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***J. Nat. Prod. Plant Resour*., 2017, 7(3): 26-39**
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Phenolic Composition, Antimicrobial and Antioxidant Properties of Apple Wood Extracts

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ABSTRACT

Each year, fruit growers have hundreds of tons of bark and pruning waste. Despite their potential for chemical recycling, residues from this waste are frequently used for applications with low added value. By converting the waste stream into a valuable source, this study contributes to a sustainable innovation. Natural phenolic compounds are an alternative to synthetic antioxidants, bacteria or mould inhibitors in food, cosmetics and possibly even in pharmaceutics. Restraining loses due to waste flow is an overall, ecological and economical inspired tendency. The significant secondary flow that exists in the fruit industry can result into a major ecological and economic value for growers. The aim of the present work is the optimization of the solvent extraction of phenolic compounds from apple trees, more specifically bark and core wood. Solvent extractions, with a varying solvent composition, are assessed based on the extraction efficiency of the total polyphenols and flavonoids (spectrophotometry). The extracts are further characterized by their antimicrobial properties and antioxidant activities. Bark extracts obtained by a 40 v/v% acetone/water mixture presented the highest phenolic (22.84 ± 0.56 mg GAE/g DW) and flavonoid (12.16 ± 0.06 mg QC/g DW) content, and antioxidant activity (1.068 ± 0.005 mM FeSO4.7H2O/g DW for the FRAP assay), while the extract also inhibited growth of the gram–positive bacteria Enterococcus faecalis and Staphylococcus aureus by 100%. Core wood extracts obtained by pure water presented the lowest phenolic (3.45 ± 0.09 mg GAE/g DW) and flavonoid (2.19 ± 0.08 mg QC/g DW) content. The results indicate that warm solvent extraction (WSE)

proved to be an efficient way to extract polyphenols with a high reducing ability from bark and core wood of apple trees.

Keywords: Apple wood, Flavonoids, Total polyphenols, Antioxidant activity, Antimicrobial properties

INTRODUCTION

Every year, 6% of the apple plantations in Belgium are renewed. This results in 30 000 ton of wood waste which is used for low added value applications, such as smoke chips, smoke wood, firewood or industrial applications with low added value (paper pulp or energy) [1]. Over the last few years, the recovery of phenolic compounds from wood wastes is getting more attention, especially with the reducing properties which the phenolic compounds entail [2,3]. Although polyphenols are the most abundant secondary metabolites of plants, they do not play an essential role in the plants' primary processes. These polyphenols do, however, act as a natural defence against diseases, or as a natural colour pigment [4,5]. Phenolic compounds are widely distributed in the plant kingdom. They have an aromatic ring with one or more hydroxyl groups and can vary from simple molecules to complex polymers. The main phenolic classes found in plants are simple phenolics, phenolic acids, flavonoids, tannins, stilbenes and lignans [6,7]. The flavonoids are a large class of secondary metabolites that includes more than 5,000 structures [3,8-12]. The classes of the flavan-3-ols, flavonols, dihydrochalcones (flavonoids) and phenolic acids predominate in apples [13]. The most common natural dietary sources of flavonoids are fruit, vegetables and cereals. The use of flavonoids in the food industry can result in improved food stability. Because of their contribution to the total antioxidant activity, flavonoids are interesting compounds to keep the presence of active oxygen species under control [14,15]. Because of their low toxic level and their biological effects, flavonoids are also interesting compounds to use as antioxidant agents in cosmetic preparations. These preparations are essentially based on lipid materials and the addition of antioxidant agents improves the quality of cosmetics because of their ability to protect the lipid phase [9]. Carola et al. [16] have already reported that oxidized and alkylated flavonoid derivatives counter drying out of the skin as well as skin ageing.

The phenolic compositions of apple fruit is fairly well know [14,17,18]. Apples are a source of phenolic compounds, which are responsible for most of the antioxidant activities of this fruit [19,20]. Next to the antioxidant activity, phenolic compounds available in apples are also involved in the natural defence mechanisms against various diseases [11,21] and are therefore of great importance for the apple tree. For instance, high amounts of phloridzin were detected when apple bark was infected with Phytophthora cactorum [22] or when leaves of an apple tree were infected with Venturia inaequalis, which manifests itself as damage to the plant on the surface of leaves or fruit [23]. Moreover, the antimicrobial effect of phloretin against Erwinia amylovora, the fire blight bacterium, has been demonstrated [24].

Next to the study about phenolic composition of apple fruit, a number of studies have been carried out on apple leaves [25-27]. Regarding polyphenols extraction from apple tree woods, only one study for phloretin extraction was published until now. Xü et al. [28] separated and purified phloretin from Fuji apple tree bark. The limited amount of

information on phenolic composition characterization from apple tree wood residues represents an interesting research field.

The objective of this study is to investigate the influence of the solvent and extraction technique on the polyphenol content and the antioxidative activity of apple wood (Malus domestica 'King Jonagold'), more specifically core wood and bark. Malus domestica, is one of the most well-known flavourful fruit trees grown in the world [27]. The bioactive compounds in apple wood are available for solvent extraction because they are present in the porous structure of wood and not bound to cellulose, lignin or hemicellulose, the structural components in wood [2]. The bioactive compounds are associated with these structural components by low-energy intermolecular interactions.

