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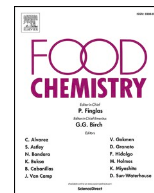

From the forest to the plate – Hemicelluloses, galactoglucomannan, glucuronoxylan, and phenolic-rich extracts from unconventional sources as functional food ingredients

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ABSTRACT

This study aimed to characterise pressurised hot water (PHW) extracts from nonconventional sources of functional carbohydrates and phenolic compounds in terms of antioxidant capacity, antiviral activity, toxicity, and human erythrocytes' protection antidiabetic potential. PHW extracts of Norway spruce bark (E1 + E2) and Birch sawdust (E3 + E4) contained mostly galactoglucomannan and glucuronoxylan. In contrast, samples E5 to E9 PHW extracted from Norway spruce, and Scots pine bark are rich sources of phenolic compounds. Overall, phenolic-rich extracts presented the highest inhibition of α-amylase and α-glucosidase and protection against stable non-enveloped enteroviruses. Additionally, all extracts protected human erythrocytes from hemolysis. Cell-based experiments using human cell lines (IMR90 and A549) showed extracts' non-toxic *in vitro* profile. Considering the relative toxicological safety of extracts from these unconventional sources, functional carbohydrates and polyphenol-rich extracts can be obtained and further used in food models.

1. Introduction

The pharmaceutical and chemical industries look for new natural materials for high value-added applications, such as drugs and cosmetics (Ragab et al., 2018). Plant cell walls are a potential source of pharmacologically active polysaccharides. Cellulose, hemicellulose, and lignin are considered the three major components of lignocellulosic biomass. More precisely, hemicellulose is the second most abundant polysaccharide class in nature, indicating the attractiveness of the exploitation of lignocellulose material as its inexpensive and plentiful natural resource (Đorđević and Antov, 2018). The new trend for extraction of hemicelluloses, galactomannans, and other potentially functional carbohydrates from renewable biomass with low cost and available industrial byproducts that can be used to produce value-added ingredients is promising (Wu et al., 2019).

Forests are essential sources of renewable materials. Forest industries such as pulp mills and sawmills provide pulp and timber used, e.g., in packages and construction. These huge industries also produce important byproducts of wood bark and sawdust mainly combusted for energy. Cascading use (e.g., biorefinery approach) would be preferable to utilise these resources fully. The cascading approach would first extract valuable compounds from either bark or sawdust, utilise extracted biomass in different applications, and finally combust or gasify the remaining matter to energy (Rasi et al., 2019). In this aspect, pressurised hot water extraction (PHWE), which uses non-toxic water and is considered a green extraction method (Teo et al., 2010), can extract compounds from woody materials. Tannins from spruce and pine bark can be extracted at lower temperatures under 100 °C. Spruce bark PHWE

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Table 1
Chemical antioxidant activity of Norway spruce galactoglucomannan, birch glucuronoxylan, Norway spruce and Scots pine phenolics-rich extracts.

Extracts	Total phenolic content (mg GAE/100 g)	Reducing capacity (mg GAE/100 g)	Cu^{2+} chelating ability (mg EDTAE/100 g)	FRAP (mg AAE/ 100 g)	DPPH (mg AAE/ 100 g)
E1 - Norway spruce galactoglucomannan	20889 ± 99^{d}	792 ± 8^{f}	ND	7446 ± 293^{h}	9230 ± 21^{c}
E2 - Norway spruce galactoglucomannan (ethanol precipitation)	2310 ± 105^{g}	142 ± 3^{g}	ND	742 ± 2^{i}	341 ± 7^{h}
E3 - Birch glucuronoxylan	18743 ± 41^{e}	1239 ± 4^{e}	414 ± 1^{g}	12590 ± 149^{f}	3377 ± 189^{g}
E4 - Birch glucuronoxylan (ethanol precipitation)	2250 ± 15^{g}	614 ± 6^{f}	ND	1238 ± 6^{i}	3899 ± 56^{f}
E5 - Norway spruce phenolics (crude extract)	45525 ± 393^{c}	5296 ± 333^{d}	1492 ± 2^{b}	23614 ± 253^{d}	9547 ± 150^{d}
E6 - Norway spruce phenolics (partial purification with XAD7HP)	50723 ± 219^{b}	6422 ± 32^{b}	1386 ± 3^{d}	40097 ± 74^{b}	10803 ± 32^{b}
E7 - Norway spruce phenolics (complete purification with XAD7HP)	45590 ± 230^{c}	5904 ± 33^{c}	1475 ± 13^{c}	33729 ± 218^{c}	10439 ± 99^{b}
E8 - Scots pine phenolics (90 °C/60 min)	22187 ± 218^{d}	1312 ± 5^{e}	1348 ± 3^{e}	16695 ± 588^{e}	5235 ± 78^{e}
E9 - Scots pine phenolics (140 °C/60 min)	13332 ± 134^{f}	1249 ± 3^{e}	1294 ± 2^{f}	9488 ± 374^{g}	3279 ± 102^{g}
E10 – Industrial tannin QS-SOL	83746 ± 2501^{a}	6749 ± 8^{a}	2316 ± 2^{a}	81642 ± 1015^{a}	38565 ± 518^{a}

Note: AAE = ascorbic acid equivalents, EDTAE = EDTA equivalents, GAE = gallic acid equivalents, ND = not detected. Different letters in the same column represent statistically different results ($p < 0.05$).

extract includes condensed tannins, stilbene glucosides, such as astringin, isorhapontin, and piceid. Pine bark PHWE extracts have – condensed tannins and sugars. Hemicelluloses of wood can be extracted at higher extraction temperatures at 160–180 °C. Increasing temperature promotes hydrolysis of hemicellulose and changes the properties of water as a solvent. PHWE extracts of hardwood birch are mainly composed of glucuronoxylans with lignin-derived phenolics (Kilpeläinen et al., 2014). Softwood spruce PHW extracts have mainly galactoglucomannans (GGM). More importantly, on an industrial scale, hemicellulose extracts can be concentrated and purified with ultrafiltration and ethanol precipitation (Bhattarai et al., 2019) or centrifugation (Valoppi et al., 2019).

When it comes to new products, compounds, and crude extracts from industrial byproducts, toxicological safety should continually be assessed to ascertain compliance with international guidelines (Pitkanen et al., 2018). As hemicellulose and other polysaccharides can be used as a thickening and stabilising agent in foods and cosmetics and as ingredients in bio-based films and coatings, it is evident that the application of hemicellulose in different products can be diverse. For this purpose, however, studies that ascertain the toxicological safety and potential bioactivities of the raw material or its extract should be meticulously tested (Li et al., 2019; Ragab et al., 2018).

For instance, cell-based experiments with different human cells have shown that crude extracts from rice straw and husk, radix (*Astragalus radix*) byproducts, bamboo (*Phyllostachys pubescens*) processing byproducts (stems), and many other sources have no deleterious effects on normal human cells (Huang et al., 2017; Li et al., 2019; Ragab et al., 2018). These practical examples indicate that these bioactive-rich extracts can be further tested to develop new technological applications in the chemical, pharma and, possibly, food sectors.

Toxicological safety is essential when new crude extracts and ingredients are developed and their bioactivity. The bioactivity study can be done using different *in vitro* chemical-based and cell-based assays. For instance, Wu et al. (2019) assessed the effects of hemicellulose and lignin fractions of mushroom (*Lentinus edodes*) on their antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays and the scavenging of hydroxyl radical assay. Results showed that the fractions (from 2 to 4%) exerted free-radical scavenging activity in a dose-dependent manner, and the activity was correlated to the hemicellulose content. Similarly, Li et al. (2019) analysed the antitumor and immunomodulatory activities of hemicellulose fractions extracted from radix (*Astragalus radix*) byproducts using a mice model of lung cancer. Fractions could inhibit tumour progression in a dose-dependent manner (50 to 200 mg/kg). Moreover, fractions increased the levels of cytokines IL-2, IL-6, and TNF-α in mice serum together with $CD4^{+}$ T cells, thus indicating an immunomodulatory stimulation in cancer-bearing mice. Pap et al. (2021) assessed the chemical and toxicological profile of macerated spruce and pine bark extract. Especially Norway spruce bark extracts showed antioxidative capacity (oxygen-radical antioxidant capacity, cupric-ion antioxidant capacity, and free radical scavenging activity in relation to DPPH) and antiviral activities against Coxsackievirus A9 (CVA9). Assessing antiviral activity has become very important due to yearly viral infections but more so due to the present pandemic caused by SARS-CoV-2.

