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Antioxidative and antibacterial activities of aqueous ethanol extracts of fruits and leaves of berry plants

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Abstract

Phenolic compounds were extracted with food grade solvent of acidified aqueous ethanol from leaves, berries, and branches of Finnish berry plants and analyzed with HPLC-DAD, UPLC-DAD-ESI-MS and NMR. The antioxidant activities of the extracts were evaluated using Folin-Ciocalteau, oxygen radical absorbance capacity (ORAC), DPPH free radical scavenging, and total radical trapping antioxidant parameter (TRAP) assays. The antibacterial activities were investigated against various Gram-negative and Gram-positive foodborne pathogens. Both antioxidative and antimicrobial activities were significantly associated with the total content and the special structure of phenolic compounds in extracts. Generally, Folin-Ciocalteu, ORAC, and TRAP assays were strongly correlated with flavonoids, the antioxidant activity of which was ranked in the order of proanthocyanins > flavan-3-ols > flavonol glycosides. Anthocyanins and non-flavonoid phenolics showed major contribution to DPPH radicals scavenging. Although the antibacterial capacity of phenolics was contributed by some flavonoids, non-flavonoid phenolics showed higher correlation with inhibition against certain bacteria species.

Keywords: Antioxidant, antibacterial, berries, leaves, phenolic compounds

1. Introduction

Phenolic compounds in berry plants have been attracting attentions in the past decades. Asides from sensory properties and cardio-protective effects, these compounds have been confirmed with significant inhibitory activities on oxidants and bacteria, suggesting potential in food protection (Fernandez-Pachon, Villano, Garcia-Parrilla, & Troncoso, 2004; Lee, Kim, Lee, & Lee, 2003).

Acting as natural antioxidants, phenolic compounds were able to scavenge free radicals, donate hydrogen, and chelate metal cations (Heim, Tagliaferro, & Bobilya, 2002). The anti-oxygenation capacity of phenolics contained in berry plants has been evaluated by previous studies. In *Rubus grandifolius* Lowe, berries presented a higher radical scavenger capacity (DPPH and ABTS) than other parts of the plant, mainly due to anthocyanins (Gouveia-Figueiraa, & Castilho, 2015). For blackberry (*Rubus fruticosus*), black raspberry (*Rubus occidentalis*), and blueberry (*Vaccinium myrtillus*), phenolic compounds showed oxygen radical scavenging activities (ORAC) in the free, soluble ester and insoluble-bound forms (Ayoub, de Camargo, & Shahidi, 2016). It is generally believed that antibacterial effect of phenolic compounds depends on cell surface structures of bacteria, substituents in the benzene ring and the length of the saturated side-chain of the phenolic acids (Das, Islam, Marcone, Warriner, & Diarra, 2017). The phenolic compounds from berries, pure compounds

and even berry products have been applied for inhibition against food-relevant bacteria, such as *Bacillus cereus*, *Escherichia coli*, *Salmonella enterica*, and *Lactobacillus rhamnosus* (Das, Islam, Marcone, Warriner, & Diarra, 2017; Salaheen, et al., 2016). Although these previous reports have shown antioxidative and antimicrobial potential of phenolic compounds of various berry species, systematic research is missing to explore the antioxidative and antibacterial activities of food grade extracts rich in phenolic compounds from fruits and leaves of various species and cultivars of berry plants, in order to evaluate their potential as natural antimicrobials and food preservatives.

In our previous research, we characterized the content and profile of phenolic compounds in food grade extracts obtained with acidified aqueous ethanol (ethanol:water:acetic acid, 70:30:1, v/v/v) from berries and leaves of a range of berry species and cultivars (Tian et al., 2017). The content and profile of phenolic compounds vary significantly among different species and cultivars and among different parts of the plant. In this study, the antioxidative and antimicrobial activities of the extracts were evaluated *in vitro*. The aim is to systematically evaluate the potential of food grade extracts from berries and leaves of edible berry species as food preservatives. Bivariate correlation and multivariate analysis were performed to find the correlation between the phenolic profiles and bioactivities of the extracts and to established structure-function relationship of phenolic compounds. Two nettle

leaves are chosen for comparison since it is used as food in the European Union and is generally considered as a food with health benefits.

2. Materials and Method

2.1 Plant materials

Twenty-four samples of berries, leaves and branches were collected in summer and autumn 2013 and stored in a freezer at -20 °C till extraction and analysis. All information of plant materials are listed in **Supplemental Table 1**.

2.2 Chemicals

Reference compounds of gallic acid, (+)-catechin, (-)-epicatechin, glycosylated flavonols (quercetin, myricetin, kaempferol, isorhamnetin, and syringetin), and anthocyanins (glycosides of cyanidin, delphinidin and malvidin) were purchased from Extrasynthese (Genay, France). 5-*O*-caffeoylquinic acid, 3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2, 2'-azobis(2-amidinopropane) dihydrochloride (AAPH), 5-amino-2,3-dihydro-1,4-phthalazinedione (luminol, 97%), gallic acid, fluorescein (98%), 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium chloride, and sodium hydroxide were purchased from Sigma-Aldrich Co. (St. Louis,

USA). Folin-Ciocalteu's phenol reagent, sodium carbonate (Na_2CO_3), monobasic potassium phosphate (KH_2PO_4), and dibasic potassium phosphate (K_2HPO_4) were from Merck Co. (Darmstadt, Germany). Boric acid (H_3BO_3), monobasic sodium phosphate (NaH_2PO_4), and dibasic sodium phosphate (Na_2HPO_4) were purchased from J.T. Baker Co. (Deventer, Holland). A B-type procyanidin dimer was prepared by the Department of Chemistry, University of Turku. Other HPLC and MS grade chemicals, such as ethanol, acetonitrile, formic acid and acetic acid, were purchased from VWR International Oy (Espoo, Finland). The bacterial strains *Staphylococcus aureus* (VTT E-70045), *Listeria monocytogenes* (VTT E-97783), *Bacillus cereus* (VTT E-93143), *Salmonella enterica* sv. Typhimurium (VTT E-95582), and *Escherichia coli* (VTT E-94564) were provided by VTT Technical Research Centre of Finland Ltd (Espoo, Finland).

2.3 Sample extraction and analyses of phenolic compounds

With a solid/liquid ratio of 1:10 (w/v, on a fresh weight basis), 40 mL of aqueous ethanol extracts (ethanol:water:acetic acid, 70:30:1, v/v/v) were prepared from 4 g berries, leaves, and branches of fifteen species of berry plants. Phenolic compounds in each extract were identified by mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR) as reported in the previous publication (Tian et al., 2017). The quantification was carried out with high performance liquid chromatography (HPLC) using an external standard method as

described previously (Tian et al., 2017).

2.4 *In vitro* study on antioxidative activities of extracts and fractions

2.4.1 Folin-Ciocalteau assay

Folin-Ciocalteau assay was performed according to ISO 14502-1 International standard method (Determination of substances characteristic of green and black tea). The extracts were mixed with Folin-Ciocalteau reagent before monitoring the absorption at 765 nm, and total phenolic content was quantified using a standard calibration curve of gallic acid. The results were expressed as gallic acid equivalents (GAE) in milligrams per hundred milliliters of extract.

2.4.2 DPPH free radical scavenging assay

DPPH assay was based on the method of Xie and Schaich with modification (Xie, & Schaich, 2014). DPPH (2,2-diphenyl-1-picrylhydrazyl) solution was dissolved in methanol and mixed with sample solution (methanol as a control), and the absorbance decrease at 515 nm was monitored for 10 min at intervals of 1 second with Ultrospec™ 7000 spectrophotometer (GE Healthcare Life Sciences, Holliston MA). The scavenging activity of DPPH radical was measured at 30 s, 1 min, 2 min and 10 min and calculated as:

$$\% \text{ DPPH scavenging activity} = (1 - [A \text{ sample} / A \text{ control}]) \times 100$$

Where "A sample" is the absorbance of the extract sample, and "A control" is the absorbance of the control.

2.4.3 Oxygen radical absorbance capacity (ORAC) assay

The ORAC procedure was conducted according to the method previously reported by Prior and Ou (Prior et al., 2003; Ou, Hampsch-Woodill, & Prior, 2001). The assay was carried out with a 96-well microplate (Greiner Bio-One, Germany) and a Hidex Sense microplate reader (Hidex, Finland) at 37 °C. For reaction, 20 μL of plant extract dilutions (or Trolox standard), 60 μL of K_2HPO_4-KH_2PO_4 buffer (75 mM, pH 7.4), and 100 μL of fluorescein solution (0.09 μM, in K_2HPO_4-KH_2PO_4 buffer) were pipetted into the wells of a microplate. The AAPH (2, 2'-azobis (2-amidinopropane) dihydrochloride, 70 μL, 300 mM) was applied as hydrophilic initiator, and fluorescence detection (ex.485 nm/em.535 nm) was recorded for 30 min. The results were calculated with the curve of relative fluorescence intensity, and expressed as Trolox equivalents (TE mg/ 100 mL).

2.4.4 Total radical trapping antioxidant parameter (TRAP) assay

TRAP assay was estimated using a Hidex Sense Microplate Reader (Hidex, Finland), coupled with 96-well microplate (Thermo Scientific, Finland). 10 μL of diluted sample was added into 140 μL of incubated AAPH (2, 2'-azobis (2-amidinopropane) dihydrochloride)–luminal

solution, consisting of NaH_2PO_4–Na_2HPO_4 buffer (pH 7.4, 0.1 M, 115 µL, in 0.9% NaCl solution), luminal solution (300 µM, 25 µL, in 0.1 M boric acid), and AAPH solution (300 mM, 10 µL, in NaH_2PO_4–Na_2HPO_4 buffer). After measurements at 37 °C for 60-70 min, the final results of extracts were presented as Trolox equivalent (TE mg/ 100 mL).

2.5 Study on antibacterial activities of the extracts

The antibacterial activities of the extracts were studied on *Escherichia coli* VTT E-94564, *Staphylococcus aureus* VTT E-70045, *Listeria monocytogenes* VTT E-97783, *Bacillus cereus* VTT E-93143 and *Salmonella enterica* sv. Typhimurium VTT E-95582 obtained from VTT Culture Collection. The growth of the target microbes and the antimicrobial efficacy of the extracts were monitored with a Bioscreen™ (Thermo Scientific, Finland) automated turbidometer and the Research Express software (Transgalactic Ltd, Finland). Briefly, the bacterial cells were grown at 37 °C overnight in Iso Sensitest Broth (Oxoid, UK) and diluted and inoculated to 10^5 cells per well (Alakomi et al., 2007). The extracts were resuspended into sterile Milli Q-water in the same volume after evaporation of the solvent. Two concentrations of each extract were examined (10 and 20 µl per well) with a total volume of 300 µl in the well. Target microbes were grown for 48 hours at 37 °C and optical density monitored with a wide band filter at 30 min intervals. Area under growth curve was calculated, and growth inhibition% (compared to the growth of control without additions of

extracts) was calculated for each sample. Each extract was examined in quadruplicates.

2.6 Statistical Analyses

All results were expressed as mean ± standard deviation (SD) using Microsoft Excel 2010 (Microsoft Corp., WA, US) and Origin Lab 8.0 software (OriginLab Corp., MA, US). To establish correlation between phenolic composition and bioactivities, bivariate Pearson correlation analysis was performed using a two-tailed test with IBM SPSS Statistics 24 for Windows (SPSS Inc., NY, US) and multivariate correlation was conducted by partial least squares regression (PLS) using Unscrambler 10.1 (Camo Process AS, Oslo, Norway). In the PLS method, the predictors (variable X) were the concentration of phenolic compounds, the responses (variable Y) being the bioactivities.