METHODS AND MATERIALS

Materials and chemicals

The apple tree used in this work is found in Haspengouw, a region in the county Limburg, in the northeast of Belgium. The tree used during this study was felled on the 18th of March 2016. The tree, 15 years old, is separated into different parts for further investigation: bark and core wood. After harvesting the apple tree, the wood was shredded and grinded. The grinded wood is dried at 60°C in an oven until constant weight. The dried wood samples are vacuum sealed and stored at -32°C until the time of extraction.

Aluminium chloride, ethanol 99 v/v%, sodium acetate and sodium carbonate (anhydrous) were purchased from Chem-Lab. Acetic acid, 2,4,6-tris(1-pyridyl)-5-triazine (TPTZ), hydrochloric acid, iron(II)sulphate heptahydrate, Folin-Ciocalteu reagent, quercetin, gallic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich. Iron (III) chloride hexahydrate was purchased from Acros. Barium chloride and the solvents for extraction-ethanol, methanol and acetone-were purchased from VWR Chemicals. Potassium acetate was purchased from Merck and sulphuric acid from Fisher chemicals. Ultrapure water was obtained from a Milli-Q System (Millipore). The spectrophotometer used in this study was the UV-1800 model from Shimadzu.

Microbial trains and media

Müller-Hinton (MH) medium (from Becton-Dickinson) and Synthetic complete (SC) medium (from Formedium) were used to grow bacteria and yeast, respectively. A 0.5 McFarland Standard was prepared: 0.5 mL 1% (w/v) barium chloride was added to 99.5 mL of 1% (v/v) cold pure sulphuric acid solution. The following Gram–positive bacteria were tested: Staphylococcus aureus Rosenbach ATCC6538, Bacillus cereus LMG9610, Bacillus subtilis ATCC6051, Enterococcus faecalis HC-1909-5, Micrococcus lysodeikticus ATCC4689, Cellulomonas hominis and Microbacterium luteus. The selected Gram-negative bacteria tested were: Escherichia coli MG1655 ATCC47076, Enterobacter aerogenes ATCC13048, *Acinetobacter baumannii* RUH134, Pseudomonas aeruginosa PAO1, Salmonella enteritidis ATCC13076, *Klebsiella pneumoniae* ATCC13883, *Shigella flexneri* LMG10472, Citrobacter freundii ATCC8090, *Brevundimonas diminuta*, Rhizobium nepotum, *Sphingomonas mucosissima* and

Stenotrophomonas maltophilia. The yeast Candida albicans SC5314 was also tested. The strains were obtained from ATCC or isolated and identified from ecological samples at KU Leuven.

Extraction technique

Different solvents were used for extraction of phenolic compounds from the grinded wood material, which included methanol, ethanol, acetone and water. The solvents were used in the pure form and also in mixtures with water (80%, 60%, 40% and 20% v/v). In general methanol, ethanol, acetone, water and ethyl acetate are used for the extraction of phenolics [29]. Flavonoids are commonly extracted from plant materials with methanol, ethanol, water or their combinations [30]. Phenolic acids are often extracted with ethanol, acetone and chloroform (in different proportions mixed with water) [29,30].

For our extractions, the method described by Meneses et al. [31] was used. This means that 1 g of sample was mixed with 100.0 mL solvent in a 250 mL conical flask, and maintained during 30 minutes on a heating plate at 60°C with magnetic stirring [32]. The extraction did not take place at room temperature but at temperatures near the boiling point of the solvents.

The produced extracts were filtered through filter paper and 0.22 µm membranes (polyvinylideenfluoride membranes were purchased from Phenomenex) and stored at -32°C until analyses. All actions happened in absence of oxygen.

Determination of the moisture content

The moisture content was determined on the raw material by the difference in weight before and after heating at 103°C for 4 hours. After the heating process, the samples were placed in a desiccator for 30 minutes at ambient temperature before weighing.

Total polyphenol content

The total phenolic content was evaluated by a modified Folin-Ciocalteu method [33] described by Dvorakova et al. [34]. The method involves the reduction of Folin-Ciocalteu reagent by phenolic compounds, with the simultaneous formation of a blue complex. The Folin-Ciocalteu reagent is a solution of complex polymeric ions, phosphomolybdic and phosphotungtic heteropoly acids. It oxidizes phenolates, reducing the heteropoly acids to a blue Mo-W complex. The phenolates are only present in alkaline solution but the reagent and products are alkali unstable, therefore a moderate alkalinity and a high reagent concentration are used in the procedure. In a test tube, 1.0 mL of the extract or standard solution was mixed with 5.0 mL of 1:10 diluted Folin-Ciocalteu reagent. After 5 minutes of incubation, 4 mL of sodium carbonate solution (7.5%, w/v) was added and mixed. The mixture was incubated for 2 h at room temperature in the absence of light, and the absorbance was read at 740 nm in a Shimadzu UV-1800 spectrophotometer. The measurement of the extract was compared to a standard curve prepared with a

gallic acid (GA) solution. The total phenolic content was expressed as milligram of GA equivalents per gram of dry matter (DM) of the sample (mg GA/g DM). All measurements were done in triplicate.

Flavonoid content

Flavonoids were measured by a colorimetric method described by Chang [35]. The principle of the aluminium chloride colorimetric method is based on the formation of a stable complex by the aluminium chloride with the C4 keto group and the C3 or C5 hydroxylgroup of the flavones and flavonols.