Coxsackievirus A9 (CVA9) belongs to the enteroviruses, which are human pathogens, causing vast amounts of acute and chronic infections worldwide (Hyöty, 2016; Marjomäki et al., 2015). These viruses are difficult to decontaminate due to their compact protein shell around the viral genome. Presently, there are no non-toxic but still effective ways to decontaminate surfaces and hands to lower the virus load from our surroundings. Nature-derived compounds contain several potential bioactive agents for their protection, which may fight microbes safely.

Several molecules bearing antioxidant and anti-cancer activities may also show antiviral capabilities. Phenolic compounds, e.g., resveratrol, have been suggested to exert several of these actions (Abba et al., 2015). Therefore, it is also relevant to assess the antiviral activity and antioxidant, anti-carcinogenic, and anti-inflammatory actions of extracts containing different bioactive compounds, mainly extracts obtained from industrial byproducts, such as sawdust and bark materials.

Taken together, it is evident that any technological application in which phenolic or functional carbohydrate-rich fractions are incorporated into product models (i.e., cosmetics or foods) should be carefully and meticulously analysed to guide the industry to develop new potentially functional ingredients. Thus, detailed chemical composition, impurity content, bioactivities, and toxicological safety profile play an essential role during the development period. Following this trend, the main objectives of this study are to characterise extracts from Norway spruce (*Picea abis*) bark and sawdust, birch (*Betula pendula*) sawdust, and Scots pine (*Pinus sylvestris*) bark, rich in polyphenols, hemicellulose, and galactomannans, in terms of antioxidant, antidiabetic, antiviral and anti-inflammatory potential, and their effects on human cell's growth and proliferation. Using industrial byproducts to recover bioactive fractions/extracts would significantly contribute to the bio circular economy concepts and align with the Sustainable Goals of the United Nations – Goal 12 – Sustainable Consumption and Production.

2. Materials and methods

2.1. Chemical reagents and cell cultures

Gallic, ascorbic and chlorogenic acids, (+)-catechin, Folin-

Ciocalteu's phenol reagent, 2,4,6-tris(2-pyridyl)-S-triazine (TPTZ), pyrocatechol violet, fluorescein, ferric chloride hexahydrate, dimethyl sulfoxide (DMSO), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) were obtained from Thermo Fischer Scientific (Ward Hill, Massachusetts, USA). Potassium hexacyanoferrate (III) was obtained from Merck (Darmstadt, Hesse, Germany). The other chemical reagents were of analytical grade. A549 – lung adenocarcinoma, HepG2 - hepatocellular carcinoma, HCT8 - human ileocecal colorectal adenocarcinoma, and IMR90 – normal human lung fibroblast cell lines were obtained from the Rio de Janeiro cell bank (Rio de Janeiro, São Paulo, Brazil). Milli-Q ultrapure water was used in the experiments.

2.2. Plant materials and extraction procedures

Ten wood hemicellulose and polyphenols samples were extracted from sawdust or bark with hot water. Samples were extracted from Norway spruce (*Picea abies*), birch (*Betula pendula*), or Scots pine (*Pinus sylvestris*), which were collected from sawmills in the centre and southern Finland. Hot water extractions were conducted on a pilot scale using a 300 L extraction system (Kilpeläinen et al., 2014). Sample amount varied from 70 to 100 kg of fresh bark or sawdust depending on the moisture content and sample density. Details of the extractions, treatments and samples are in Table 1 **- SM.**

Sawdust samples were stored in a freezer at −20 °C before extractions and used as such without milling or other pretreatments. Birch and spruce sawdust samples were extracted in flow-through mode with a continuous flow of hot water through the extraction vessel during the whole 60 min extraction time (Table 1**-SM**). Hemicellulose samples from sawdust were concentrated with ultrafiltration (Bhattarai et al., 2019) and spray dried or purified with ethanol precipitation. Ethanol precipitation was used to remove lignin-derived phenols from hemicellulose. Spruce galactoglucomannan containing samples E1 and E2 mainly consisted of carbohydrates (710–780 mg/g). Birch gluclucurunoxylan samples E3 and E4 contained 580 – 690 mg/g of carbohydrates. The composition of carbohydrates, acetyl groups and extractives of the hemicellulose samples is fully described by Mikkonen et al. (2019). For more information, detailed structural characterisation of the typical hot water extracted glucuronoxylan and galactoglucomannan by nuclear magnetic resonance (NMR) and analytical pyrolysis was carried out elsewhere (Lahtinen et al., 2019).

Spruce and pine bark samples were extracted in batch mode, where the bark was placed inside the reactor and kept for the whole extraction time. In both cases, the extract was collected in a 1000 L container after the extraction. Spruce bark extracts were dried under vacuum in a rotary evaporator (E5), or extract was further purified with XAD7HP adsorbent (E6 and E7) and dried. Details of extraction, treatments, and chemical composition can be found (Varila et al., 2020). Briefly, the extract was eluted through an adsorbent to remove simple sugars. The adsorbent then contained polyphenols that were eluted out with ethanol. Samples E5 – E7 mainly consisted of polyphenols (430–760 mg/g) and carbohydrates (170 – 310 mg/g). Pine bark samples E8 - E9 are from two-stage extraction. The first bark was extracted at 90 °C and then at 140 °C. Samples were concentrated and freeze-dried in the laboratory. As a reference, there was an industrial purified tannin sample Tannino QS-SOL from Silvateam, Italy (Sample E10).

2.3. Total phenolics and chemical antioxidant activity

Freeze-dried extracts (100 mg) were dissolved in water (100 mL) and analysed for total phenolic content and *in vitro* antioxidant activity. The total phenolic content was assayed according to a standardised spectrophotometric Folin-Ciocalteu method, and results were expressed as mg of gallic acid equivalents (GAE) per 100 g (Granato et al., 2015). The DPPH free-radical scavenging activity was assayed using a methanolic DPPH solution at 0.10 mmol/L. Results were expressed as mg of ascorbic acid equivalent per 100 g of extract (mg AAE/100 g). The extracts' ferric reducing antioxidant power (FRAP) was assessed under acidic conditions (pH 3.6), and results were expressed as mg AAE/100 g. The reducing power of extracts was evaluated using the Prussian Blue assay, and results were expressed as mg of gallic acid equivalent per 100 g of extract (mg GAE/100 g). The Cu^{2+} chelating ability was evaluated using pyrocatechol violet as the chromogen agent, and results were expressed as mg of disodium ethylenediaminetetraacetic acid (EDTA) per 100 g of extract (mg EDTA/100 g). All these methods were conducted using the experimental conditions described in our previous works (Fidelis et al., 2020; Santos et al., 2018), and analyses were performed in triplicate.

2.4. Cell viability and proliferation tests and cellular antioxidant activity

Cells were cultured in Dulbecco's modified Eagle's medium F12 Ham (DMEM, Sigma, Germany) with 10% FBS and 1% penicillin/streptomycin, at 37 °C under 5% CO_2. To assess the viability of cells in relation to each extract, A549 (human lung cancer cells), HCT8 (human colorectal cancer cell), HepG2 (human liver cancer cell), and IMR90 (lung normal cell) were seeded into 96 well plates with a density of 1×10^4 cells/well. Twenty-four hours later, cells were treated with the extracts at different concentrations (50, 250, 500, 1000 μg/mL) for 48 h. At the end of the incubation period, the cell viability was evaluated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay, as described by Zhang et al. (2019).

For the cellular antioxidant activity assay, the DCFH-DA (2',7'-dichlorofluorescein diacetate) was used to detect oxidative products from the cell lines (Li et al., 2020). Briefly, all cell lines (6×10^4 cells/well) were treated for 1 h with the extracts previously diluted in DCFH-DA solution (25 mmol/L) at different concentrations (100, 500, and 1000 μg/mL). Cells were treated with or without 15 μmol/L H_2O_2 to induce reactive oxygen species (ROS) and assess ROS induction caused by the extracts. For the positive control, cells were treated with 15 μmol/L H_2O_2. The cells were only treated with a culture medium for the negative control which was taken to reference the percentage of protection against H_2O_2. Intracellular ROS generation was measured as described by do Carmo et al. (2019) using a spectrophotometer (excitation, 485 nm; emission, 530 nm).

2.5. Antihemolytic activity

Extracts were tested to protect human erythrocytes against mechanical hemolysis following the procedures adopted elsewhere (Fidelis et al., 2020). Extracts were solubilised in phosphate saline buffer (PBS, 5 mmol/L, pH 7.35, with NaCl 0.85% w/v) until 1, 5, and 10 μg GAE/g was achieved. Then, extracts were tested under harsh hypotonic (0.1% w/v NaCl), hypotonic (0.4% w/v NaCl), and isotonic conditions (0.85% w/v NaCl). Ultrapure water was used as the positive control for total hemolysis and quercetin at 5 μg/mL as a negative control for comparison purposes. Analyses were performed in triplicate, and results were expressed as a percentage of hemolysis. The ethics committee approved the biological protocol (CAAE 94830318.1.0000.0105).