3. Results and Discussion

3.1 Phenolic compounds in extracts analyzed by NMR, UPLC-MS and HPLC-DAD

Various profiles of phenolic compounds are found in the extracts of Finnish berry plants, the detail information of which has been reported by our previous research (Tian et al., 2017). The HPLC chromatograms of the extracts are present in **Supplemental Fig. 1** showing highly diversified profiles of phenolic compounds among the extracts. The concentration of phenolics in the extracts is shown in **Table 1** and **Supplemental Table 2**.

Leaf and branch extracts. In **Table 1**, leaf extracts were quantified as abundant sources of different groups of phenolics. Two most commonly found flavan-3-ols, (+)-catechin and (-)-epicatechin, were present at the highest level in lingonberry (*Vaccinium vitis-idaea*) leaf extract (118 mg/100 mL). The extracts of two sea buckthorn (*Hippophaë rhamnoides* ssp.) leaves also contained high levels of flavan-3-ols ranging from 22 to 26 mg/100 mL, followed by hawthorn leaf (19 mg/100 mL), the latter being known as species rich in these compounds (Chai et al., 2013). Proanthocyanidins, primarily as procyanidin dimers and trimers, were richer in the extracts of lingonberry leaf (85 mg/100 mL), hawthorn (*Crataegus* spp.), leaf (24), and saskatoon (*Amelanchier alnifolia*) leaf (23). Eight ellagitannins were identified as galloyl glucose or hexahydroxydiphenoic acid (HHDP) esters of glucose. In sea buckthorn leaf extracts, seven ellagitannins accounted for over 90% of total content of phenolics. The major phenolic acids in Finnish berry plants have been reported to be esters of hydroxycinnamic acids (Kylli, 2011). In bilberry (*Vaccinium myrtillus*) leaf extract, hydroxycinnamic acid derivatives represented 82% of the total content of phenolics, mostly as 3-*O*-caffeoylquinic acid. Other hydroxycinnamic acids (coumaric acid, caffeic acid and ferulic acid) were identified as esters of acids or hexoses in some extracts. Flavonols in leaf extracts were present mainly as 3-*O*-glycosides or acylated 3-*O*-glycosides of quercetin, isorhamnetin, and kaempferol (**Supplemental Table 2**). The extracts rich in glycosylated

flavonol glycosides were from lingonberry leaf (100 mg/100 mL), raspberry (*Rubus idaeus*) leaf (69), saskatoon leaves (67), and red currant (*Ribes rubrum 'Red Dutch'*) leaves (52). For flavones, the hawthorn leaf extract contained *C*-glycosides of apigenin and luteolin at a total level of 16 mg/100 mL extract, which was in accordance with the report of a previous study (Kirakosyan, Seymour, Kaufman, Warber, Bolling, & Chang, 2003). A trace quantity of flavanones (eriodictyol 7-*O*-glucoside) was detected only in the extract of saskatoon branches. In addition, other phenolic compounds were also quantified, such as *β*-*p*-arbutin accounting for 44% of total phenolics in the lingonberry leaf extract (Tian et al., 2017).

Berry extracts. Compared to the extracts from leaves and branches, the berry extracts had simpler composition of phenolics (**Table 1**). Anthocyanins were the major phenolic compounds in dark-skinned berries, mostly present as 3-*O*-glycosides of cyanidin, delphinidin, peonidins, petunidin and malvidin (**Supplemental Table 2**) (Tian et al., 2017). Anthocyanins accounted for 95% of total phenolics in bilberry, 89% in black currant (*Ribes nigrum 'Mortti'*) press cake, 81% in crowberry (*Empetrum nigrum*), and 57% in chokeberry (*Aronia melonocarpa*). Higher levels of phenolic acid derivatives were found in the extracts of saskatoon berry (27 mg/100 mL), chokeberry (25), rowanberry (*Sorbus aucuparia*) (24), and lingonberry (21). Compared with others, 1-*O*-benzoyl-*β*-glucose was the main derivative of phenolic acid in lingonberry extract, whereas caffeoylquinic acids dominated in others.

The lingonberry extract was also rich in flavan-3-ols and proanthocyanidins (**Table 1**). Flavonols in the berry extracts represented a low concentration ranging from 3 to 9 mg/ 100 mL. The major flavonol aglycones were quercetin and isorhamnetin; however, in certain extracts, glycosides of myricetin, laricitrin, and syringetin were also found in trace amounts (**Supplemental Table 2**).

3.2 Antioxidative activities of phenolic compounds in extracts

The antioxidative activities of the extracts were evaluated in four different assays. Folin-Ciocalteau and DPPH assays associated with delivering single-electron (ET); ORAC and TRAP evaluations were based on hydrogen atom transfer (HAT) (Badarinath, Mallikarjuna Rao, Madhu Sudhana Chetty, Ramkanth, Rajan, & Gnanaprakash, 2010). The results of antioxidant activities were present in **Table 2**.

3.2.1 Folin-Ciocalteau assay

Folin-Ciocalteau assay is widely applied to estimate total phenols in the samples. The mechanism is to test any compounds with reducing hydroxyl group (-OH), causing some differences compared to HPLC analysis results. Nevertheless, the Folin-Ciocalteau results were generally in accordance with total concentration of phenolics determined with HPLC-DAD. As shown in **Table 2**, higher value of Folin-Ciocalteau was found in the extracts from

leaves than in the extract from berries and branches, the highest level (860 GAE mg/100 mL) found in the lingonberry leaf extract. In the two leaf extracts of sea buckthorn, the value ranged from 407 to 453 GAE mg/100 mL. Among the berry extracts, chokeberry extract showed strongest activity of electron-transferring (105 GAE mg/100 mL), and the lowest activity was present in sea buckthorn berry extracts (21-25 GAE mg/100 mL).

3.2.2 DPPH assay

To stimulate the reaction between antioxidant and unstable radicals (such as HO•, HOO•, and NO•), Reşat Apak et al. (2013) suggest that DPPH reaction is preferably recorded over 4 min but no more than 6-10 min (Apak, Gorinstein, Böhm, Schaich, Özyürek, & Güçlü, 2013). Our study showed that the leaf extracts were more active in trapping DPPH radicals than the berry extracts. Within 10 min, all the leaf extracts succeeded to capture over 80% of DPPH radicals except the extracts from chokeberry leaves (60%) and nettle leaves (8-25%). The extracts of sea buckthorn leaves were surprisingly active, trapping approximately 90% of DPPH radicals during the first 30 seconds. In contrast with other extracts, the trapping rate of the extracts of sea buckthorn leaves was increased within 1 min, and then became steady until 10 min with 95 % of DPPH radicals scavenged in total. The same trend was also observed in the extracts of sea buckthorn berries, although the radical scavenging capacity was significantly lower (23-30%). The DPPH radical scavenging capacity of the berry extracts varied among species

and cultivars, ranging 30% to 80% at the end point of the 10 min. For saskatoon, the branch extract was equally effective as the berry extract in quenching DPPH radicals.

3.2.3 ORAC assay

In ORAC assay, overall, the leaf extracts showed higher peroxyl-radical scavenging capacities than the berry extracts, probably due to the higher phenolic concentration (**Table 2**). The lingonberry leaf extract had an extremely high ORAC activity (4627 TE mg/100 mL) which was three or four times stronger than the following leaf extracts: hawthorn (1427), bilberry (1213), saskatoon (1015). The antioxidant ability of the extract of saskatoon branches (697 TE mg/100 mL) was between the activity of the extracts from the corresponding leaves (1015) and berries (365). Among the berry extracts, chokeberry (464 TE mg/100 mL) and lingonberry (420) extracts showed the highest ORAC values, whereas the lowest was found in two extracts of sea buckthorn berries (101-130 TE mg/100mL). Compared to the extracts of berries, leaves and branches, the nettle leaf extracts showed lower peroxyl-radical scavenging capacity, especially in leaves collected in October (74 TE mg/100 mL).

3.2.4 TRAP assay

TRAP measurement showed similar results with ORAC assay (**Table 2**). The extract of

lingonberry leaf exhibited the best ability to donate hydrogen, based on the highest TRAP value of 1077 TE mg/100 mL among the extracts studied. Other potent hydrogen donators were confirmed as the leaf extracts of bilberry (648 TE mg/100 mL), hawthorn (613), sea buckthorn (Terhi, 549) and saskatoon (424). Two berry extracts of sea buckthorn presented the lowest TRAP activity (19-24 TE mg/100 mL), which might be explained by the lower content of total phenolics and the lack of anthocyanins. Petko Denev et al. (2010) evaluated antioxidant properties of solid-phase extracted anthocyanins from chokeberry, elderberry (*Sambucus nigra*), black currant, blackberry and blueberry. Chokeberry anthocyanins showed the highest TRAP value, which is in agreement with our study (Denev, Ciz, Ambrozova, Lojek, Yanakieva, & Kratchanov, 2010).

3.2.5 Correlation among phenolic compounds and antioxidant activities

3.2.5.1 Bivariate Pearson's correlation

Pearson's correlation was applied to measure the linear relationship between different groups of phenolics and antioxidant activities. Higher correlation coefficient values suggested more contribution of phenolic compounds to antioxidant capacity of the extracts. In **Supplemental table 3**, the total phenolics were calculated as the sum of concentration of each phenolic compound quantified by HPLC-DAD. Correlated very strongly with the antioxidative activities measured by Folin-Ciocalteau assay (**Fig. 1a**), the total phenolic content showed

somewhat weaker correlation with antioxidative actitivities measured in other assays (**Fig. 1b&c**), suggesting the importance of specific profile of phenolic compounds for the activities measured in these assays. Also, some non-phenolic compounds might have also contributed to the antioxidant capacity in Folin-Ciocalteau assays. Flavonoids represented stronger correlation with Folin-Ciocalteu (R = 0.888, p = 0.01), ORAC (R = 0.961, p = 0.01), and TRAP (R = 0.835, p = 0.01) assays than the total content of phenolic compounds indicating less activities of non-flavonoid phenolic compounds (ellagitannins, phenolic acids and other phenolics) (**Fig. 1d, e&f**). Among flavonoids, the order of correlation coefficients with Folin-Ciocalteu, ORAC, and TRAP was proanthocyanidins (mainly as procyanidin dimers and trimers, **Fig. 1g, h&i**) > flavan-3-ols (catechin and epicatechin, **Fig. 1j, k&l**) > flavonols (glycosides of quercetin) (**Fig. 1m, n&o**). Significantly high correlation was also found between isorhamnetin glycosides and DPPH assays. The difference in antioxidant capacity among different phenolics has been discussed in some previous studies. Based on the data of pure reference compounds measured by TEAC, FRAP and hypochlorite scavenging, Soobrattee et al. (2005) ranked the antioxidant activity in the order of procyanidin dimer > flavan-3-ols > flavonols > hydroxycinnamic acids > simple phenolic acids (such as gallic acid and ellagic acid) (Soobrattee, Neergheen, Luximon-Ramma, Aruoma, & Bahorun, 2005). Gangopadhyay and co-workers (2016) evaluated phenolic fractions of barley (*Hordeum vulgare*) grain with DPPH, FRAP, and ORAC assays, and confirmed B-type procyanidin

dimers and flavan-3-ols as the strongest antioxidants followed by quercetins and ferulic acids (Gangopadhyay, Rai, Brunton, Gallagher, & Hossain, 2016). According to structure-activity relationship (SAR) of polyphenols, three essential structural features affect antioxidant properties of flavonoids: a catechol group (ortho-dihydroxyl group) in the B ring, a C_2–C_3 double bond conjugated with 4-oxo group, and hydroxyl groups in C_3 and C_5 of C ring. The antioxidant activity of flavan-3-ols was attributed to the catechol group in the B ring and C_3-OH in C ring. As oligomers and polymers of flavan-3-ols, proanthocyanidins exhibit higher radical scavenging capacity owing to the presence of more catechol groups, coupled with C_3-OH and C_4-C_8 linkage (Heim, Tagliaferro, & Bobilya, 2002). The effect of polymerization on antioxidative activities may vary depending on the antioxidative assays used (Lotito et al., 2006). Flavonols contain a C_2-C_3 double bond and a 4-oxo group, but *O*-glycosylation in C_3 will interfere the planarity of rings, leading to suppression of antioxidant capacity (Balasundram, Sundram, & Samman, 2006).