Five hundred µL of the extract or a standard solution of quercetin at different concentrations (between 0 and 100 µg/mL) were mixed with 1.5 mL of ethanol (80 v/v%), 100 µL 1 mol/L potassium acetate, 2.8 mL distilled water and 100 µL of 10% (w/v) aluminium chloride. This mixture was incubated for 30 minutes at room temperature, after which the absorbance of the mixture was measured at 415 nm with a Shimadzu UV-1800 spectrophotometer. The measurement of the extract was compared to a standard curve prepared with quercetin (QE) solution. The flavonoid content was expressed as milligram of QE equivalent per gram of dry matter (DM) of the sample (mg QE/g DM). All measurements were done in triplicate.

Determination of the antioxidant potential of the extracts

The antioxidant activity of the extracts obtained from the apple tree material was measured by the DPPH radical scavenging activity according to Brand-Williams et al. [36] and the Ferric Reducing Antioxidant Power (FRAP) assay, as described by Benzie and Strain [37].

DPPH-test: DPPH is a stable free radical and is used to test phenolic compounds containing antioxidant potential [31,38]. The extract (150 µL) was added to 2.85 mL of a 1.30×10^{-4} mol/L daily made methanol DPPH solution, and was mixed. After an incubation period of 90 minutes at room temperature in the dark, the decrease in absorbance was determined at 515 nm against a blank. Methanol was used as a blank or control solution. The decrease of the absorbance of a reaction mixture is a measure of antioxidative potential. The radical scavenging activity was expressed as a percentage of inhibition calculated by:
$(1-(AE/A0)) \times 100$, where A0 and AE are the absorbance of the blank solution and the absorbance of the extract, respectively.

The FRAP assay was performed as described by Benzie and Strain [37]. It is an inexpensive and rapid method for determination of the “antioxidant power”. One hundred µL of extract was mixed with 3 mL of FRAP reagent and 300 µL of milli-Q water. This mixture was incubated at 37°C for 15 minutes. After that, the absorbance was determined at 593 nm against a blank prepared with milli-Q water. The FRAP reagent was freshly prepared: 300 mmol/L acetate buffer (pH 3.6); 10 mmol/L TPTZ solution in 40 mmol/L HCl and 20 mmol/L $FeCl_3.6H_2O$ solution in a ratio of 10:1:1 (v/v/v).

Aqueous solutions of $FeSO_4 \cdot 7H_2O$ in a range from 200-1000 μmol/L were used for generating a calibration curve. FRAP values were expressed as mmol/L of ferrous equivalent per gram of dry matter of the material (mM Fe (II)/g DM).

Antimicrobial testing

The growth of a panel of microorganisms was evaluated in a 384-well plate based on CLSI antimicrobial susceptibility testing guidelines [39]. The growth was assessed in the presence of a selection of extracts obtained from bark and core wood extracted with 40 and 60 v/v% ethanol or acetone. The OD630 of each plant extract by itself was measured after 18 or 24 hours incubation in MH broth or SC medium for the bacterial and yeast assay, respectively.

After overnight growth of the bacterial target, the inoculum was diluted with broth to McFarland 0.5 standard (i.e. OD625=0.08-0.10; 1.5×10^8 CFU/mL), followed by another dilution of 150 times (1×10^6 CFU/mL). An aliquot of 85.5 µL (approximately 1×10^5 CFU) was added to the wells of a 384-well plate. For yeast, the cell amounts were adjusted such that approximately 5×10^3 CFU were assayed. To each well, 4.5 µL plant extract was added for a final concentration of 0.5 mg/mL. The plate was closed with BreathSeal (a gas-permeable sealer from Greiner) to permit aeration, and placed in a microplate shaker (VWR, 750 rpm) for 18 or 24 hours at 37°C or 35°C for bacteria or yeast, respectively. The endpoint OD630 was measured in a Multiskan microplate spectrophotometer. The result was expressed as a percentage:

%inhibition=((OD630 (bacteria + plant extract)-OD630 (plant extract))/OD630 (solvent control)) × 100.

Statistical analysis

The results of the spectrophotometric analysis reported in this publication are the average of three measurements, and the coefficients of variations are expressed as the percentage ratio between standard deviations (SD) and the mean values.

Data analysis was carried out with the software IBM SPSS Statistics 23. This software processes, analyses and submits data to statistical tests. During this study the one-way between-groups analysis of variance (ANOVA) is used. One-way analysis of variance involves one independent variable which can assume a number of different levels. The dependent variable is a continuous variable. A significance level, p-value, less than 0.05 ($p<0.05$) was considered statistically significant.

RESULT AND DISCUSSION

The moisture content present in the different parts of apple wood: bark and core wood is 46.9% and 43.5%, respectively. After pre-treatment, the moisture content is lower than 10%. Dry matter for bark and core wood was 100% and 96.3%, respectively.

Effect of the extraction solvent on the polyphenol content, the antioxidant activity and antimicrobial properties of extracts derived from core wood.

Solvent extractions with varying solvent compositions were assessed based on the extraction efficiency of the total polyphenols and the flavonoid content (spectrophotometry). The extracts were assessed for their antioxidant activity, measured by the DPPH and FRAP assay (spectrophotometry). Tables 1 and 2 show the results of the analyses performed on the extracts obtained from core wood. The results indicate that, in general, extracts from core wood obtained with 40% up to 80% v/v acetone contain the highest amounts of total polyphenols and flavonoids. The same extracts also show the highest antioxidant activity, suggesting that the phenolic compounds recovered from this by-product can be linked to the high antioxidant properties. As shown in Figure 1, there is a good correlation between the total polyphenols and the results of the FRAP and DPPH test, with a correlation coefficient of 0.9423 and 0.8836, respectively. Also the correlation between the flavonoid content and reducing power measured by the FRAP and DPPH assay is high (Figure 2) with a correlation coefficient of 0.9007 and 0.9058, respectively. Because of the high correlation between the reducing power and the amount of total polyphenols and flavonoids, the large reducing power could be due to the polyphenols in core wood.