2.6. Inhibition of α-amylase and α-glucosidase

The inhibition of α-amylase and α-glucosidase activities of the freeze-dried extracts was determined using the methods proposed elsewhere (Johnson et al., 2011). Extracts were diluted in ultrapure water and tested at different concentrations (α-amylase: 1, 5, 10, 15, and 20 mg/mL; α-glucosidase: 10, 25, 50, 100, 200 and 500 μg/mL). For the α-amylase assay, acarbose (1 mmol/L) was used as a positive control. The inhibition of the enzyme activity was expressed in percentage, and, when applicable, IC_{50} values (the concentration required to inhibit enzyme activity by 50%) were obtained in triplicate.

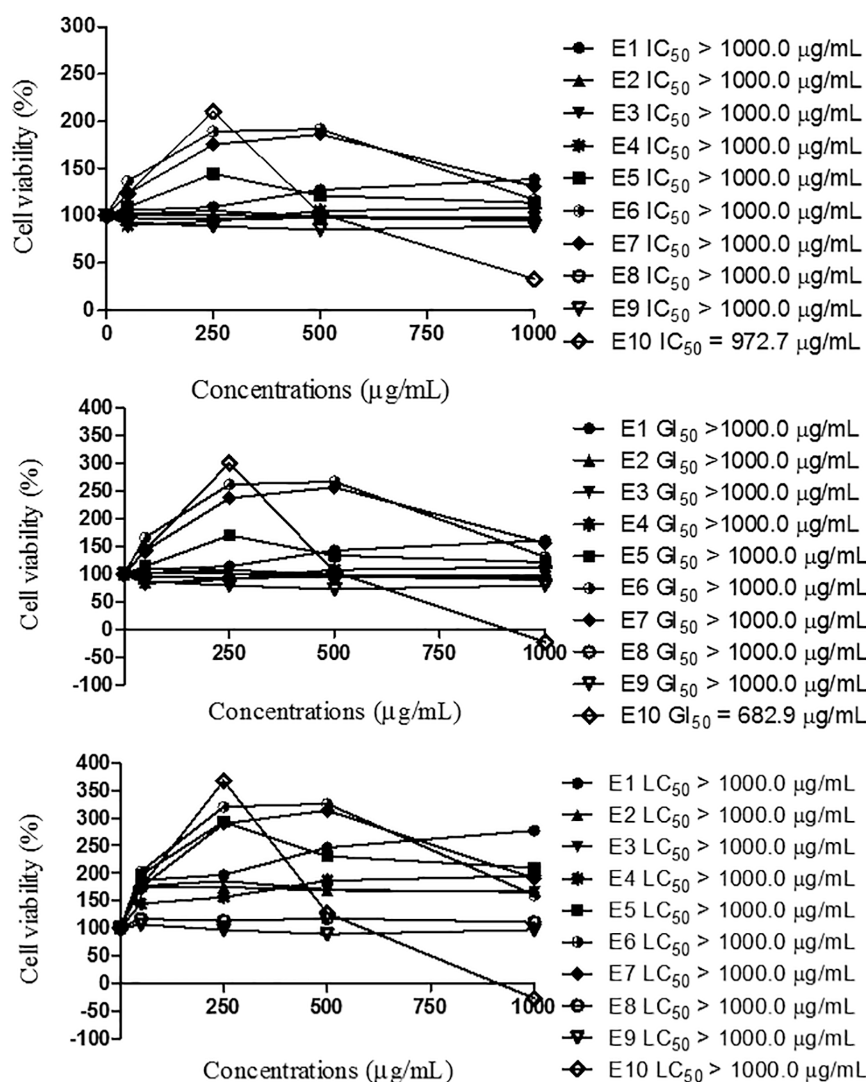


Fig. 1. Cell viability and representative evaluation of the concentration-dependent impact after 48 h exposure of A549 (A, B, C), HepG2 (D, E, F), HCT8 (G, H, I), and IMR90 (J, K, L) cell lines. Values correspond to the IC_{50}, GI_{50}, and LC_{50}. Note: E1 = Norway spruce hemicellulose galactoglucomannan (GGM); E2 = concentrated and ethanol precipitated spruce hemicellulose GGM; E3 = birch hemicellulose xylan; E4 = concentrated and ethanol precipitated xylan; E5 = spruce bark tannin extract; E6 = XAD7HP partially purified spruce bark tannin extract; E7 = XAD7HP completely purified spruce bark tannin extract; E8 = pine bark tannin extract; E9 = pine hemicellulose extract; E10 = Purified industrial tannin. Concentrations are expressed in µg/mL of lyophilized extracts.

2.7. Antiviral effects

Adenocarcinomic human alveolar basal epithelial (A549) cells and Coxsackievirus A9 strain (CVA9; Griggs strain, ATCC) were used in the assays. CVA9 was produced and purified as described before (Ruokolainen et al., 2019). The antiviral activity was assessed using a cytopathic effect (CPE) inhibition assay, modified by Schmidtke et al. (2001). Briefly, A549 cells were plated in 96-well plates at a density of 12,000 cells/well in DMEM supplemented with 10% FBS, 1% GlutaMAX, and 1% penicillin/streptomycin antibiotics. The following day, 2 X 10^8 PFU/mL of CVA9 was pre-incubated with different extracts concentrations for 1 h at 37 °C. The virus-compound mixture was further diluted and added to cells for 24 h incubation at a 5% CO_2 incubator. A similar amount of virus without the extract and mock infection (without the virus and extract) were used as two controls for the experiments. The development of cytopathic effect (CPE) was monitored using light microscopy. For quantification of the CPE, cells were fixed and stained for 10 min with CPE dye (0.03% crystal violet, 2% ethanol, and 36.5% formaldehyde), washed twice with water, and then lysed using a lysis buffer (0.8979 g of sodium citrate and 1 N HCl in 47.5% ethanol) to elute the crystal violet. The absorbance was measured spectrophotometrically at 570 nm using the PerkinElmer VICTOR^{TM} X4 multilabel reader. For the antiviral assay, cytotoxicity was also evaluated using the CPE Inhibition assay, where the compounds (without any virus) were tested for their toxicity on A549 cells for 24 h. Mock infection was used as a control for the assay.

2.8. Statistical analysis

Experimental data are expressed as means ± standard deviation. Multiple comparisons between extractives were performed using one-way analysis of variances (ANOVA) followed by Duncan's multiple range test, considering $p < 0.05$ as significant (Granato, de Araujo Calado, & Jarvis, 2014). Correlation analysis was based on Pearson's correlation coefficient. The software TIBCO Statistica v. 13.3 (TIBCO Software Ltd, Palo Alto, CA, USA) was used in the analyses.

3. Results and discussion

3.1. Total phenolics and antioxidant activity

Table 1 shows that the TPC ranged from 2250 to 83746 mg GAE/100 g, in which samples E5, E6, E7, and E10 presented the highest values. These values corroborate with the data presented by Neiva et al. (2018) and Spinelli et al. (2019). Tannins and stilbenes, phenolic acids, and some flavonoids have already been found from spruce and pine species

