidant capacity (expressed as milligrams of ascorbic acid), ILP_{50} – lipid peroxidation activity, OH_{50} – hydroxy radical-scavenging activity, IC_{50} – DPPH radical-scavenging activity, *cis*-DDP – *cis*-diaminedichloroplatinum.

Tab. 3. Antimicrobial activity of tart cherry twig extracts.

Strain	Minimum inhibitory concentration [$\mu g \cdot ml^{-1}$]						
	O1	O2	M1	M2	S1	S2	A
Staphylococcus saprophyticus ATCC 15035	7.81	125.00	62.50	250.00	62.50	250.00	0.24
Staphylococcus aureus ATCC 25923	62.50	250.00	250.00	250.00	15.82	125.00	0.97
Listeria ivanovii ATCC 19119	31.25	250.00	125.00	500.00	31.25	62.50	0.49
Listeria innocua ATCC 33090	125.00	500.00	125.00	125.00	7.81	250.00	0.97
Eneterococcus faecalis ATCC 2912	62.50	250.00	250.00	125.00	15.82	7.81	0.49
Listeria monocytogenes ATCC 19112	125.00	15.82	500.00	62.50	31.25	15.82	0.49
Bacillus subtilis ATCC 6633	125.00	250.00	125.00	250.00	7.81	62.50	0.24
Eneterococcus faecium ATCC 6057	250.00	500.00	62.50	125.00	31.25	125.00	0.97
Escherichia coli ATCC 25922	7.81	125.00	15.82	250.00	62.50	62.50	0.49
Salmonella enterica serovar Enteritidis ATCC 13076	125.00	500.00	250.00	62.50	250.00	250.00	0.97
Enterobacter aerogenes ATCC 13048	62.50	500.00	7.81	250.00	15.82	62.50	0.49
Citrobacter freundii ATCC 43864	125.00	250.00	7.81	31.25	15.82	500.00	0.49
Salmonella enterica serovar Typhimurium ATCC 14028	31.25	500.00	15.82	250.00	7.81	62.50	0.24
Pseudomonas aeroginosa ATCC 27853	125.00	62.50	125.00	62.50	15.82	31.25	0.97
Proteus mirabilis ATCC 35659	31.25	500.00	15.82	250.00	7.81	31.25	0.49

O1 – macerate of Oblačinska tart cherries; O2 – Soxhlet extract of Oblačinska tart cherries; M1 – macerate of Mađarska tart cherries; M2 – Soxhlet extract of Mađarska tart cherries; S1 – macerate of Šumadinka tart cherries; S2 – Soxhlet extract of Šumadinka tart cherries, A – amracin.

cherries [31–34]. Comparing previous results with those reported in this study it might be concluded that twigs may be a significant secondary source of phytochemicals.

Data on antioxidant and cytotoxic activities are given in Tab. 2. Antioxidant activity was determined using four different assays, while cytotoxic activity was investigated on three different cell lines. The results were compared with cytotoxic activity of standard (*cis*-DDP). Observed activities followed the previously described trend [35] for the results in Tab. 3. All tests showed that the highest activity was expressed by S2 sample, while the lowest was noticed for O1 sample. Results for

Tab. 4. Pearson's correlation coefficients among investigated parameters of twig extracts.

Test	*TPC*	*TFC*	*CT*	*GA*	*TAC*	*TA*	ILP_{50}	OH_{50}	IC_{50}	Hep2c	RD	L20B
TPC	1											
TFC	0.9560^{c}	1										
CT	0.9577^{c}	0.9737^{c}	1									
GA	0.9759^{c}	0.9822^{c}	0.9421^{c}	1								
TAC	0.2825^{a}	0.3337^{a}	0.2248^{a}	0.3518^{a}	1							
TA	0.9571^{c}	0.9555^{c}	0.9804^{c}	0.9400^{c}	0.3666^{a}	1						
ILP_{50}	–0.9826^{c}	–0.9858^{c}	–0.9927^{c}	–0.9726^{c}	–0.2503^{a}	–0.9762^{c}	1					
OH_{50}	–0.9777^{c}	–0.9906^{c}	–0.9797^{c}	–0.9885^{c}	–0.2745^{a}	–0.9664^{c}	0.9938^{c}	1				
IC_{50}	–0.9484^{c}	–0.9747^{c}	–0.9727^{c}	–0.9621^{c}	–0.1334^{a}	–0.9311^{c}	0.9809^{c}	0.9869^{c}	1			
Hep2c	–0.9686^{c}	–0.9343^{c}	–0.9644^{c}	–0.9313^{c}	–0.3676^{a}	–0.9922^{c}	0.9670^{c}	0.9517^{c}	0.9071^{b}	1		
RD	–0.9693^{c}	–0.9168^{c}	–0.9506^{c}	–0.9386^{c}	–0.1942^{a}	–0.9604^{c}	0.9622^{c}	0.9601^{c}	0.9405^{c}	0.9665^{c}	1	
L20B	–0.9336^{c}	–0.9733^{c}	–0.9895^{c}	–0.9252^{c}	–0.1869^{a}	–0.9471^{c}	0.9805^{c}	0.9648^{c}	0.9701^{c}	0.9244^{c}	0.9035^{b}	1