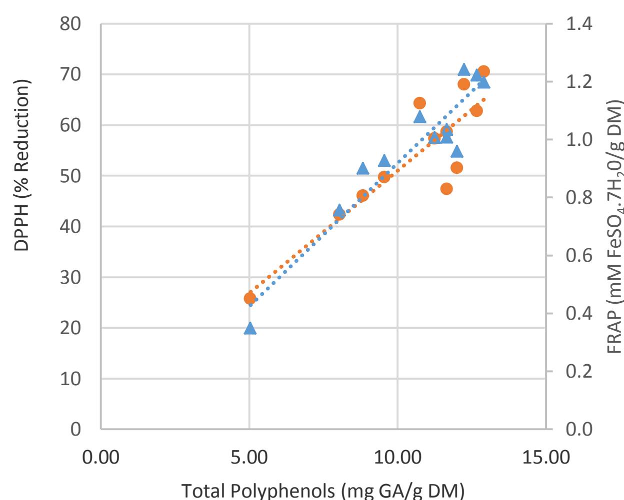

Figure 1: Correlation between the amount of total polyphenols and the reducing power measured by the FRAP (▲) and DPPH (●) test in extracts obtained from apple tree core wood

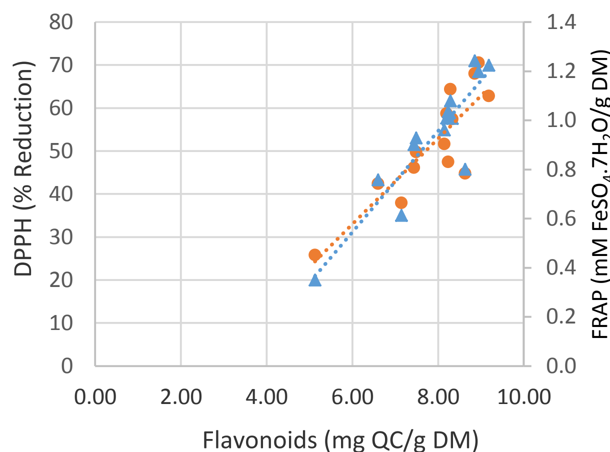

Figure 2: Correlation between the amount of flavonoids and the reducing power measured by the FRAP (▲) and DPPH (●) test in extracts obtained from apple tree core wood

A one-way between-group ANOVA was conducted to explore the impact of the solvent mixtures based on acetone and methanol on the total amount of extracted polyphenols, the content of flavonoids, as well as the FRAP and DPPH value in core wood. There was a significant interaction effect of the solvent mixture on the total amount of polyphenols ($p<0.0001$), flavonoids ($p<0.0001$), FRAP-values ($p<0.0001$) and DPPH values ($p<0.0001$). The one-way between-group ANOVA was not performed for the solvent mixtures based on ethanol because of the lack of homogeneity of variances. The graphs shown in Figures 3 through 6 illustrate the correlation between the independent variable, solvent mixture, on the dependent variable, more specifically total polyphenols, and flavonoids; FRAP value and DPPH value, respectively.

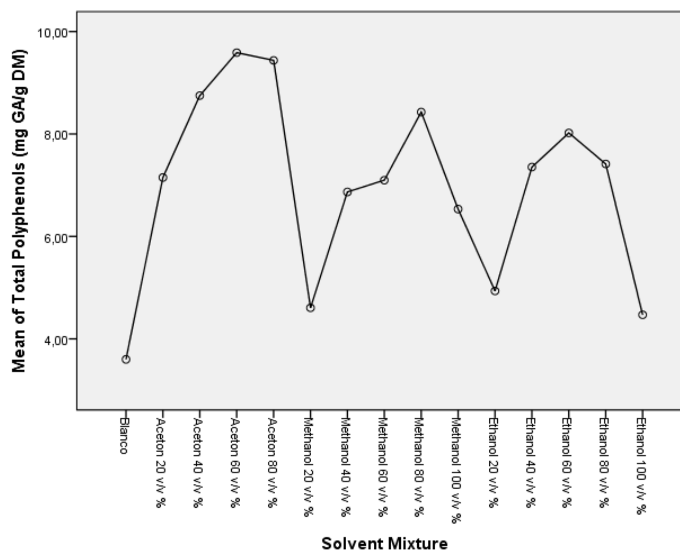

Figure 3: Influence of the solvent mixture on the amount of total polyphenols in extracts obtained from apple tree core wood

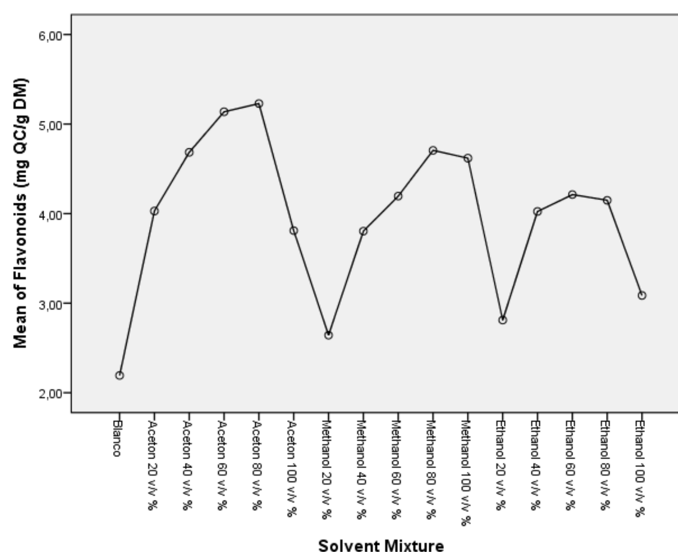

Figure 4: Influence of the solvent mixture on the amount of flavonoids in extracts obtained from apple tree core wood

Figures 3 and 4 suggest that the extracts from core wood obtained with 60% and 80% v/v acetone contain the highest amounts of total polyphenols and flavonoids. It can generally conclude that the extracts obtained with acetone solutions contain the highest levels of polyphenols and flavonoids.