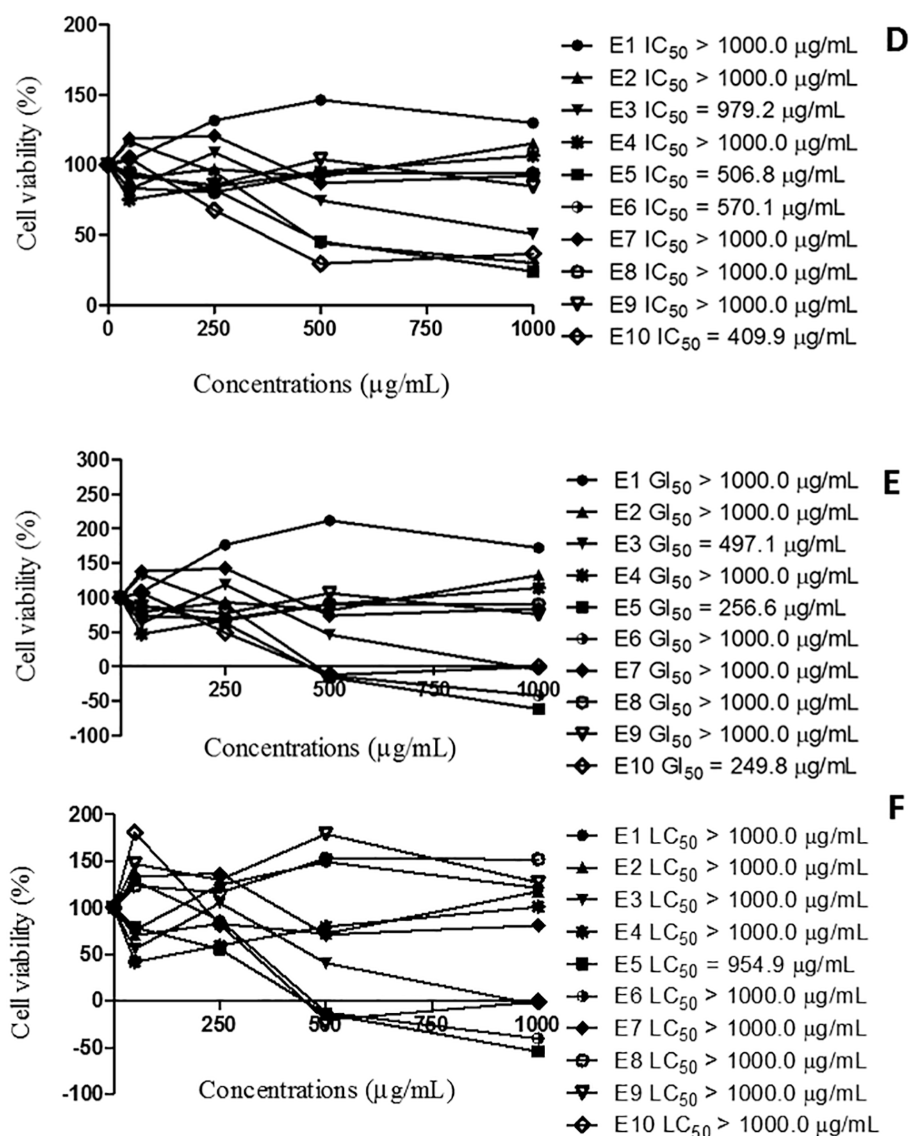


Fig. 1. (*continued*).

(Metsämuuronen and Sirén, 2019; Spinelli et al., 2019; Kim et al., 2020). Regarding the reducing capacity, values ranged from 142 to 6749 mg GAE/100 g, in which phenolic rich-extracts E5, E6, E7, and E10 presented the highest values. FRAP values followed the same trend – phenolic-rich extracts, namely E5 to E8 and E10, gave the highest mean values, while samples E2 and E4, containing carbohydrates, showed the lowest mean values. For the DPPH free radical scavenging activity, the industrial tannin sample, E10, presented the highest antioxidant activity, followed by samples E6/E7, E1, and E5.

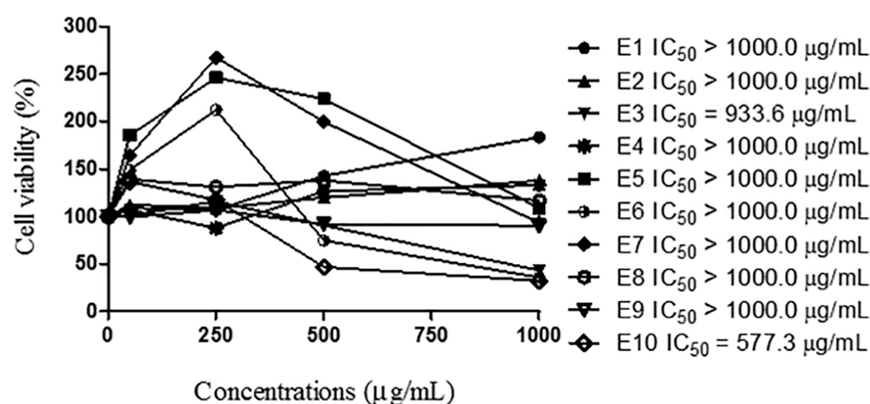
Regarding the Cu^{2+} chelating ability, only sample E3 had a chelation effect from the carbohydrate-rich samples. On the contrary, samples E5-E10 had a clear and pronounced copper chelating effect. From all samples, E10 (purified industrial tannin) presented the highest antioxidant activity using all methodologies, while sample E2 (concentrated and ethanol precipitated spruce hemicellulose GGM) had the opposite effect. Correlation analysis showed that the TPC correlated significantly with DPPH ($r = 0.888$; $p = 0.001$), FRAP ($r = 0.966$; $p < 0.001$), Cu^{2+} chelating ability ($r = 0.846$; $p = 0.002$), and slightly correlated with reducing power ($r = 0.626$; $p = 0.053$), which is in-line with previous studies that aimed to correlate the TPC and *in vitro* antioxidant activity assays (Granato et al., 2015; Santos et al., 2018; Fidelis et al., 2020).

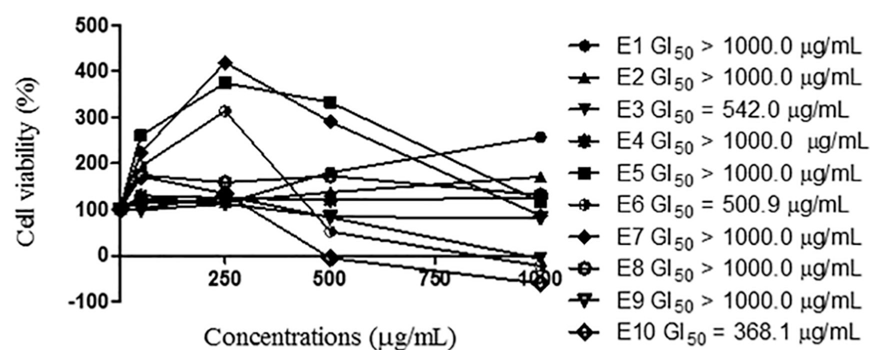
Studies reported that aqueous Norway spruce bark extracts (without further purification) obtained with maceration or ultrasound-assisted extraction (UAE) presented a lower TPC ($TPC_{maceration} = 113.5$ mg GAE/g and $TPC_{UAE} = 84.3$ mg GAE/g) than the values observed for samples E5-E7, implying that the purification procedures using resins/adsorbents are effective in eliminating compounds that do not react with Folin-Ciocalteu. Authors also observed that the aqueous spruce extract (0.5 to 10 mg/mL) presented free-radical scavenging activity towards DPPH (maceration = 42.4 to 84.4% inhibition; UAE = 51.5 to 83.4% inhibition). Similarly, Ferreira-Santos et al. (2020) extracted pine (*Pinus pinaster*) bark with ethanolic solutions ranging from 30 to 90% (v/v) (maceration method). They found TPC ranging from 450 to 700 mg GAE/g, FRAP values ranging from 100 to 140 mmol Fe^{2+}/g, and DPPH values ranging from $IC_{50} = 49$ to 100 μg/mL.

Korean red pine (*Pinus densiflora* Sieb. et Zucc.) bark extracted with water showed to be a source of protocatechuic acid, procyanidin B1, catechin, caffeic acid, vanillin, and taxifolin, totalling 44 mg/g of freeze-dried extract (Kim et al., 2020). This phenolic-rich extract showed antihypertensive and antioxidant in Wistar-Kyoto rats.

3.2. Cell viability tests and cellular antioxidant activity

Different concentrations of the extracts (from 50 to 1000 μg/mL) were used to investigate the relative potential of cytotoxicity and growth inhibition against cancer (A549, HCT8, and HepG2) and normal





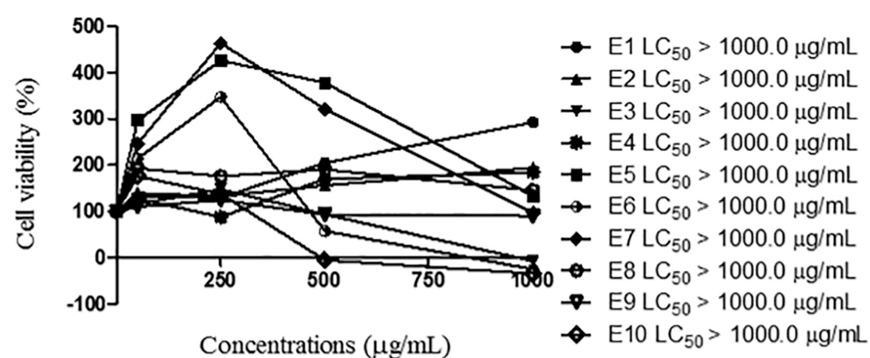


Fig. 1. (*continued*).