Although considerably high antioxidant capacities were found in berry extracts, no significant bivariate correlations between total content of anthocyanins and antioxidant activities were established, except for DPPH scavenging activities (R = 0.778-0.802, n = 8, p = 0.05) (**Supplemental table 3**). On the contrary, cyanidin glycosides showed significant correlations with Folin-Ciocalteu (R = 0.763, p = 0.05) and ORAC (R = 0.751, p = 0.05) assays. This

result can be explained by the different profiles of anthocyanins, especially the different structures of anthocyanidins, in berry extracts. Also, the antioxidant property of anthocyanins might be interfered by the structural rearrangement from flavylium cation to carbinol pseudo-base responding to increase in pH from acidic extract to neutral buffer reaction media (Clifford, 2000). Previously, Feng et al. (2016) characterized anthocyanins (mainly as cyanidin glycosides) in Chinese wild berries and pointed out no significant bivariate correlation between total anthocyanins and the antioxidant activity measured by DPPH and FRAP assays, whereas Wang et al. (2014) confirmed total anthocyanins in *Vaccinium uliginosum* berry (mainly as 3-*O*-glucosides of delphinidin, petunidin and malvidin) had strong correlations with antioxidant activities measured in DPPH, ABTS (2,2' – azinobis-(3-ethyl-benzothiazoline-6-sulphonic acid)) and FRAP assays (Feng et al., 2016; Wang et al., 2014).

As shown in **Supplemental table 3**, the content of non-flavonoid phenolics (ellagitannins, phenolic acids and other phenolics) exhibited significant correlation (R = 0.682-0.839, p = 0.01) with DPPH radical-scavenging activity. The antioxidative activities of hydroxycinnamic acids and hydroxybenzoic acids were associated with the numbers of hydroxyl groups and their positions relative to the carboxyl functional group, such as -COOH and -CH=CH-COOH (Rice-Evans, Miller, & Paganga, 1996). Despite the weaker antioxidant ability than

the flavonoids, phenolic acid derivatives should be taken in to account due to their abundance in the extracts. Overall, there was only a moderate correlations between the total content of phenolic acid derivatives and the antioxidative activities in TRAP assays (R = 0.520, p = 0.05); however, with higher level of 3-*O*-caffeolquinic acid, the bilberry leaf extract showed more potent ability of transferring both hydrogen and electron shown by ORAC and DPPH assays. Aside from caffeoylquinic acids, the content of derivatives of other phenolic acids surprisingly represented strong correlations with Folin-Ciocalteu (R = 0.672, p = 0.05) and ORAC assays (R = 0.707, p = 0.05). Ellagitannins containing more hydroxyl groups have been shown to be efficient in quenching DPPH radicals (Moilanen, 2015). In our study, ellagitannins seemed to have a moderate ability of donating hydrogen considering the high concentrations in the extracts from sea buckthorn leaves.

A consistency among the antioxidative activity assays has been extensively discussed. The correlation among antioxidant assays were also investigated in this study. As presented in **Supplement table 3**, Folin-Ciocalteau exhibited very strong correlations with HAT methods (R = 0.895 with ORAC and R = 0.918 with TRAP, p = 0.01) as well as a strong correlation with DPPH (R = 0.606-0.728, p = 0.01). Strong correlation were found between the activities in ORAC and TRAP assays (R = 0.889, p = 0.01). For DPPH assay, a strong correlation was present with TRAP (R = 0.604-0.658, p = 0.01), but only a moderate correlation with ORAC

(R = 0.421-0.478, p = 0.05).

3.2.5.2 Multivariate correlation by PLS

PLS regression models were applied to determine the multivariate correlation of various groups of phenolics as well as individual phenolic compounds with the antioxidative capacities of the extracts. As shown in **Supplemental Fig. 2**, non-flavonoid phenolics were positively associated with DPPH assay, whereas Folin-Ciocalteu, ORAC, and TRAP assays were correlated with flavonoids. This was in agreement with the results of bivariate correlation analysis. Considering the diversity of phenolic content and composition among leaves and berries, two separate models were built to explain the major contribution of individual phenolic compounds to the antioxidative activities in leaf and berry extracts, respectively (**Fig. 2**). The PLS plots of the berry extracts were shown in **Fig. 2a** where 63% of the chemical variables explained 89% of the variation in the antioxidative activities in four factors. Total content of phenolics, cyanidin glycosides (mainly cyanidin 3-*O*-galactoside), and quercetin 3-*O*-galactoside positively correlated with Folin-Ciocalteu, ORAC and TRAP assays. Some moderate contributions were also found in non-flavonoid phenolics, primarily phenolic acids. Interestingly, the negative correlations to these three assays were shown in some quercetins and isorhamnetins with di- and tri-saccharide as sugar moieties, such as quercetin 3-*O*-sophoroside-7-*O*-rhamnetin (Q-SopRha) and isorhamnetin 3-*O*-rutinoside (I-

Rut). Due to the presence of 3-*O*-glucosides of cyanidins (Cy-Glu) and delphenidins (De-Glu), DPPH assay were positively associated with total concentration of anthocyanins; however, some negative correlation was seen between the DPPH radical scavenging activity and flavan-3-ols and proanthocyanidins as well as some mono-glycosides of quercetins. In PLS model of the leaf extracts, 69% of the chemical variables explained 95% of the variation among the antioxidative data in five factors (**Fig. 2b**). The activities measured in Folin-Ciocalteu, ORAC and TRAP assays were highly correlated with flavonoids, such as flavan-3-ols, proanthocyanidins, and quercetins with mono-saccharide as sugar moieties (**Fig. 2b**). For DPPH assays, ellagitannins contributed positively to quenching DPPH radicals. It is acknowledged that more sugar moieties in flavonols may reduce the radical scavenging activity by diminishing co-planarity of the B ring and by occupying more hydroxyl groups (Heim, Tagliaferro, & Bobilya, 2002). In our study, a positive correlation was found between DPPH radical scavenging activity and flavonol di- and tri-glycosides. This result might be caused by co-existence compounds with high DPPH scavenging activities, for example ellagitannins in the sea buckthorn leaf extracts. Also the type and position of sugar substituents in the molecule may have significant influence on the antioxidative activities.

3.3 Antibacterial activities of phenolic compounds in berry plants

The antibacterial activities of twenty-four raw extracts were evaluated against five foodborne

pathogens including both Gram-negative and Gram-positive bacteria. The growth inhibiting effect of the extracts against the target microbes is presented in **Table 3**.

3.3.1 Antibacterial activities of extracts

As shown in **Table 3**, *Escherichia coli*, a Gram-negative bacterium represented higher resistance to the extracts of berry plants, compared to other tested bacteria. At the low dose (10 µL in 300 µL culture medium), no inhibition was observed in the extracts of bilberry leaves, chokeberry leaves, nettle leaves and sea buckthorn berries (Terhi). Clear growth inhibition was observed when 20 µL of the extracts of saskatoon leaves (75%), saskatoon branches (68%), and two berry press cakes (67%) was used in 300 µL of culture medium. *Staphylococcus aureus* exhibited sensitivity to the low dose of sea buckthorn leaf extracts, as well as to the leaf extracts of lingonberry and hawthorn. Ellagitannins were the major phenolic compounds in sea buckthorn leaf extracts, whereas flavan-3-ols and proanthocynidins dominated in hawthorn leaf extracts. These three extracts also inhibited over 90% of the growth of *Bacillus cereus* when only 10 µL of volume added into culture medium. Other extracts, such as those from lingonberry, bilberry, bilberry leaf, saskatoon berry, and rowan berry, had weak efficacy against *Bacillus cereus*. Stronger inhibitory effect on *Listeria monocytogenes* was present in the leaf extracts of sea buckthorn (100%) and raspberry (80%) at the low dose (10 µL), suggesting active role of ellagitannins in the growth

inhibition. Except the extracts from nettle leaves, lingonberry leaves, and bilberry leaves, most of the extracts exhibited strongest inhibition against *L. monocytogenes* (72-100%) at high dose (20 μL). For *Salmonella enterica* sv. Typhimurium, the growth inhibition was 33-54% at the low dose and 17-100% at the high dose. The inhibition was generally stronger with the increase in the dose of the extracts; however, the inhibitory effect of the nettle leaf extracts dropped to the half of the value observed at low dose when the high dose was used.

The compositional profiles of phenolic compounds play a major role in the anti-bacterial activities of the extracts from fruits and leaves of berry plants. Puupponen-Pimiä et al. (2001) evaluated the anti-bacterial activities of several Finnish berry extracts with selected Gram-positive and Gram-negative bacteria species. The results showed that the number of hydroxyl groups in the molecules might affect the antimicrobial activity of phenolic compounds, which may explain the strong inhibition on Gram-positive bacteria caused by ellagitannins and proanthocyanidins in our study (Puupponen-Pimiä et al., 2001). Compared to Gram-positive bacteria, Gram-negative microbes presented stronger resistance to the extracts rich in ellagitannins (sea buckthorn leaves) and proanthocyanidins (hawthorn leaves). This might have been due to the hydrophilic surface of outer membrane in these bacteria, and the presence of certain enzymes in the periplasmic space, which broke down the molecules introduced from outside (Gao, van Belkum, & Stiles, 1999; Shan, Cai, Brooks, & Corke,

2007). The anti-bacterial capacity of phenolic acids has been reported to be mainly dependent on the presence of carboxyl group (-COOH). The substitution pattern of the benzene ring also influence the activity, such as two hydroxyl groups (-OH) in para- and ortho-positions, or a methoxyl group (-OCH_3) in meta-position of benzene ring (Alves, Ferreira, Froufe, Abreu, Martins, & Pintado, 2013). Hydroxyl groups have also been reported to play a role in the weakening the outer membrane of Gram-negative bacteria (Alakomi et al., 2007). In our experiments minor growth inhibition against *E. coli* was observed, whereas growth of *Salmonella* was significantly affected by several berry and leaf extracts. This indicates differences in the outer membrane (OM) structures of the cells of different bacterial species. Phenolic extracts of cloudberry and raspberry have previously been reported to disintegrate the OM of *Salmonella* (Nohynek et al., 2006). In the present study, low anti-bacterial capacities of the bilberry leaf extracts suggested that all of the target bacteria may have strong tolerance to 3-*O*-caffeoylquinic acid.