A similar observation can be made concerning the reducing power as shown in Figures 5 and 6. It is worth noting that the level of polyphenols extracted by acetone: water mixtures is more efficient than pure acetone or pure water. Additionally, there is an increase in the amount in polyphenols, flavonoids and FRAP value with an increase of solvent in the mixtures till 60% v/v is reached: this is the case for all the solvents tested. The addition of water to organic solvents such as acetone, methanol or ethanol creates a more polar medium that facilitates the extraction of phenolic compounds [31]. Like shown in Figure 3 through Figure 6, also in Tables 1 and 2, the use of pure water as a solvent is not efficient for extracting these compounds because they are more soluble in solvents less polar than water. This is in agreement with the results obtained in this study.

Table 1: The amount of total polyphenols and flavonoids in the extracts produced by WSE of apple tree core wood using different solvents

Solvent (v/v%)		Total polyphenols (mg GA/g DM)			Flavonoids (mg QC/g DM)		
		Mean	**SD**	**VC (%)**	**Mean**	**SD**	**VC (%)**
Acetone	20	7.15	0.45	6.34	4.03	0.1	2.59
	40	8.75	0.11	1.23	4.68	0.06	1.29
	60	9.59	0.08	0.82	5.14	0.06	1.18
	80	9.44	0.23	2.4	5.23	0.08	1.61
	100	-	-	-	-	-	-
Methanol	20	4.61	0.12	2.62	2.64	0.08	2.98
	40	6.87	0.01	0.21	3.8	0.02	0.43

	60	7.1	0.08	1.08	4.2	0.13	3.05
	80	8.43	0.12	1.42	4.71	0.05	1.07
	100	6.53	0.02	0.26	4.62	0.06	1.29
Ethanol	20	4.94	0.19	3.88	2.81	0.15	5.22
	40	7.35	0.06	0.85	4.02	0.03	0.76
	60	8.02	0.02	0.22	4.21	0.04	0.85
	80	7.41	0.05	0.67	4.15	0.17	4.11
	100	4.47	0.25	5.58	3.81	0.09	2.25
H2O	100	3.45	0.09	2.65	2.19	0.08	3.64

Table 2: The antioxidant capacity determined by FRAP and DPPH of the extracts of apple tree core wood produced by WSE using different solvents

(v/v%)		FRAP (mM FeSO4.7H20/g DM)			DPPH (Reduction (%))		
		Mean	**SD**	**VC (%)**	**Mean**	**SD**	**VC (%)**
Acetone	20	0.643	0.016	2.41	42.45	1.42	3.34
	40	0.849	0.033	3.81	48.32	0.41	0.85
	60	0.836	0.025	2.96	49.79	1.24	2.49
	80	0.813	0.027	3.38	47.42	0.74	1.56
	100	0.22	0.005	2.16	-	-	-
Methanol	20	0.405	0.012	3.04	25.44	0.34	1.33
	40	0.581	0.024	4.08	35.76	0.94	2.64
	60	0.59	0.004	0.69	32.37	0.79	2.43
	80	0.638	0.009	1.44	37.56	0.9	2.4
	100	0.46	0.025	5.44	29.23	0.58	2
Ethanol	20	0.424	0.009	2.1	27.34	2.22	8.12
	40	0.601	0.015	2.42	34.36	0.58	1.67
	60	0.629	0.013	2.07	38.9	3.2	8.22
	80	0.569	0.032	5.68	32.23	0.58	1.79
	100	0.296	0.006	1.99	21.52	0.37	1.7
H2O	100	0.304	0.004	1.44	19.21	0.43	2.26

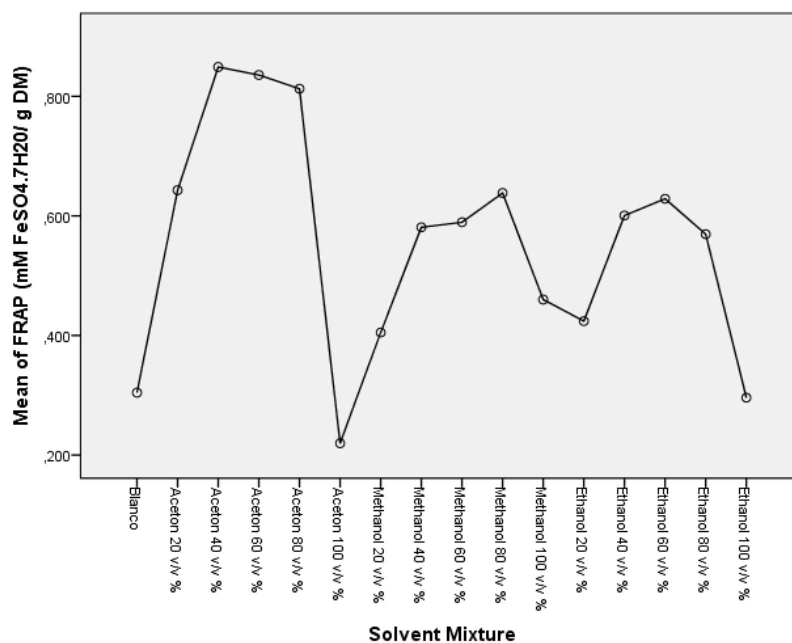

Figure 5: Influence of the solvent mixture on the reducing power determined by FRAP assay in extracts obtained from apple tree core wood