(IMR90) cell lines (Fig. 1). In general, the extracts presented non-toxic *in vitro* profiles. For A549 cells, only E10 (pure tannin extract) caused growth inhibition, with a GI_{50} value of 682.9 μg/mL. In contrast, the HepG2 cell line seemed to be more sensitive to the extract effects since E3, E5, E6, and E10 revealed antiproliferative effects (GI_{50} values from 249.8 to 497.1 μg/mL). In comparison, E5 exhibited cytotoxic activity with a lethal concentration (LC_{50}) of 954.9 μg/mL. The GI_{50} values for HCT8 varied between 368.1 and 542 μg/mL for E3, E6, and E10. Considering the normal cell (IMR90), only E9 showed an anti-proliferative effect in lower concentrations (GI_{50} = 153 μg/mL), although this sample presented no cytotoxic activity (LC_{50} > 1000 μg/mL). *Lunasia amara* wood extracts, which contained alkaloids and lunacrine in their main chemical composition, showed cytotoxicity (IC_{50} from 71.1 to 412.4 μg/mL) against T47D (human breast cancer cell) and HeLa (cervix adenocarcinoma) cells. Thus, it is possible to infer that the effects of different extracts from woods are highly associated with their chemical composition.

Overall, considering the three cancer cell lines used in the present study, E10 displayed a higher potential in interfering with cell viability, followed by E3. Our results can be of technological importance, as they can be the basis for developing food and cosmetic models.

In a previous work conducted by Coșarcă et al. (2018), Spruce bark extract and beech bark (TPC = 40.2 mg GAE/g) did not show cytotoxicity of A375 (human melanoma), A549 (lung carcinoma), and normal human keratinocytes (HaCaT), implying the extracts show no cytotoxicity at a maximum concentration of 2.5 mg/mL. Similarly, *Pinus pinaster* bark extracts (water and ethanol mixtures) at concentrations up to 1000 μg/mL stimulated the growth of murine fibroblast cells (L929) and embryonic kidney cells (HEK293T) – and inhibited the growth of A549 cancerous cells (Ferreira-Santos et al., 2020).

Concerning the intracellular antioxidant activity data shown in Fig. 2, the extracts presented different results concerning cell viability when distinct cell lines were analysed. Briefly, for HCT8 cells, E1, E3, E5-E10 exhibited pro-oxidant effects, while E2 and E4 protected the cells against H_2O_2 action at 100 μg/mL. For A549 cells, E1-E3, E6, E7, and E10 increased the ROS generation when associated with H_2O_2. In contrast, E8 and E9 exerted antioxidant effects by decreasing ROS generation. Herein, in HepG2 cells, E3 and E4 decreased the ROS levels induced by H_2O_2. Interestingly, the same structural features responsible for the antioxidant activity of phenolic compounds are also related to their pro-oxidant properties, showing a selective behaviour (Fidelis et al., 2020; de Mejia et al., 2010). Moreover, our findings displayed that

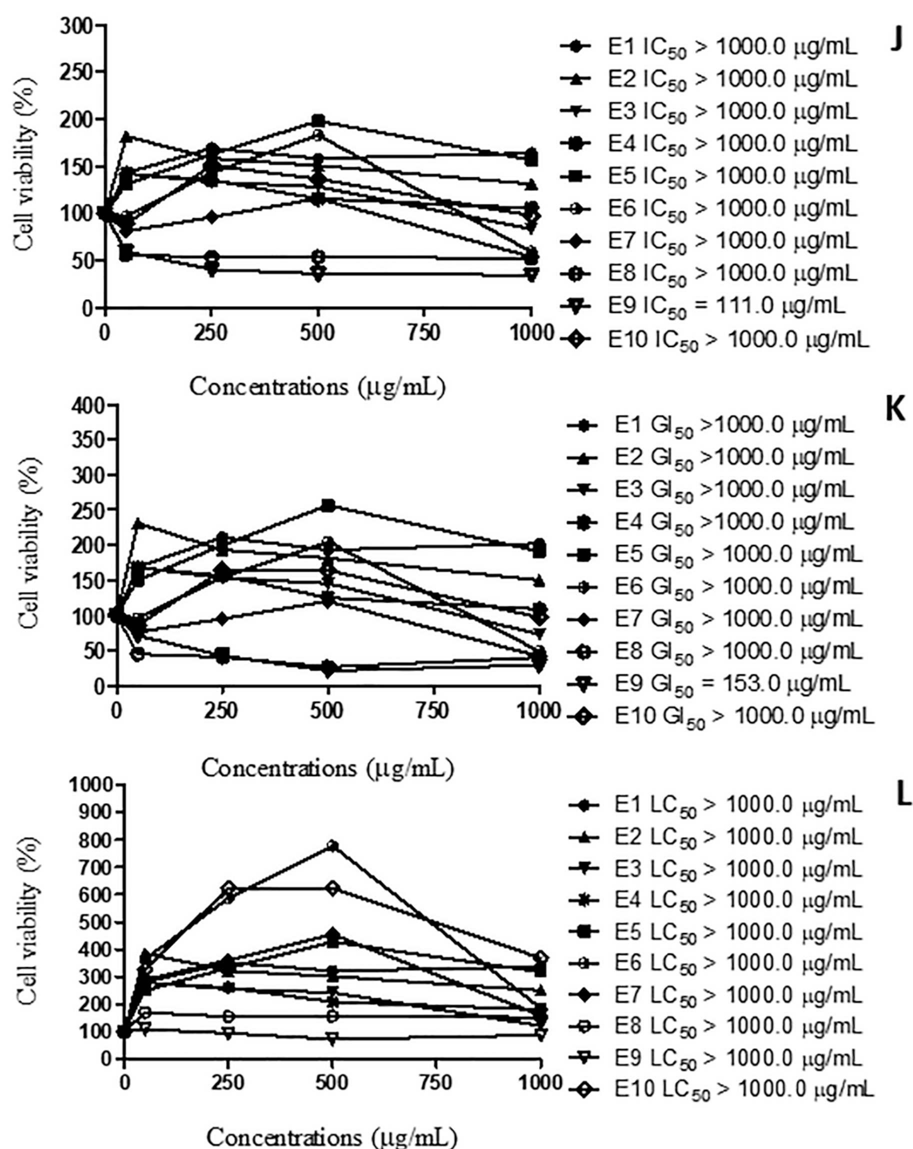


Fig. 1. (*continued*).

the same extract exerted different behaviour acting as an antioxidant or pro-oxidant depending on the cell line. This behaviour could be attributed to the variation in the specific levels of oxygen species that each cell line deals with to maintain its homeostasis, besides peculiarities such as origin, mutations and genetic variability, and microenvironment (Carvalho et al., 2019). This ROS variation strongly impacts cellular metabolism by regulating signalling, proliferation, and differentiation (Harris and DeNicola, 2020). Correlation analysis was performed and we verified that the GI_{50} values of HCT8 and A549 were significantly correlated with the antioxidant activity measured by the DPPH ($r = -0.644$; $p = 0.045$ and $r = -0.944$; $p < 0.001$, respectively), FRAP ($r = -0.721$; $p = 0.019$ and $r = -0.842$; $p = 0.002$, respectively), and TPC ($r = -0.640$; $p = 0.046$ and $r = -0.731$; $p = 0.016$, respectively). No significant correlation ($p > 0.05$) was found between TPC, antioxidant activity, and the GI_{50} values of IMR90 and HepG2 cell lines (data not shown).

Indeed, we remarked that for normal IMR90, extracts did not induce ROS generation, and they were able to reestablish the ROS levels to the same magnitude as that of the negative control. Normal cells adaptations may drive these findings and different results among cell lines in overcoming the damaging effects of ROS through the balanced generation of these species, sufficient antioxidant activity, and cellular repair, which result in low concentrations of ROS, toward to limited cell survival and proliferation (do Carmo et al., 2018).

3.3. Antihemolytic activity

In the isotonic condition ([NaCl] = 0.8% w/v), none of the samples presented hemolytic property (Fig. 3**A**) since these compounds (5 µg GAE/mL) did not cause a significant increase in the hemolysis rate. Contrariwise, the samples significantly ($p \leq 0.05$) decreased the hemolysis rate, presenting efficiency similar to that achieved with quercetin 5 µg/mL, excepting the E3 and E9 samples. E1, E4, and E9 reached the highest protective effect, at this osmotic condition and among the tested concentrations, at 1 µg GAE/Ml (Fig. 3**B**). The maximum erythrocyte protection of the other samples was achieved at 5 µg GAE/mL (E2, E5, E7 and E10) or 10 µg GAE/mL (E3, E6, and E8), thus showing a dose-dependent behaviour.

In the hypotonic condition ([NaCl] = 0.4% w/v), the erythrocytes were osmotically fragilised and, compared to the positive control (PBS), all samples (5 µg GAE/mL) reduced the hemolysis rate significantly ($p \leq 0.05$) (Fig. 3**C**).