3.3.2 Correlation between the content of phenolic compounds and anti-bacterial activities

The contribution of phenolic composition is determined with bivariate Pearson's correlation (**Fig. 3**, **Supplemental Table 4**). A strong correlation (R = 0.772, p = 0.01, n = 22) was observed between the total content of phenolics and the growth inhibition on *Staphylococcus*

475 *aureus* strain. The antibacterial activity on *Staphylococcus aureus* showed stronger
476 correlation with the content of non-flavonoid phenolic compounds in berry and leaf extracts,
477 compared to flavonoids. For flavonoids, both proanthocyanidins (primarily as procyanidin
478 dimmers and trimers) and glycosylated flavonols (quercetin glycosides) exhibited high
479 coefficient value of 0.761 (p = 0.05, n = 22) and 0.647 (p = 0.01, n = 19), respectively (**Fig.
480 3a-f**). The inhibition on *Listeria monocytogenes* strains had strong correlation with the total
481 content of phenolics (R = 0.609, p = 0.01), as well as total content of non-flavonoid phenolics
482 (R = 0.594, p = 0.01) (**Fig. 3g&h**). The highest correlation coefficient value of 0.825 (p =
483 0.01) indicated phenolic compounds to be the main inhibitor against *Bacillus cereus* stains.
484 The correlation with antibacterial activity against *Bacillus cereus* was stronger for non-
485 flavonoid phenolic compounds than flavonoids. The content of flavonol glycosides correlated
486 strongly with the inhibition of *Bacillus cereus* strains, especially that of glycosides of
487 quercetin (R = 0.617, p = 0.01, n =22) and isorhamnetin (R = 0.705, p = 0.05, n =12) (**Fig. 3i-
488 n**). Phenolic compounds were also found to have positive correlation with antibacterial
489 activity against *Salmonella enterica* sv. Typhimurium (R = 0.665, p = 0.01. n =15), mainly
490 due to the total content of flavonol glycosides in the extracts (**Fig. 3o-q**). No significant
491 bivariate correlation was present between phenolics and inhibition of *Escherichia coli*.
492
493 Successful multivariate correlation was established only between phenolic compounds in the

leaf extracts and antibacterial activities against *Staphylococcus aureus* (E-70045) and *Bacillus cereus* (E-93143). The PLS plots were shown in **Supplemental Fig. 3**, where 74% of the chemical variables explained 97% of the variation among the percentage of growth inhibition in five factors. In **Supplemental Fig. 3**, the total content of phenolics was strongly associated with the inhibition of both *Staphylococcus aureus* and *Bacillus cereus* strains. Non-flavonoid phenolic compounds (especially ellagitannins) and isorhamnetin di- and tri-glycosides were the main inhibitors, as well as quercetin 3-*O*-glucoside-7-*O*-rhamnoside (Q-GluRha), quercetin 3-*O*-(6-*O*-feruloylglucoside)-glucoside-7-*O*-rhamnoside (Q-feGluGluRha), and kaempferol 3-*O*-neohesperidoside (K-Neo).

4. Conclusions

The antioxidative and anti-bacterial activities of aqueous ethanolic extracts of leaves, berries and branches of berry species were evaluated with multiple antioxidant assays and a variety of bacteria. In the present study, phenolic compounds were characterized primarily as flavan-3-ols, proanthocynidins, ellagitannins, phenolic acid derivatives, flavonols, flavones, flavanones, anthocyanins, and other phenolics. The structures and total concentration of phenolic compounds were major factors determining both the antioxidative and the anti-bacterial activities of the extracts. Most of flavonoids exhibited potent antioxidative activities in Folin-Ciocalteu, ORAC, and TRAP assays but not in scavenging DPPH radicals. Non-

flavonoid phenolic compounds mainly contributed to the growth inhibition of selected foodborne pathogens. These results could be applied in selection of optimal antioxidant and antibacterial efficacies based on the specific group of phenolics presented in the raw materials and ingredients. Future studies related to efficacy of Gram-negative bacteria and weakening of the Gram-negative bacteria should be performed.

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Conflict of interest

The authors in this manuscript have no conflict of interest.

Appendix A. Abbreviations used

All abbreviations of phenolic compounds used in this study are listed as below:

(+)-catechin (**(+)-Cat**), (-)-epicatechin (**(-)-Epic**), A/B-type procyanidin dimers/trimers (**A/B-PC di/tri**), bis(hexahydroxydiphenoyl)-hexoside (**bisHHDP-Hex**), ellagitannin (**Et**), galloyl-bis(hexahydroxydiphenoyl)-hexoside (**G-bisHHDP-Hex**), 4-(2-hydroxyethyl)phenol-hexoside (**HP-Hex**) vanillic acid-hexoside (**VA-Hex**), coumaric acid-hexoside (**CoA-Hex**), caffeic acid-hexoside (**CaA-Hex**), coumaroylquinic acid (**CoQA**), ferulic acid-hexoside (**FA-Hex**), cafferol-hexose-hydrophenol (**Ca-Hex-H**), caffeic acid (**CaA**), *p*-coumaric acid (***p*-CoA**), 5/3/4-*O*-caffeoylquinic acid (**5/3/4-CQA**), dicaffeoylquinic acid (**diCQA**), caffeoylmalic acid (**CaMA**), caffeoylglyceric acid (**CaGA**), 1-*O*-benzoyl-β-glucose (**BA-Glu**), quercetin (**Q**), myricetin (**M**), isorhamnetin (**I**), kaempferol (**K**), laricitrin (**La**), syringetin (**S**), apigenin (**A**), luteolin (**Lu**), eriodictyol (**E**), cyanidin (**Cy**), delphinidin (**De**), petunidin (**Pt**), peonidin (**Po**), malvidin (**Ma**), rutinoside (**Rut**), galactoside (**Gal**), glucoside (**Glu**), hexoside (**Hex**), rhamnoside (**Rha**), deoxyhexoside (**Deox**), xyloside (**Xyl**), arabinoside (**Ara**), arabinofuranoside (**Araf**), pentoside (**Pent**), glucuronide (**Gluc**),

coumaroyl-glucoside (**coGlu**), hydroxy-methylglutaroyl-galactoside (**hmgGal**), hydroxy-methylglutaroyl-galactoside (**hmgRha**), benzoyl-galactoside/glucoside (**beGal**/**Glu**), malonyl-galactoside/ glucoside (**maGal**/**Glu**), feruloyl-glucoside (**feGlu**), acetyl-glucoside (**acGlu**), methoxyhexoside (**meHex**), methyl-hexoside (**mtHex**), dihexoside (**diHex**), neohesperidoside (**Neo**), and β-arbutin (**Arb**).

Appendix B. Supporting information description

The supporting information is provided: (1) HPLC chromatograms of the extracts of raw materials studied (**Supplemental Fig. 1**). Abbreviations of phenolic compounds refer to Appendix A. (2) PLS plots of the correlations between phenolic compounds and antioxidative activities in all samples studied (**Supplemental Fig. 2**). The antioxidative assays are in red bold font and the main groups of phenolic compounds are in blue bold font (**The color should be used in print**). (3) PLS plots of the correlations between phenolic compounds and antibacterial activities of leaf samples against *Staphylococcus aureus* (E-70045) and *Bacillus cereus* (E-93143) (**Supplemental Fig. 3**). The bacteria are in red bold font. The main groups of phenolic compounds are in blue bold font and individual phenolics are in blue font with a smaller letter size (**The color should be used in print**). Abbreviations of phenolic compounds refer to Appendix A. (4) Names and sources of plant materials studied (**Supplemental Table 1**). (5) Concentrations (mg/100 mL, n=4) of flavonol glycosides and

anthocyanins in extracts of berry plants by HPLC-DAD (**Supplemental Table 2**). (6) Pearson's correlation coefficients of antioxidative assays (**Supplemental Table 3**). (7) Pearson's correlation coefficients of antibacterial assays (**Supplemental Table 4**).

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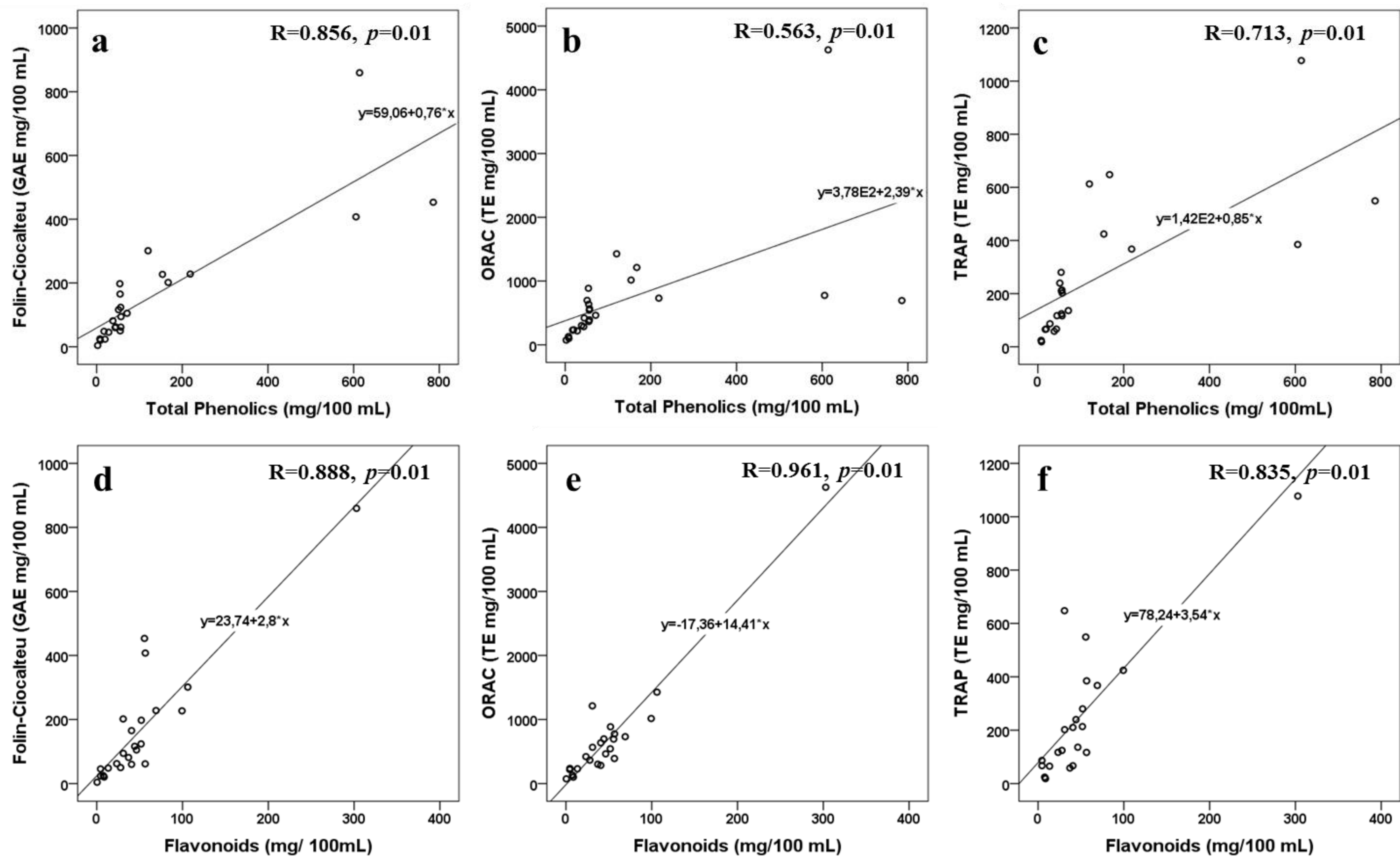
Figure captions

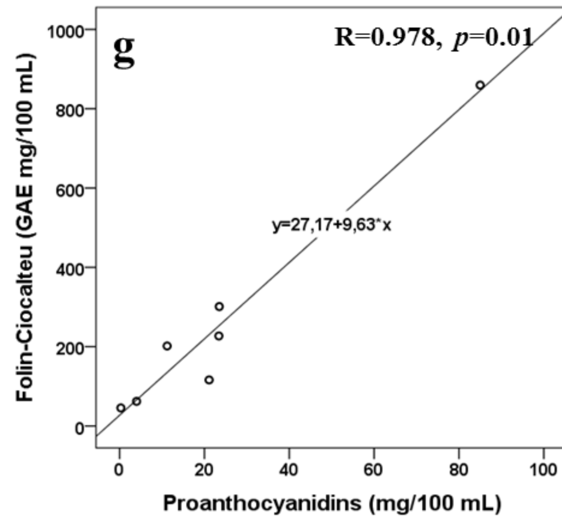
Fig. 1: Bivariate correlation between phenolic composition and antioxidative assays (Folin-Ciocalteu, ORAC, and TRAP)

Fig. 2: PLS plots of the correlations between phenolic composition and antioxidative assays in berry samples (**a**) and leaf samples (**b**). The antioxidative assays are in red bold font. The main groups of phenolic compounds are in blue bold font and individual phenolics are in blue font with a smaller letter size (**The color should be used in print**). Abbreviations of phenolic compounds refer to Appendix A.