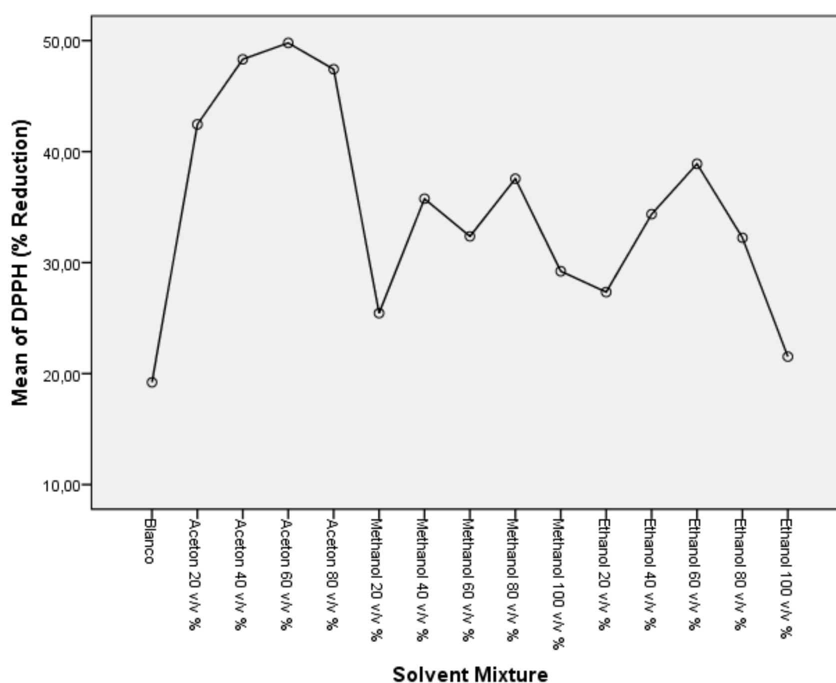

Figure 6: Influence of the solvent mixture on the reducing power determined by the DPPH test in extracts obtained from apple tree core wood

Antimicrobial testing-The extract obtained with 60 v/v% acetone showed inhibition on Enterococcus faecalis with 61% inhibition.

Effect of the extraction solvent on the polyphenol content, the antioxidant activity and antimicrobial properties of extracts derived from bark.

Tables 3 and 4 show the results of the analyses performed on the extracts obtained from bark. Extracts from bark obtained with acetone: water mixtures clearly contain the highest amounts of total polyphenols and flavonoids. Also 60 v/v% ethanol extracts show high amounts of total polyphenols and flavonoids (Table 3, Figures 7 and 8). The acetone extracts show the highest antioxidant activity (Table 4, Figures 9 and 10).

Table 3: The amount of total polyphenols and flavonoids in the extracts of apple tree bark produced by WSE using different solvents

Solvent (v/v%)		Total polyphenols (mg GA/g DM)			Flavonoids (mg QC/g DM)		
		Mean	SD	VC (%)	Mean	SD	VC (%)
Acetone	20	18.9	0.29	1.55	10.97	0.67	6.09
	40	22.8	0.56	2.43	12.16	0.06	0.46
	60	22.2	1.35	6.08	12.07	0.64	5.33
	80	19.3	0.13	0.67	10.35	0.18	1.75
	100	9.84	0.32	3.27	5.17	0.25	4.77
Methanol	20	13.7	0.53	3.88	6.56	0.09	1.31
	40	15.8	0.71	4.52	8.2	0.19	2.37
	60	14.3	0.27	1.88	7.78	0.09	1.13
	80	15.5	0.23	1.49	9.78	0.21	2.13
	100	15.7	0.26	1.65	9.09	0.29	3.2
Ethanol	20	15.1	0.11	0.7	6.89	0.14	2.1
	40	17.3	0.32	1.84	9.13	0.18	1.97
	60	19.8	0.33	1.65	10.57	0.23	2.17
	80	18.1	0.33	1.82	9.53	0.12	1.29
	100	11	0.43	3.87	5.06	0.31	6.04
H20	100	10.2	0.1	0.99	4.58	0.27	5.94

Table 4: The antioxidant capacity determined by FRAP and DPPH of the extracts of apple tree bark produced by WSE using different solvents

(v/v%)		FRAP (mM FeSO4.7H20/g DM)			DPPH (Reduction (%))		
		Mean	SD	VC (%)	Mean	SD	VC (%)
Acetone	20	0.927	0.018	1.96	59.5	0.019	3.13
	40	1.068	0.005	0.43	69.6	0.006	0.81
	60	0.97	0.068	6.97	71.27	0.017	2.41
	80	0.872	0.004	0.49	62.42	0.011	1.76
	100	0.275	0.007	2.59	-	-	-
Methanol	20	0.519	0.025	4.72	43.28	0.006	1.47
	40	0.713	0.037	5.23	51.03	0.005	1.06

	60	0.658	0.021	3.19	48.34	0.007	1.49
	80	0.752	0.038	5.01	56.58	0.012	2.2
	100	0.589	0.019	3.25	-	-	-
Ethanol	20	0.266	0.005	1.86	42	0.004	0.89
	40	0.376	0.018	4.67	54.22	0.006	1.08
	60	0.425	0.009	2.14	61.24	0.008	1.36
	80	0.323	0.005	1.67	53.99	0.011	1.99
	100	0.145	0.008	5.71	29.24	0.003	1.14
H2O	100	0.343	0.02	5.89	32.49	0.007	2.13

The graphs shown in Figures 7 through Figure 10 illustrate the correlation between the independent variable: the solvent mixture, and one dependent variable, more specifically total polyphenols, flavonoids, FRAP value and DPPH value, respectively.