Although carbohydrates have already been demonstrated (Escher et al., 2019), anti-hemolytic activity is more commonly attributed to phenolic compounds (Olchowik et al., 2012). In this work, tannin-rich

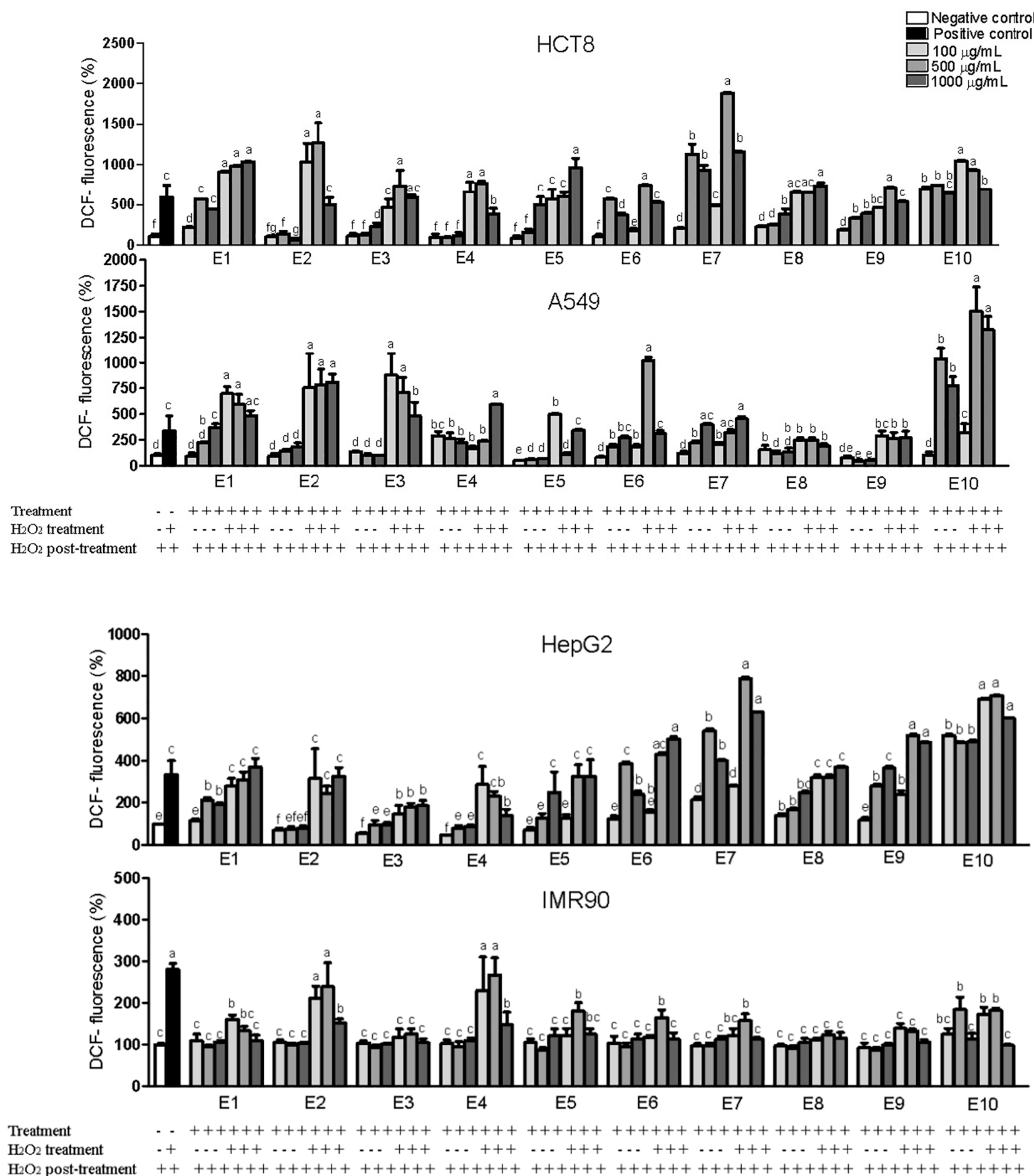


Fig. 2. Results of intracellular ROS measurement in HCT8, A549, HepG2, and IMR90 cell lines. Treatments tested at 10 – 100 μg/mL: E1 = Norway spruce hemicellulose galactoglucomannan (GGM); E2 = concentrated and ethanol precipitated spruce hemicellulose GGM; E3 = birch hemicellulose xylan; E4 = concentrated and ethanol precipitated xylan; E5 = spruce bark tannin extract; E6 = XAD7HP partially purified spruce bark tannin extract; E7 = XAD7HP completely purified spruce bark tannin extract; E8 = pine bark tannin extract; E9 = pine hemicellulose extract; E10 = Purified industrial tannin. Quantitative data are expressed as the mean ± standard deviation and different letters represent statistically different results ($p < 0.05$).

extracts displayed anti-hemolytic efficiency similar to the hemicellulose-rich extracts. This similarity occurs because, although the tannins are phenolic compounds, their rigid structure makes it difficult to penetrate the cell membrane and offer protection (Weber-Lotfi et al., 2002). The antihemolytic effect was already reported for needle spruce extracts (Rasouli et al., 2017), but our paper is the first report involving sawdust and bark extracts. As for wild pine, a previous study indicates the possibility of bark extracts acting as antihemolytic agents (Harris et al., 2008), which was confirmed herein.

Hemolysis rate was not correlated to any of the antioxidant activity assays ($r < 0.500$, $p > 0.340$) and TPC ($r = -0.360$, $p = 0.308$), thus implying that the protective action of human erythrocytes is not only associated with phenolic compounds or functional carbohydrates separately. Two possible hypotheses emerge from the data: carbohydrates, in solution, may form a colloidal system that exerts a physical barrier that impedes hemolysis; phenolic compounds and carbohydrates interact, creating a physicochemical barrier that protects erythrocytes from hemolysis.