Fig. 3: Bivariate correlations between phenolic composition and antibacterial assays

Figure 1

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R=0.978, p=0.01
y=27,17+9,63*x
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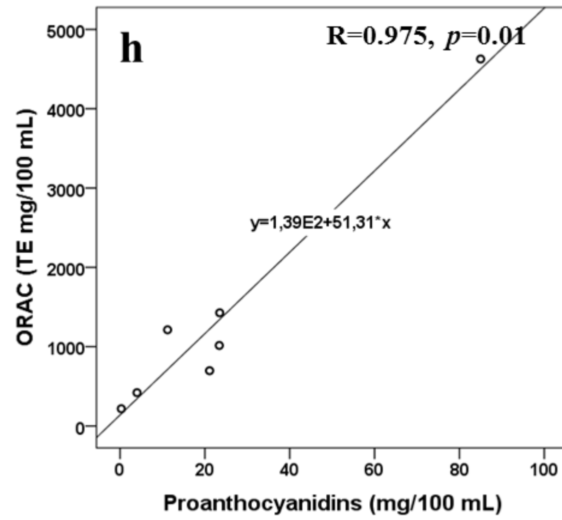
Proanthocyanidins (mg/100 mL)

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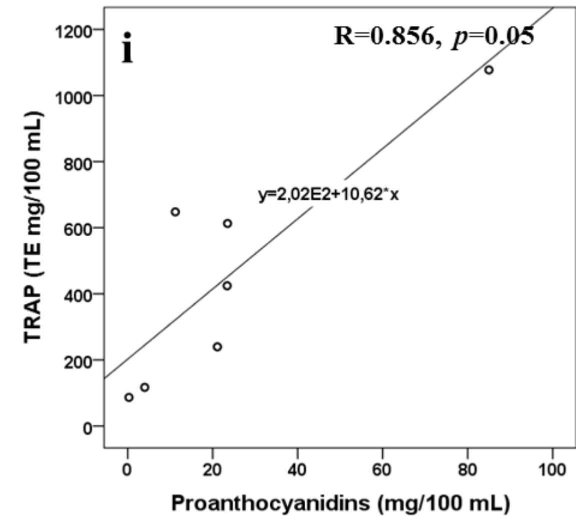
y=1,39E2+51,31*x
ORAC (TE mg/100 mL)
Proanthocyanidins (mg/100 mL)

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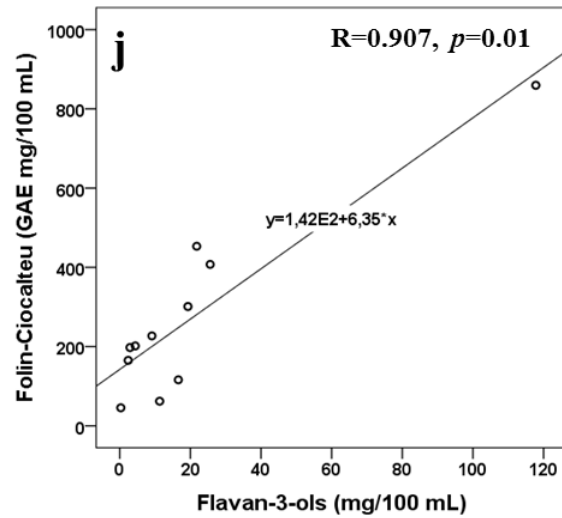
R=0.856, p=0.05
y=2,02E2+10,62*x
TRAP (TE mg/100 mL)
Proanthocyanidins (mg/100 mL)

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R=0.907, p=0.01
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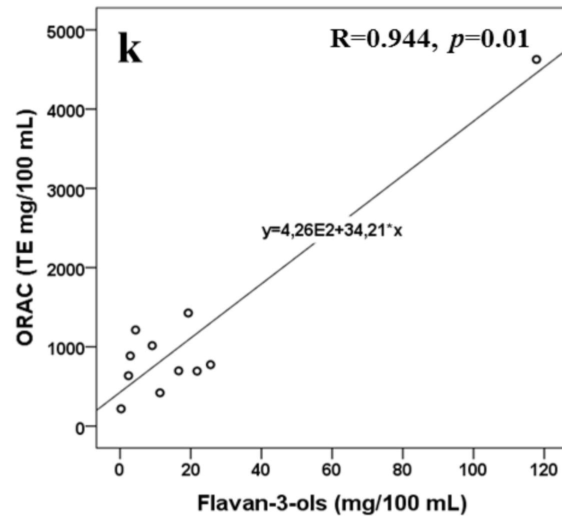
Folin-Ciocalteu (GAE mg/100 mL)
Flavan-3-ols (mg/100 mL)

k
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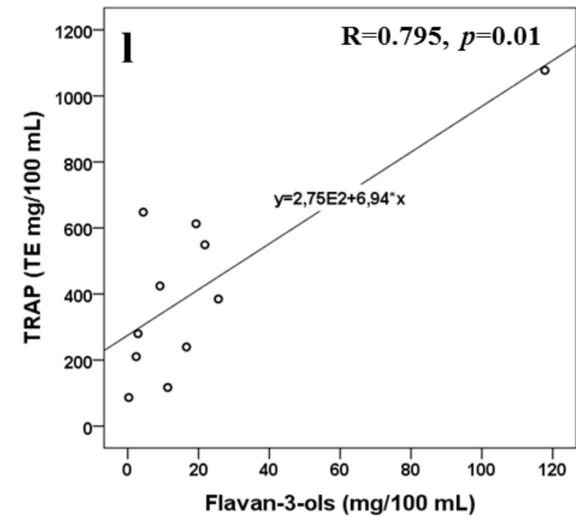
ORAC (TE mg/100 mL)
Flavan-3-ols (mg/100 mL)

l
R=0.795, p=0.01
y=2,75E2+6,94*x
TRAP (TE mg/100 mL)
Flavan-3-ols (mg/100 mL)

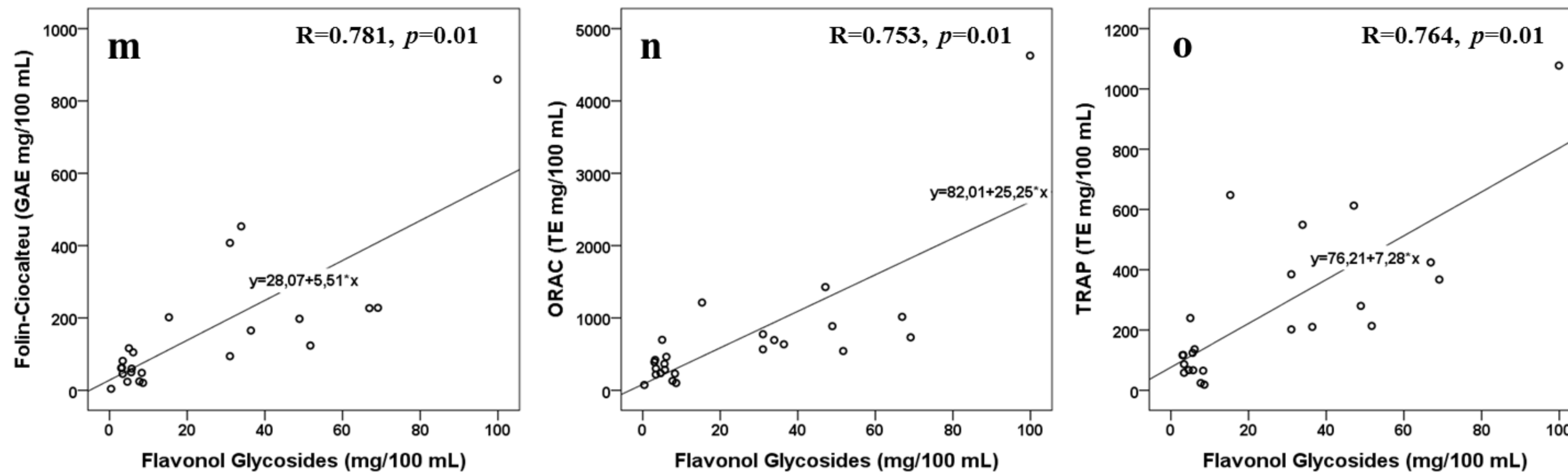
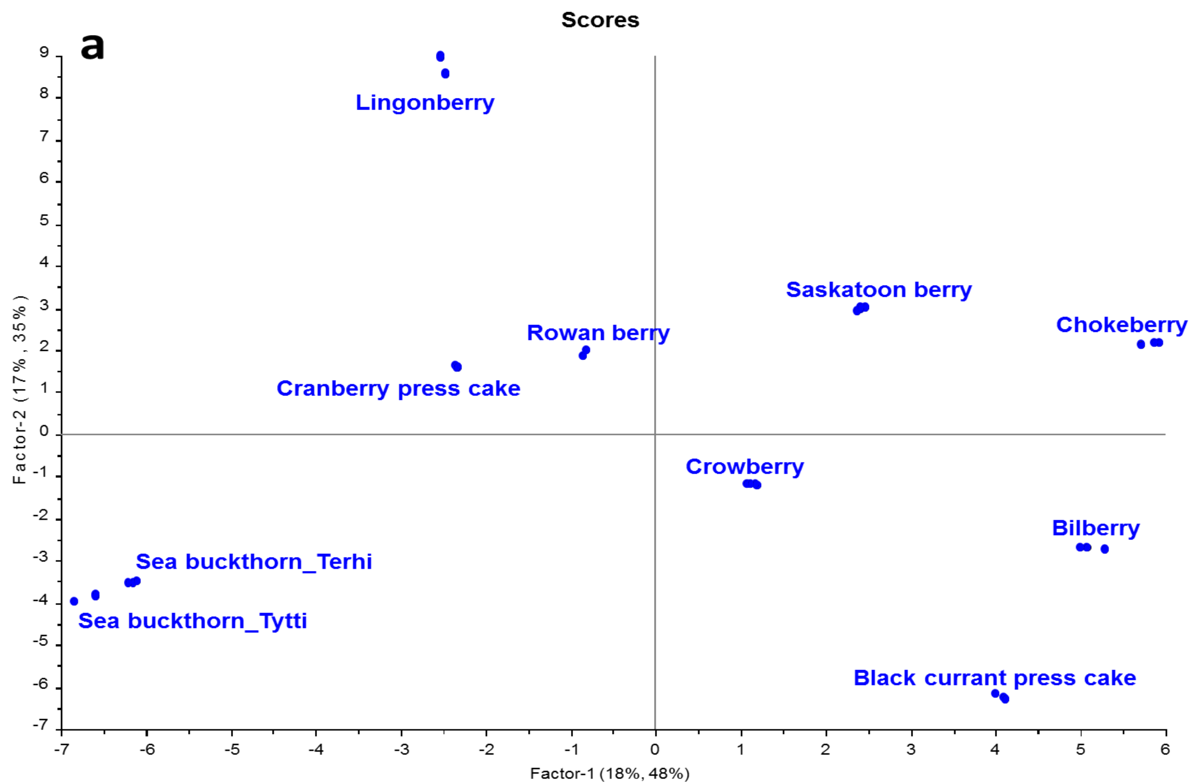
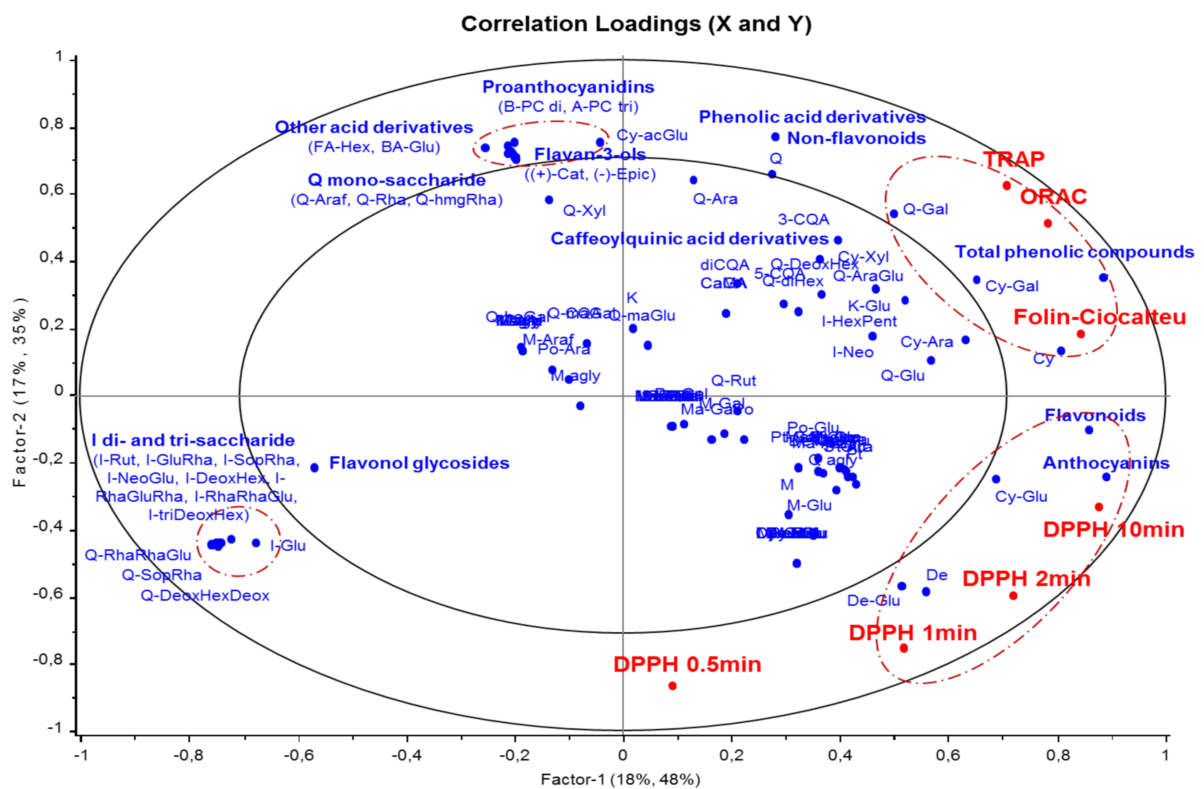