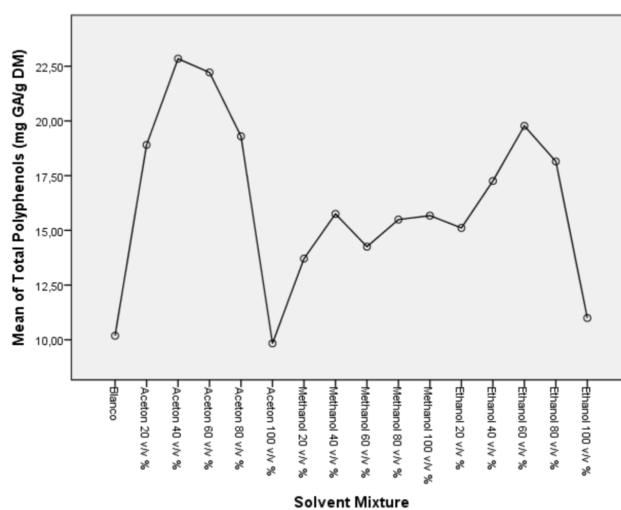

Figure 7: Influence of the solvent mixture on the amount of total polyphenols in extracts obtained from apple tree bark

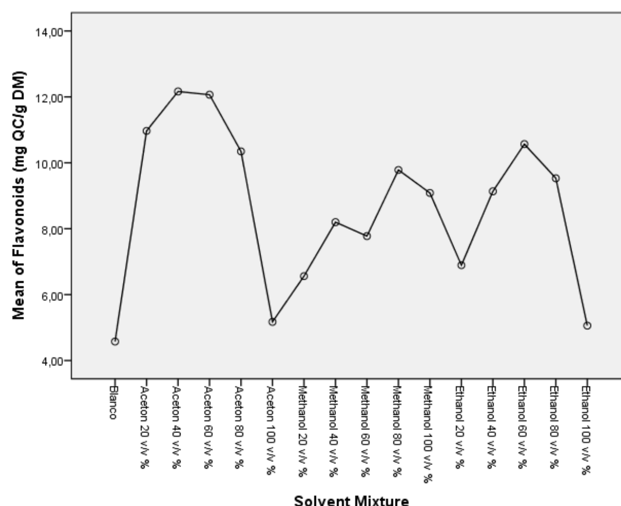

Figure 8: Influence of the solvent mixture on the amount of flavonoids in extracts obtained from apple tree bark

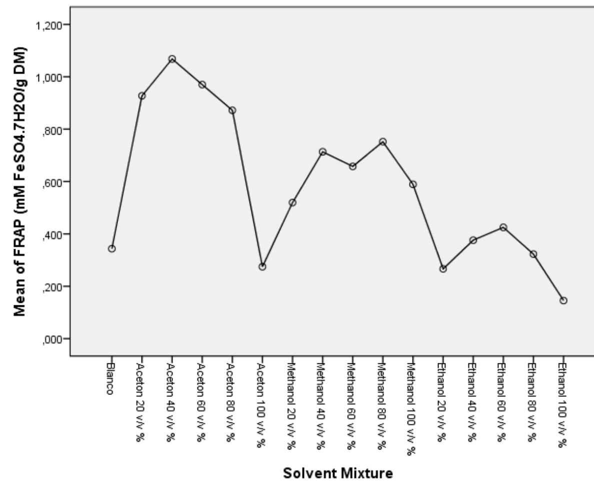

Figure 9: Influence of the solvent mixture on the reducing power determined by FRAP assay in extracts obtained from apple tree bark

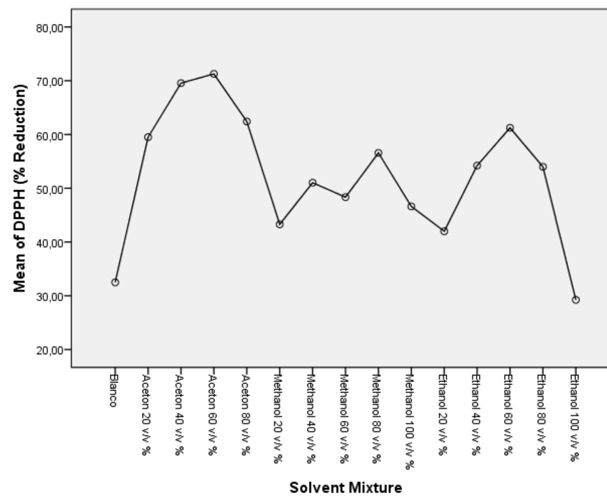

Figure 10: Influence of the solvent mixture on the reducing power determined with DPPH test in extracts obtained from apple tree bark