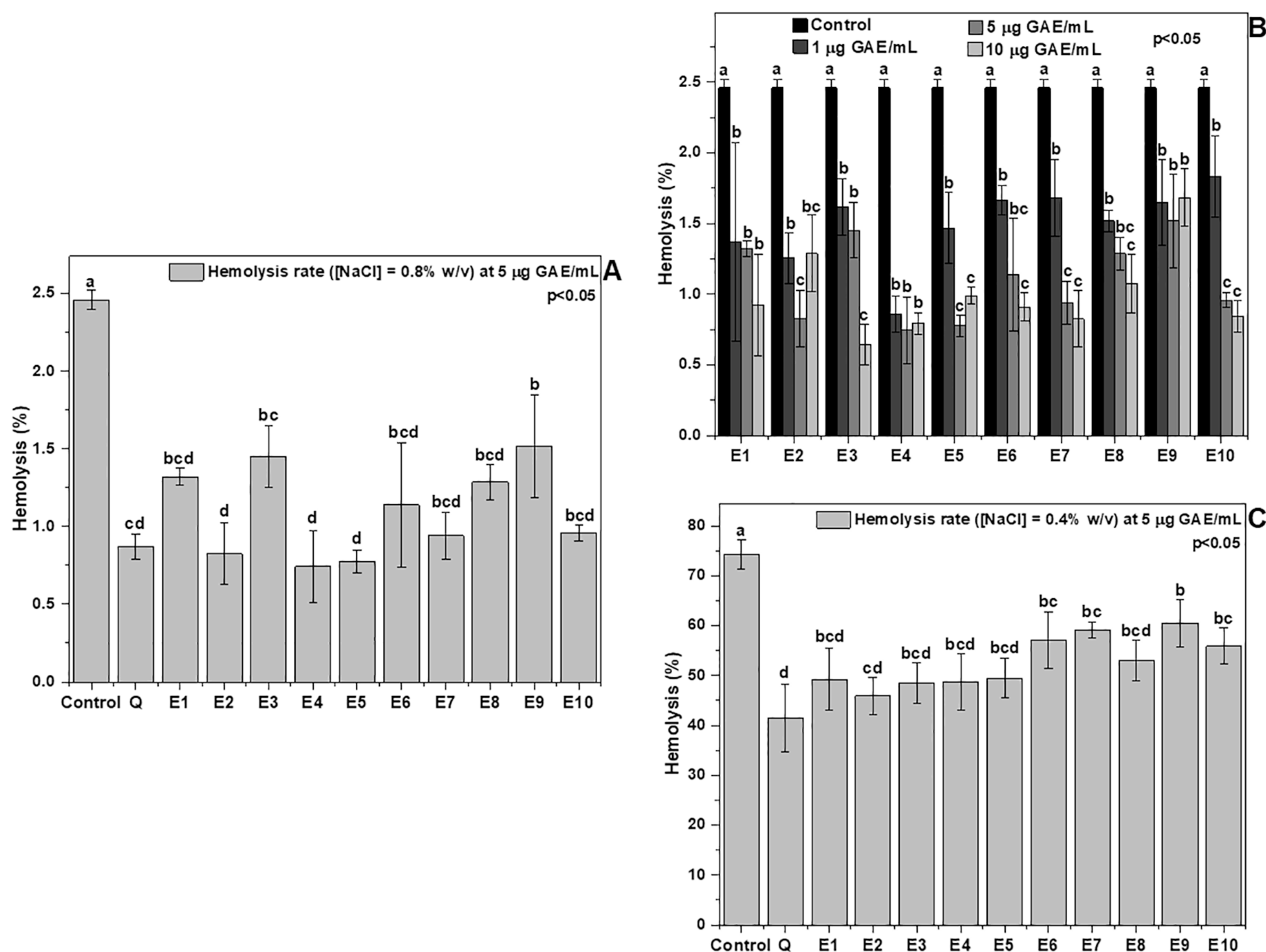


Fig. 3. Antihemolytic behavior of extracts (5 μg/mL) compared to quercetin (5 μg/mL) in different osmolarity conditions: NaCl at 0.8% w/v (E, isotonic condition), NaCl at 0.4% w/v (C, hypotonic condition), and 0.1% w/v (A, harsh hypotonic condition). The dose-dependent effect of the samples in all osmotic conditions is shown in B, D and F. Note: E1 = Norway spruce hemicellulose galactoglucomannan (GGM); E2 = concentrated and ethanol precipitated spruce hemicellulose GGM; E3 = birch hemicellulose xylan; E4 = concentrated and ethanol precipitated xylan; E5 = spruce bark tannin extract; E6 = XAD7HP partially purified spruce bark tannin extract; E7 = XAD7HP completely purified spruce bark tannin extract; E8 = pine bark tannin extract; E9 = pine hemicellulose extract; E10 = Purified industrial tannin. Different letters represent statistically different results ($p < 0.05$).

3.4. Inhibition of α-amylase and α-glucosidase

Regarding the inhibition of α-amylase (Table 2), it is possible to note that samples E1, E2, and E4 presented the highest IC_{50} values (α-amylase: 22 to > 500 mg/mL). In contrast, samples E5 to E7 and E10 gave the opposite behaviour (α-amylase: 0.46 to 2.68 mg/mL). Regarding the inhibition of α-glucosidase, samples E10, E5 to E8 presented the highest enzyme inhibition (IC_{50} values ranging from 6.7 to 137 μg/mL), while samples E2 to E4 gave the lowest enzyme inhibition (IC_{50} values ranging from 1159 to 3821 μg/mL). Sample E10, which corresponds to the phenolic-rich fraction, presented the highest inhibition of the enzymes, proving that phenolic compounds are very active in inhibiting these key enzymes' activity (Harris et al., 2008). Similarly, Ferreira-Santos et al. (2020) extracted *Pinus pinaster* bark with ethanolic solutions ranging from 30 to 90% (v/v). They found that the extract inhibited both α-amylase (IC_{50} ranging from 250 to 537 μg/mL) and α-glucosidase (IC_{50} ranging from 122 to 11,000 μg/mL). Our results corroborate the findings obtained with bark extracts from *Picea glauca* (Jiang et al., 2017) and *Eucalyptus grandis* and *E. urophylla* (Vadivel and Biesalski, 2011).

Recent evidence supports specific non-covalent bonds between different polyphenols (mainly flavonoids and tannins) and carbohydrate polymers via hydrogen bonds and hydrophobic interactions, so it is not surprising that samples E5-E10 showed the highest potential as enzyme inhibitors (Vadivel and Biesalski, 2011). Correlation analysis showed that the inhibition of α-glucosidase (IC_{50}) was significantly associated with TPC ($r = -0.675$, $p = 0.032$), Cu^{2+} chelating ability ($r = -0.723$, $p = 0.018$) and reducing power ($r = -0.868$, $p = 0.001$). Similarly, the inhibition of α-amylase (IC_{50}) was significantly associated with the reducing power ($r = -0.846$, $p = 0.002$) but not with TPC ($r = -0.486$, $p = 0.155$). These results are in-line with those obtained earlier (Deby-Dupont et al., 2005) with extracts of Karanja (*Pongamia pinnata*), rich in phenolics. Extracts with higher TPC presented higher antioxidant activity (FRAP and DPPH) and higher inhibition of α-glucosidase and α-amylase activities.

3.5. Antiviral activity

Herein, the potential of bark extracts was tested for their capability to rescue cells from infection against CVA9, and results are shown in Fig. 4. Samples E1 to E4 are spruce (E1-E2) and birch-derived extracts (E3-E4) enriched in hemicellulose only (E2, E4) or also containing lignin-derived phenols (E1, E3). To our surprise, none of the hemicellulose extracts with or without lignin showed potency to protect against

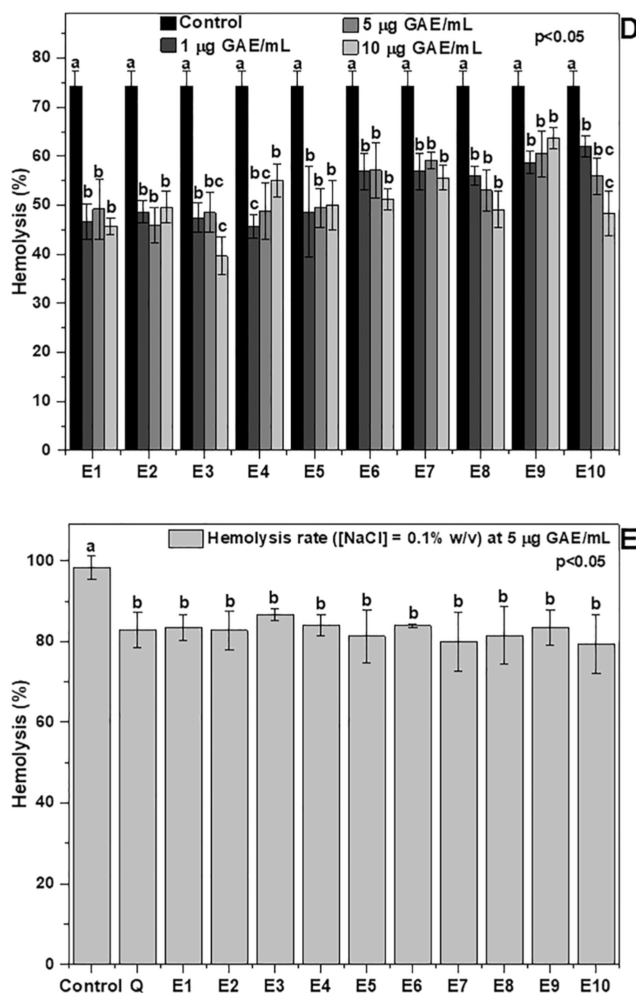


Fig. 3. (*continued*).

Table 2
In vitro antidiabetic potential of Norway spruce galactoglucomannan, birch glucuronoxylan, Norway spruce and Scots pine phenolic-rich extracts.

Samples	α-Glucosidase (IC_{50} μg/mL)	α-Amylase (IC_{50} mg/mL)
E1 - Norway spruce galactoglucomannan	274 ± 12	58.17 ± 6.07
E2 - Norway spruce galactoglucomannan (ethanol precipitation)	3821 ± 931	>500
E3 - Birch glucuronoxylan	1159 ± 172	22.98 ± 2.70
E4 - Birch glucuronoxylan (ethanol precipitation)	3493 ± 881	46.24 ± 6.94
E5 - Norway spruce phenolics (crude extract)	37.39 ± 0.72	2.68 ± 0.05
E6 - Norway spruce phenolics (partial purification with XAD7HP)	18.43 ± 0.96	1.06 ± 0.02
E7 - Norway spruce phenolics (complete purification with XAD7HP)	19.90 ± 4.20	2.27 ± 0.03
E8 - Scots pine phenolics (90 °C/60 min)	137 ± 7	31.46 ± 0.77
E9 - Scots pine phenolics (140 °C/60 min)	381 ± 38	35.19 ± 1.01
E10 – Industrial tannin QS-SOL	6.73 ± 0.45	0.46 ± 0.01

CVA9 infection with used concentrations from 1 to 200 μg/mL. In contrast, all the samples containing tannin fractions, from spruce (E5-E7) and pine (E8 and E9), were very effective against CVA9. Total rescue of cells against viral infection was gained with 50 μg/mL, and even 1 μg/mL showed>50% rescue for most samples. The purified tannin showed somewhat better recovery at 1 μg/mL concentration, altogether confirming that tannins show excellent antiviral efficacy against enterovirus infection. In addition, the charged sugars present in the pine sample E8 showed somewhat better antiviral potential than E9 without the sugars at 1 μg/mL concentration, suggesting that the presence of sugars could be beneficial in some bark preparations. It is noteworthy that none of the tested samples was cytotoxic at used concentrations during 24 h tested in A549 cells.