Fig. 1 Bivariate correlation between phenolic composition and antioxidative assays (Folin-Ciocalteu, ORAC, and TRAP)

Figure 2





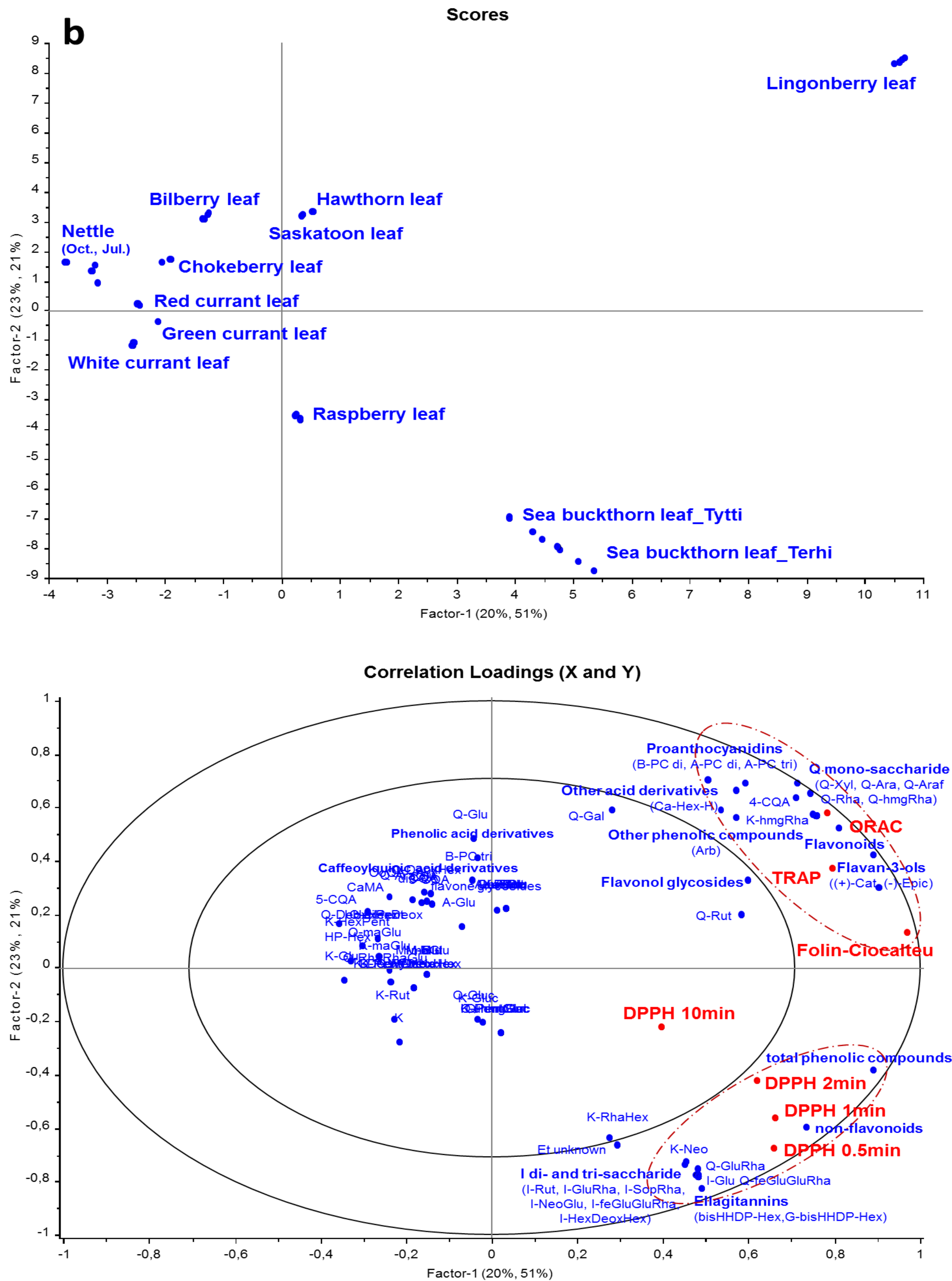
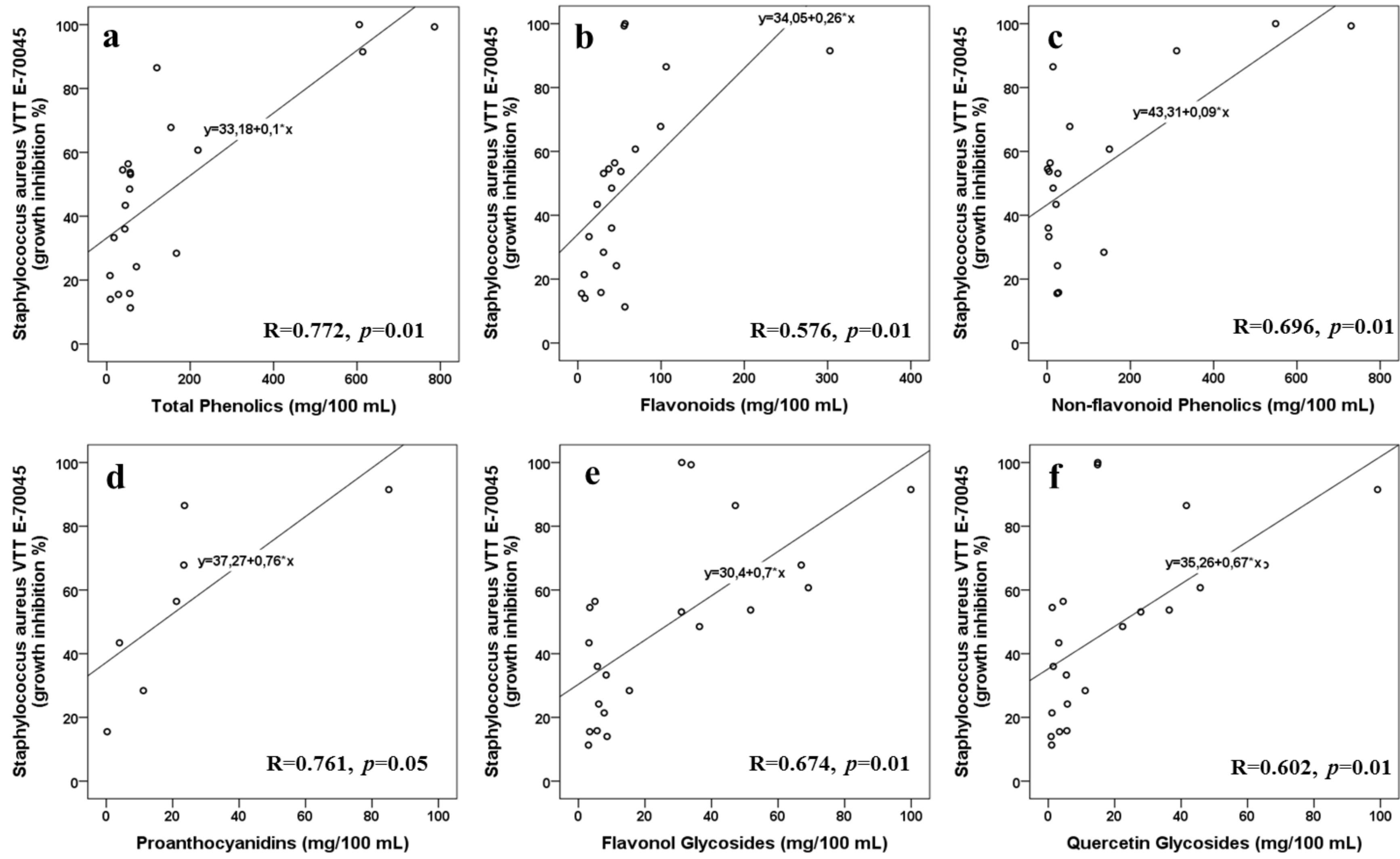
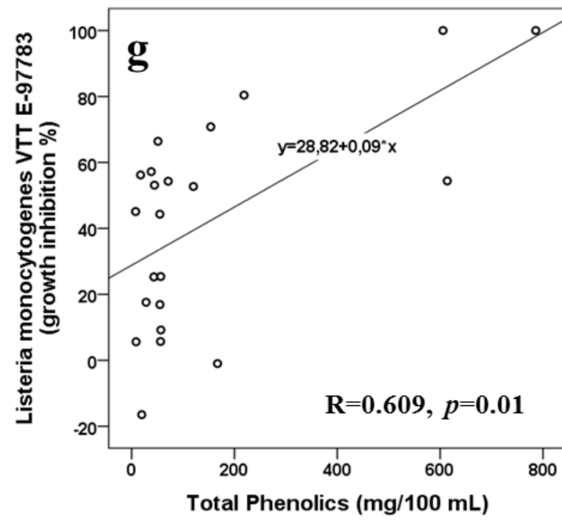
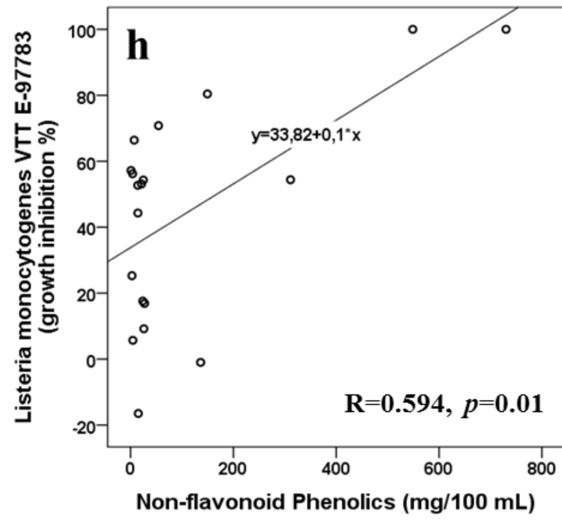