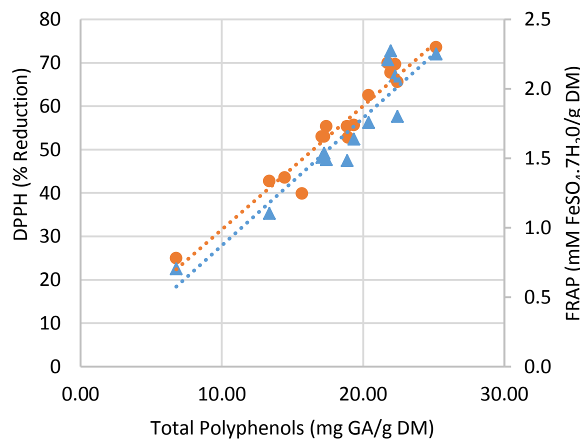

Figure 11: Correlation between the amount of total polyphenols and the reducing power measured by the FRAP (▲) and DPPH (●) test in extracts obtained from apple tree bark

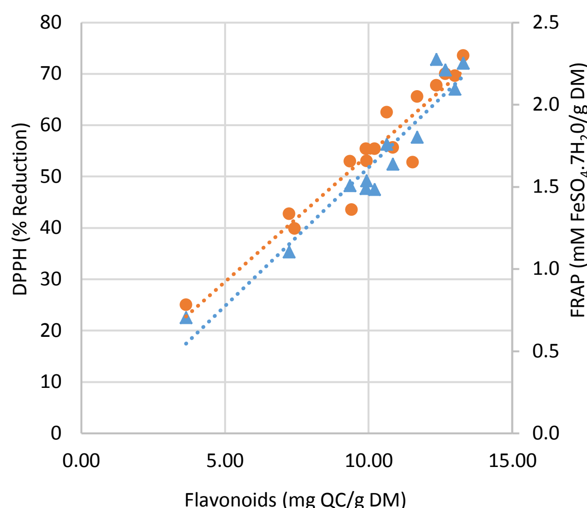

Figure 12: Correlation between the amount of flavonoids and the reducing power measured by the FRAP (▲) and DPPH (●) test in extracts obtained from apple tree bark

A one-way between-group ANOVA was conducted to explore the impact of the solvent mixtures, with exception of acetone 60 v/v%, on the total amount of polyphenols, the content of flavonoids, as well as the FRAP and DPPH value in bark. There was a significant interaction effect of the solvent mixture on the total amount of polyphenols ($p<0.0001$), flavonoids ($p<0.0001$), FRAP-values ($p<0.0001$) and DPPH values ($p<0.0001$).

In analogy with the results obtained on core wood, the highest values of polyphenols and reducing power are found in extracts prepared with acetone solutions.

A comparison between Tables 3 and 4 shows that the highest levels of polyphenols corresponds to the highest FRAP and DPPH values. The same tendency is noticeable in Figures 7 through Figure 10, where the highest amounts of polyphenols are correlated with the highest amounts of flavonoids and reducing power determined by the FRAP and DPPH assay. As shown in Figure 11, there is a strong correlation between the total polyphenols and the FRAP and DPPH assay with a correlation coefficient of 0.9179 and 0.9696, respectively. Also the correlation between the flavonoids and reducing power measured by the FRAP and DPPH assay is high (Figure 12) with a correlation coefficient of 0.9066 and 0.9549, respectively. Following the analysis on core wood, there is a high correlation between the tests to determine the reducing power and the amount of polyphenols. Because of the high correlation a large reducing power could be due to the polyphenols detected in bark.

Antimicrobial testing-the extract obtained with 40% v/v acetone inhibited growth of Pseudomonas aeruginosa, Acinetobacter baumanji, Stenotrophomonas maltophilia, Brevundimonas diminuta and Salmonella enteritidis by 64%, 27%, 32%, 22% and 57%, respectively. The gram-positive bacteria Enterococcus faecalis and Staphylococcus aureus were inhibited by 100%. The same extract inhibited growth of the yeast Candida albicans by 40%. The extract obtained with 60% v/v acetone selectively inhibited growth of Enterococcus faecalis by 68%.

CONCLUSION

Generally, solid-liquid extraction is a simple technique of mass transfer for the recovery of polyphenols. Our study corroborates that this type of extraction, WSE, is an efficient way for the extraction of polyphenols from bark and core wood from the apple tree. The spectrophotometric analysis of extracts derived from both core wood and bark shows the highest amount of total polyphenols, flavonoids and reducing power when the extractions are carried out with acetone: water mixtures. In addition, extractions with acetone: water mixtures show higher extraction efficiency than those conducted with pure acetone or pure water. When comparing the spectrophotometric data for the amount of total polyphenols, flavonoids and reducing power in core wood and bark, the data for bark are generally higher. However, this study shows that apple wood extracts contain a considerable amount of phenolic compounds with antioxidant activity. This creates opportunities to valorize apple wood by-products as natural source of functional substances for further use in different fields like e.g. food, feed and cosmetics.

FUNDING SOURCES

Annick Boeykens is a beneficiary of a PWO (‘Projectmatig Wetenschappelijk Onderzoek’-Project Scientific Research) Grant, provided to Odisee by the Flemish Government, for the investigation project ‘Phenolic compounds in by-products’. The determination of the antimicrobial activity was funded by TETRA project 150158 from VLAIO (‘Vlaams Agentschap Innoveren en Ondernemen’ - Flemish Agency for Innovation and Entrepreneurship). Walter Luyten largely funded himself.

ACKNOWLEDGEMENTS

The authors are grateful to Valerie Van Vooren for the provided language help and writing assistance regarding this paper.

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