Different letters in superscript represent statistical significance (a – at $p < 0.1$, b – at $p < 0.05$, c – at $p < 0.01$).
TPC – total phenolics content, *TFC* – total flavonoids content, *CT* – condensed tannins, *GA* – gallotannins, *TAC* – total anthocyanins content, *TA* – total antioxidant capacity, ILP_{50} – lipid peroxidation activity, OH_{50} – hydroxy radical-scavenging activity, IC_{50} – DPPH radical-scavenging activity, Hep2c – IC_{50} of Hep2c cells, RD – IC_{50} of RD cells, L20B – IC_{50} of L20B cells.

DPPH assay (IC_{50} values, Tab. 2) were quite comparable to the activity expressed by fruit, where methanolic extract of fruit showed IC_{50} value between 12.5 mg·l^{-1} and 25 mg·l^{-1} [13].

Trend of activities and chemical profile of twigs was confirmed by Pearson's correlation test (Tab. 4). According to the coefficients presented in Tab. 4, correlation among *TPC, TFC, CT, GA, TA,* ILP_{50}, OH_{50}, IC_{50} and cytotoxic activity against all three cell lines was particularly high ($r > 0.9$). Such correlation indicated that same class or classes of compounds were responsible for the demonstrated activities. On the other hand, correlation between *TAC* and other investigated parameters was poor ($r < 0.5$).

Regarding the criterion of American National Cancer Institute (Rockville, Maryland, USA) for cytotoxic activity of plant extracts ($IC_{50} < 30$ μg·ml^{-1}) [36], it might be noticed that most of the samples fulfilled this criterion. Even the activities which were above 30 μg·ml^{-1} were close to this value. Such results confirmed the possibility for utilization of twigs in food and/or pharmaceutical industries. Results for antimicrobial activity (Tab. 3) also supported these possibilities. Although activities of samples were quite different, it might be noticed that generally sample O1 showed the highest activity, while sample M2 demonstrated the lowest antimicrobial activity. The highest activity of O1 extract was noticed against *Staphylococcus saprophyticus* and *Escherichia coli*. M1 expressed the strongest influence on *Enterobacter aerogenes* and *Citrobacter freundii*, while *Listeria innocua*, *Bacillus subtilis* subsp. *spizizenii, Salmonella enterica* serovar Typhimurium and *Proteus mirabilis* were quite sensitive to S1 extract. S2 extract was proven to be highly potent against *Enterococcus faecalis*. Comparing to the standard of amracin and previous results [35], all samples investigated in this study expressed high and significant activity.

CONCLUSIONS

Previous studies showed that tart cherry represents significant source of biologically active compounds. It has been also widely used as a food as well as in food industry for preparation of different products such as juices or jams but, at exploitation of cherry fruits, a lot of waste is produced. This study investigated the possibility of plant waste utilization. Results showed that extracts of tart cherry twigs of three cultivars may be exploited as a secondary source of phytochemicals, which are important for human health. Chemical profile proved significant contents of those compounds in extracts from tart cherry twigs. Assays demonstrated high antioxidant, cytotoxic and antimicrobial activities of the prepared extracts, which was positive in particular because the source were twigs that are considered as waste. Results of this study can be of interest for the food or cosmetic industries, where this secondary source of phytochemicals may be utilized.

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