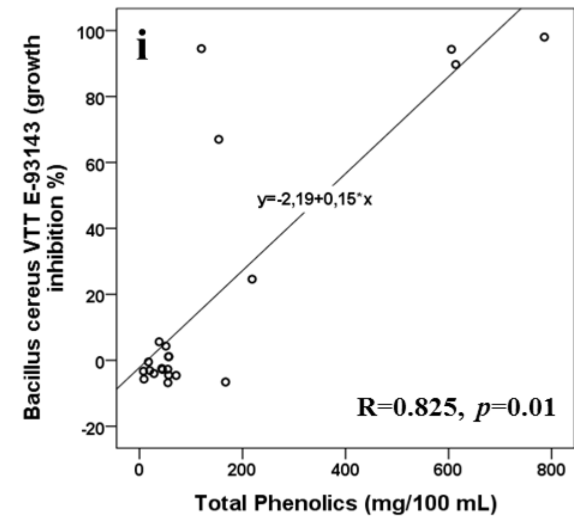
Fig. 2 PLS plots of the correlations between phenolic composition and antioxidative assays

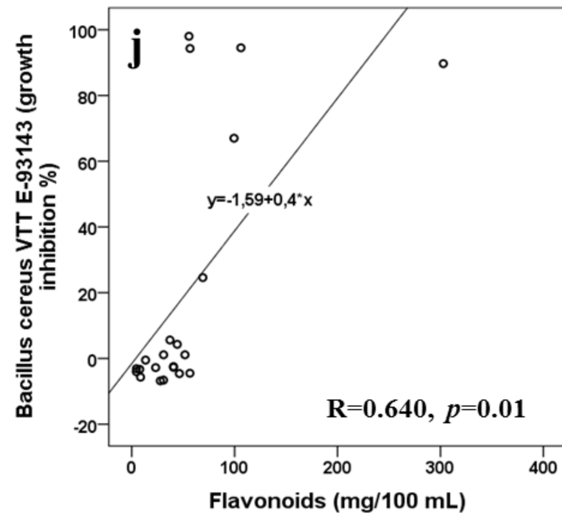
Figure 3

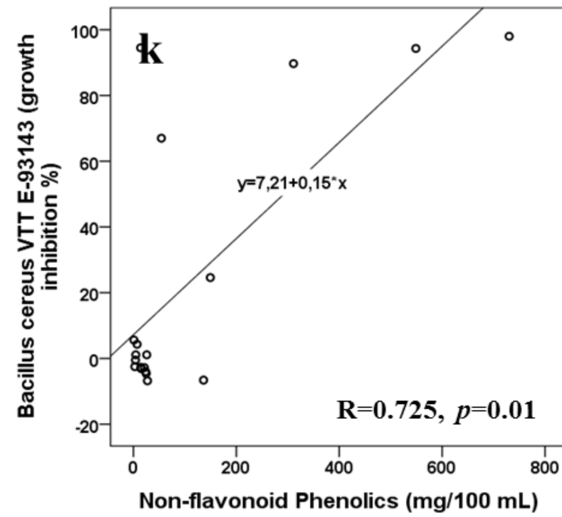


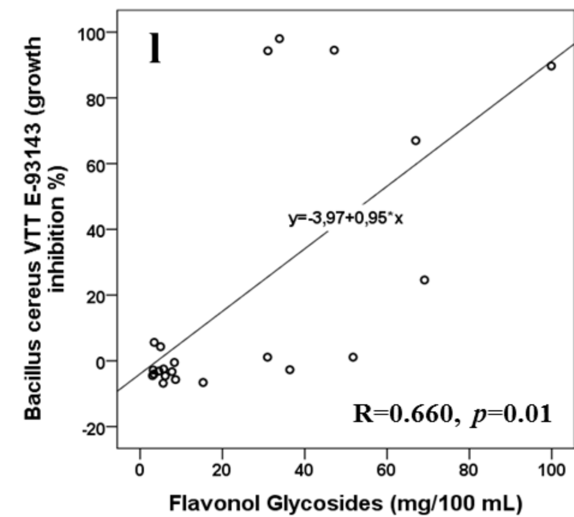

g
Listeria monocytogenes VTT E-97783 (growth inhibition %)
Total Phenolics (mg/100 mL)
y=28,82+0,09*x
R=0.609, p=0.01

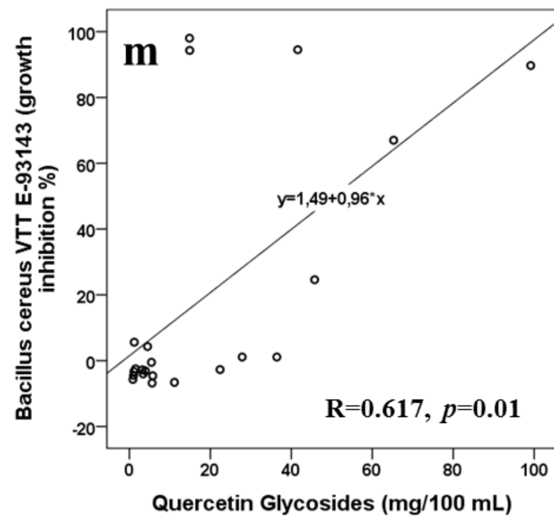

h
Listeria monocytogenes VTT E-97783 (growth inhibition %)
Non-flavonoid Phenolics (mg/100 mL)
y=33,82+0,1*x
R=0.594, p=0.01

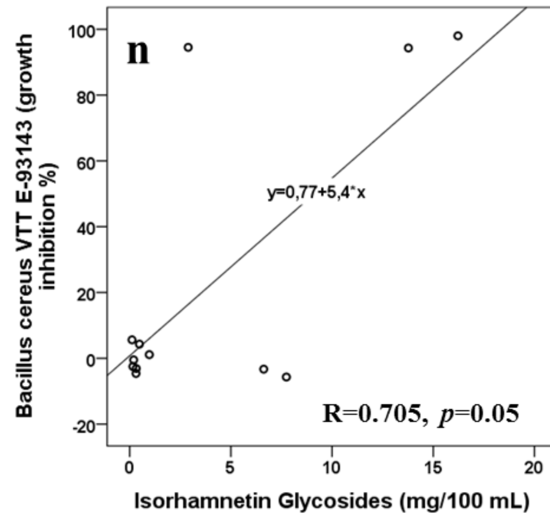

i
Bacillus cereus VTT E-93143 (growth inhibition %)
Total Phenolics (mg/100 mL)
y=-2,19+0,15*x
R=0.825, p=0.01

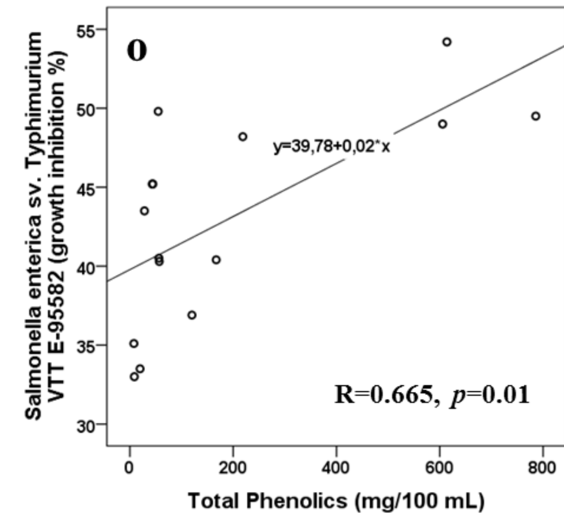

j
Bacillus cereus VTT E-93143 (growth inhibition %)
Flavonoids (mg/100 mL)
y=-1,59+0,4*x
R=0.640, p=0.01

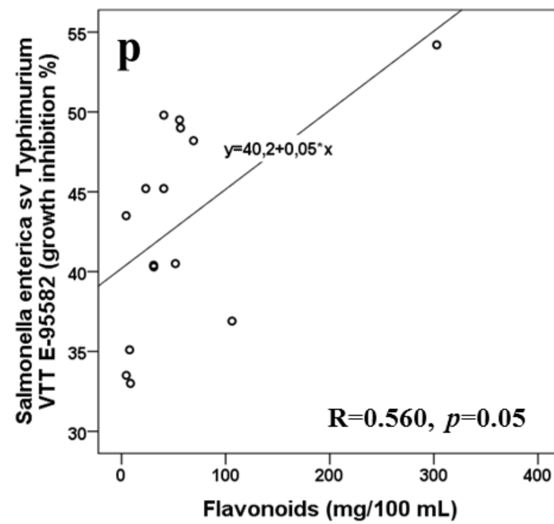

k
Bacillus cereus VTT E-93143 (growth inhibition %)
Non-flavonoid Phenolics (mg/100 mL)
y=7,21+0,15*x
R=0.725, p=0.01


l
Bacillus cereus VTT E-93143 (growth inhibition %)
Flavonol Glycosides (mg/100 mL)
y=-3,97+0,95*x
R=0.660, p=0.01


m
Bacillus cereus VTT E-93143 (growth inhibition %)
Quercetin Glycosides (mg/100 mL)
y=1,49+0,96*x
R=0.617, p=0.01


n
Bacillus cereus VTT E-93143 (growth inhibition %)
Isorhamnetin Glycosides (mg/100 mL)
y=0,77+5,4*x
R=0.705, p=0.05


o
Salmonella enterica sv. Typhimurium VTT E-95582 (growth inhibition %)
Total Phenolics (mg/100 mL)
y=39,78+0,02*x
R=0.665, p=0.01


p
Salmonella enterica sv Typhimurium VTT E-95582 (growth inhibition %)
Flavonoids (mg/100 mL)
y=40,2+0,05*x
R=0.560, p=0.05

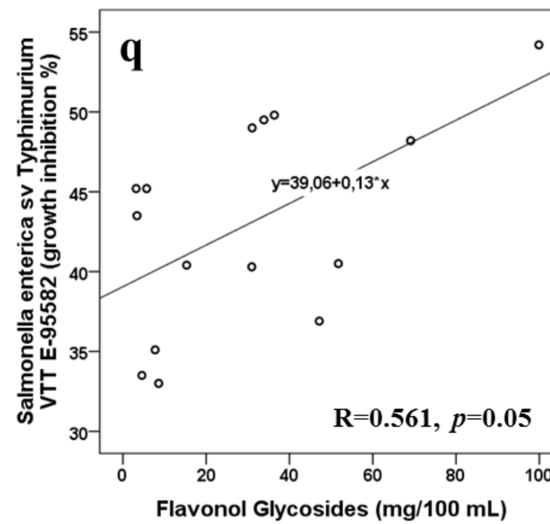

q
Salmonella enterica sv Typhimurium VTT E-95582 (growth inhibition %)
Flavonol Glycosides (mg/100 mL)
y=39,06+0,13*x
R=0.561, p=0.05

Fig. 3 Bivariate correlations between phenolic composition and antibacterial assays

Table 1

Table 1 Concentrations (mg/100 mL, n=4) of phenolic compounds in extracts of berries, leaves and branches by HPLC-DAD

extract name	**Flavan-3-ols**	**Proanthocyanidins**	**Ellagitannins**	**Phenolic acid derivatives**	**Flavonol glycosides**	**Flavone glycosides**	**Flavanone glycosides**	**Anthocyanins**	**Other phenolic compounds**	**Total**
Lingonberry	11.3±1.2	4.0±0.3	-	21.2±1.1	3.2±0.1	-	-	4.8±0.1	-	44.5±2.8
Lingonberry leaf	117.8±3.2	85.1±2.3	-	39.9±1.5	99.9±2.3	-	-	-	271.1±2.0	613.8±11.3
Bilberry	-	-	-	-	3.0±0.0	-	-	53.5±1.4	-	56.5±1.4
Bilberry leaf	4.4±1.0	11.2±1.0	-	136.3±5.8	15.3±0.8	-	-	-	-	167.2±8.6
Red currant leaf	-	-	-	4.5±0.1	51.7±1.2	-	-	-	-	56.2±1.3
White currant leaf	2.4±0.1	-	-	6.5±0.6	36.4±0.8	1.9±0.3	-	-	7.8±0.1	55.0±1.9
Green currant leaf	2.9±0.0	-	-	2.4±0.0	49.0±0.5	-	-	-	-	54.3±0.5
Hawthorn leaf	19.3±1.9	23.5±1.6	-	14.0±1.0	47.1±0.4	16.1±0.4	-	-	-	120.0±5.3
Chokeberry	-	-	-	24.8±0.3	6.1±0.1	-	-	40.2±0.9	-	71.1±1.3
Chokeberry leaf	-	-	-	18.2±0.2	31.0±0.3	-	-	-	7.7±0.1	56.9±0.6
Sea buckthorn (Terhi)	-	-	-	-	8.6±0.1	-	-	-	-	8.6±0.1
Sea buckthorn leaf (Terhi)	21.8±1.1	-	730.2±25.2	-	33.9±1.3	-	-	-	-	785.9±27.6
Sea buckthorn (Tytti)	-	-	-	-	7.7±0.2	-	-	-	-	7.7±0.2
Sea buckthorn leaf (Tytti)	25.6±0.7	-	548.9±19.5	-	31.1±1.4	-	-	-	-	605.6±21.6
Saskatoon berry	-	-	-	27.3±0.5	5.6±0.1	-	-	22.2±0.5	-	55.1±1.1
Saskatoon leaf	9.1±0.5	23.4±1.7	-	54.4±3.8	67.0±0.8	-	-	-	-	153.9±6.8
Saskatoon branch	16.6±0.9	21.1±0.6	-	7.0±0.3	5.0±0.2	-	1.6±0.0	-	-	51.3±2.0
Nettle (Oct.)	-	-	-	2.2±0.1	0.4±0.0	-	-	-	-	2.6±0.1
Nettle (Jul.)	-	-	-	15.1±3.1	4.6±1.8	-	-	-	-	19.7±4.9
Raspberry leaf	-	-	149.5±4.5	-	69.1±2.6	-	-	-	-	218.6±7.1
Crowberry	-	-	-	2.7±0.1	5.7±0.2	-	-	34.9±0.5	-	43.3±0.8
Rowan berry	0.3±0.0	0.3±0.0	-	23.7±0.6	3.4±0.1	-	-	0.5±0.0	-	28.2±0.7
Black currant press cake	-	-	-	1.0±0.0	3.4±0.0	-	-	33.7±0.5	-	38.1±0.5
Cranberry press cake	-	-	-	4.0±0.1	8.3±0.1	-	-	5.1±0.1	-	17.4±0.3

Table 2

Table 2 Antioxidant activity of phenolic compounds in extracts of berries, leaves, and branches

sample name	**Folin-Ciocalteu** (GAE mg/100mL)	**ORAC** (TE mg/100mL)	**DPPH** (scavenging %)				**TRAP** (TE mg/100mL)
			0.5 min	**1 min**	**2 min**	**10 min**	
Lingonberry	62.1±2.7	419.9±20.6	7.0±2.2	9.6±2.4	13.1±2.6	26.5±3.5	116.9±9.4
Lingonberry leaf	859.5±9.9	4626.6±138.6	52.0±1.7	68.9±2.3	81.5±2.7	86.3±2.5	1077.4±140.2
Bilberry	61.4±0.6	390.8±7.3	22.2±1.1	32.7±1.2	44.7±1.4	66.3±1.7	116.6±6.4
Bilberry leaf	201.7±18.2	1212.5±32.9	27.7±1.7	43.3±0.8	61.2±0.6	91.8±0.2	647.8±40.0
Red currant leaf	123.7±1.2	542.7±15.9	28.8±3.2	41.4±3.8	55.6±4.4	83.1±3.1	213.6±7.9
White currant leaf	165.2±2.7	636.2±44.3	46.7±2.5	60.7±3.1	75.2±3.2	94.2±0.7	210.3±8.1
Green currant leaf	197.6±3.2	886.2±33.7	42.0±2.0	60.3±1.8	77.4±1.8	95.3±0.2	280.0±18.6
Hawthorn leaf	301.2±5.0	1427.0±72.1	30.8±1.1	46.1±1.5	63.8±1.9	92.1±0.4	612.8±48.0
Chokeberry	104.9±0.8	463.7±17.1	18.3±1.7	30.8±1.7	46.7±2.5	80.0±4.3	136.0±19.7
Chokeberry leaf	94.2±1.0	566.4±16.6	15.3±1.5	23.3±1.7	33.6±2.6	59.4±6.6	201.9±27.7
Sea buckthorn (Terhi)	20.5±0.9	100.6±1.4	23.5±2.2	24.6±2.1	26.5±2.0	29.6±1.9	18.6±1.6
Sea buckthorn leaf (Terhi)	453.2±12.7	694.1±39.4	88.8±1.5	93.6±0.1	94.3±0.1	94.9±0.2	549.1±30.0
Sea buckthorn (Tytti)	24.5±3.7	130.0±1.5	23.7±5.2	25.0±5.2	27.2±5.1	31.2±6.0	24.0±2.1
Sea buckthorn leaf (Tytti)	407.4±8.4	775.1±25.2	89.8±0.7	93.7±0.1	94.3±0.0	94.8±0.1	384.9±37.0
Saskatoon berry	49.8±1.4	365.4±21.5	13.9±0.7	22.0±1.1	31.4±2.1	52.1±4.6	124.1±11.5
Saskatoon leaf	227.1±0.7	1015.2±29.7	29.9±3.3	43.1±3.4	58.8±3.0	88.8±0.8	424.3±33.2
Saskatoon branch	116.1±2.5	697.1±33.8	9.8±1.2	15.7±1.2	24.2±1.3	56.2±1.1	239.6±24.6
Nettle (Oct.)	4.0±0.0	73.5±2.0	5.0±3.7	5.7±3.7	6.3±3.7	7.7±3.6	-
Nettle (Jul.)	23.6±5.1	236.5±7.8	14.0±3.5	17.7±3.6	21.4±3.8	25.3±5.0	67.1±7.1
Raspberry leaf	228.1±4.7	731.4±22.3	71.2±1.5	86.2±0.8	92.3±0.1	94.2±0.2	367.6±31.5
Crowberry	59.9±2.6	283.7±13.7	17.8±2.7	25.7±2.6	34.0±2.3	51.1±2.3	66.6±6.0
Rowan berry	45.6±2.6	217.5±28.7	15.0±5.8	18.1±6.0	23.0±5.9	38.7±5.3	86.4±7.9
Black currant press cake	81.4±1.4	301.6±15.8	29.4±3.4	41.7±2.9	56.8±2.5	83.2±2.3	58.1±6.9
Cranberry press cake	48.2±0.5	230.1±12.6	14.6±3.2	20.0±3.5	26.7±4.2	44.2±5.4	65.0±7.3

Table 3

Table 3 Antibacterial activities (growth inhibition %) of phenolic extracts of berry plants (10 µL or 20 µL of extracts in 300 µL of media)

sample name	*Escherichia coli* (E-94564)		*Staphylococcus aureus* (E-70045)		*Listeria monocytogenes* (E-97783)		*Bacillus cereus* (E-93143)		*Salmonella enterica* sv. Typhimurium (E-95582)	
	10 µL	**20 µL**	**10 µL**	**20 µL**	**10 µL**	**20 µL**	**10 µL**	**20 µL**	**10 µL**	**20 µL**
Lingonberry	23±1	43±3	43±4	90±2	53±1	92±1	-3±2	-3±0	45±1	84±17
Lingonberry leaf	26±2	50±3	92±1	100±0	54±2	37±1	90±3	100±0	54±5	71±4
Bilberry	38±3	58±2	11±2	33±3	25±1	77±2	-5±2	-4±1	-	-
Bilberry leaf	-2±0	16±3	28±3	40±2	-1±0	43±1	-7±3	6±1	40±0	58±8
Red currant leaf	8±2	36±3	54±4	77±4	6±2	83±2	1±1	26±2	41±1	67±4
White currant leaf	12±1	39±1	49±5	91±3	44±3	73±3	-3±1	90±3	50±2	78±12
Hawthorn leaf	20±1	40±2	87±3	100±0	53±1	100±0	95±4	100±0	37±8	86±4
Chokeberry	40±2	59±4	24±1	74±2	54±13	99±3	-	82±20	-	-
Chokeberry leaf	0±0	23±2	53±4	72±4	9±4	89±1	1±1	98±1	40±2	68±15
Sea buckthorn_Terhi	1±0	32±1	14±6	48±3	6±1	43±3	-6±2	27±3	33±5	34±0
Sea buckthorn leaf_Terhi	24±4	55±5	99±1	100±0	100±0	100±0	98±2	100±0	50±1	100±5
Sea buckthorn_Tytti	4±0	42±1	21±2	64±2	45±3	92±1	-3±1	90±2	35±1	98±0
Sea buckthorn leaf_Tytti	26±4	47±3	100±0	100±0	100±0	100±0	94±1	100±0	49±1	87±12
Saskatoon berry	42±4	57±5	16±0	31±6	17±3	74±1	-7±0	-6±2	-	-
Saskatoon leaf	53±3	75±4	68±6	100±0	71±7	100±0	67±21	89±16	-	-
Saskatoon branch	38±3	68±4	56±2	100±0	66±3	100±0	4±5	84±19	-	-
Nettle_Jul.	-4±1	20±1	-	26±3	-17±1	10±1	-3±0	46±4	34±4	17±0
Raspberry leaf	16±4	43±7	61±5	95±3	80±2	100±0	25±3	96±2	48±3	81±5
Crowberry	14±2	33±1	36±2	66±3	25±1	84±0	-3±1	89±4	45±0	77±6
Rowan berry	22±1	47±3	16±3	61±3	18±1	72±2	-4±1	-4±1	44±0	50±1
Black currant press cake	43±1	67±2	55±7	100±0	57±4	100±0	6±7	77±37	-	-
Cranberry press cake	38±3	67±4	33±2	97±1	56±8	100±0	-1±2	89±14	-	-

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*Graphical Abstract

Antioxidative assays

Antibacterial assays