Correlation analysis showed that the antiviral activity is closely associated with FCRC ($r = 0.709$, $p = 0.032$) and Cu^{2+} chelating ability ($r = 0.916$, $p = 0.001$). The antiviral activity of extracts was significantly correlated to TPC ($r = 0.750$, $p = 0.020$), which implies that phenolic compounds are the main responsible antiviral agents. Another observation should be pondered: the higher the antioxidant activity, the higher the antiviral and anti-inflammatory activity of the extracts from the species studied. This work can be the basis for future works dedicated to isolating specific bioactive compounds or standardising extracts with specific bioactivity.

4. Conclusions

Different extracts from Norway spruce birch sawdust and Scots pine bark were analysed for their bioactive potential. Results showed that the antidiabetic potential, antioxidant activity, in terms of the free-radical scavenging activity, reducing effect, and metal chelation mainly were associated with the total phenolic content of the extracts. None of the

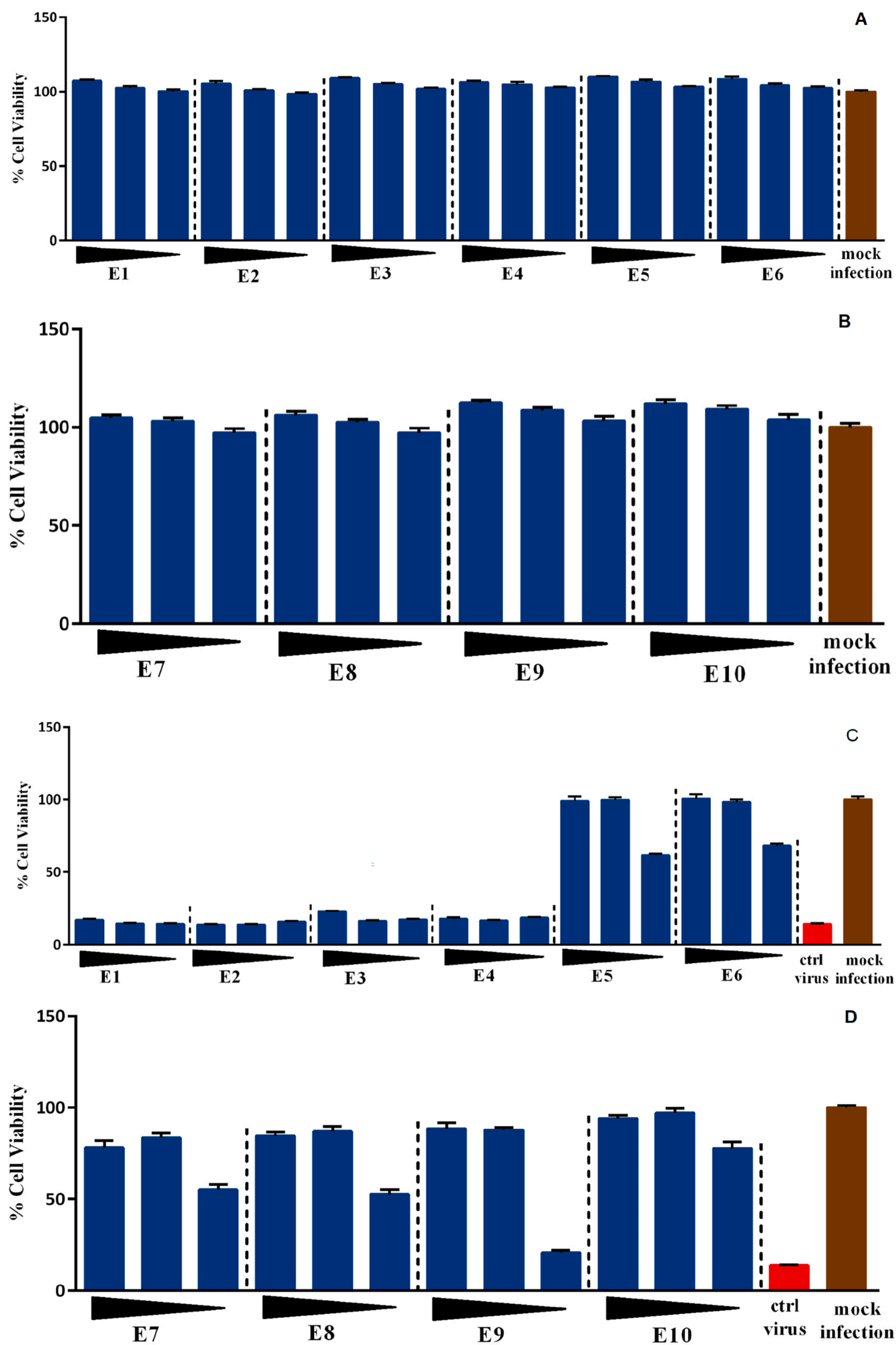


Fig. 4. Cytotoxicity (A, B), and antiviral efficacy (C, D) of the samples E1 - E10 (1, 50 and 200 μg/mL) tested on human A549 cells. For the antiviral assessment, CoxsackievirusA9 (2×10^8 PFU/mL) was treated with increasing concentrations (1, 50 and 200 μg/mL) of E1-E10 samples. Note: E1 = Norway spruce hemicellulose galactoglucomannan (GGM); E2 = concentrated and ethanol precipitated spruce hemicellulose GGM; E3 = birch hemicellulose xylan; E4 = concentrated and ethanol precipitated xylan; E5 = spruce bark tannin extract; E6 = XAD7HP partially purified spruce bark tannin extract; E7 = XAD7HP completely purified spruce bark tannin extract; E8 = pine bark tannin extract; E9 = pine hemicellulose extract; E10 = Purified industrial tannin.

extracts was toxic to normal human IMR90 cells, and the other cell lines observed low (or inexistent) toxicity. On the contrary, pronounced protection of human erythrocytes was observed for all the extracts. Considering the relative toxicological safety of the extracts using different biological assays and human cells, food models, and cosmetic applications using the extracts coming from forest industry byproducts represent a green-based approach to produce new functional ingredients for high value-added applications.

CRediT authorship contribution statement

Daniel Granato: Data curation, Formal analysis, Writing – original draft, Methodology. **Dhanik Reshamwala:** Investigation, Formal analysis, Writing – original draft. **Risto Korpinen:** Investigation, Formal analysis, Writing – original draft. **Luciana Azevedo:** Investigation, Formal analysis, Writing – original draft. **Mariana Araújo Vieira do Carmo:** Investigation, Formal analysis, Writing – original draft. **Thiago Mendanha Cruz:** Investigation, Formal analysis, Writing – original draft. **Mariza Boscacci Marques:** Investigation, Formal analysis, Writing – original draft. **Mingchun Wen:** Investigation, Formal analysis. **Liang Zhang:** Investigation, Formal analysis, Writing – original draft. **Varpu Marjomäki:** Investigation, Formal analysis, Writing – original draft. **Petri Kilpeläinen:** Conceptualization, Methodology, Project administration, Funding acquisition, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.132284.

References

Abba, Y., Hassim, H., Hamzah, H., & Noordin, M. M. (2015). Antiviral activity of resveratrol against human and animal viruses. *Advances in Virology, 2015*, 1–7. https://doi.org/10.1155/2015/184241

Bhattarai, M., Pitkänen, L., Kitunen, V., Korpinen, R., Ilvesniemi, H., Kilpeläinen, P. O., … Mikkonen, K. S. (2019). Functionality of spruce galactoglucomannans in oil-in-water emulsions. *Food Hydrocolloids, 86*, 154–161. https://doi.org/10.1016/j.foodhyd.2018.03.020

Carvalho, T. M. A., Cardoso, H. J., Figueira, M. I., Vaz, C. V., & Socorro, S. (2019). The peculiarities of cancer cell metabolism: A route to metastasization and a target for therapy. *European Journal of Medical Chemistry, 171*, 343–363. https://doi.org/10.1016/j.ejmech.2019.03.053

Coşarcă, S.-L., Moacă, E.-A., Tanase, C., Muntean, D. L., Pavel, I. Z., & Dehelean, C. A. (2018). Spruce and beech bark aqueous extracts: Source of polyphenols, tannins and antioxidants correlated to *in vitro* antitumor potential on two different cell lines. *Wood Science and Technology, 53*(2), 313–333. https://doi.org/10.1007/s00226-018-1071-5

Deby-Dupont, G., Mouithys-Mickalad, A., Serteyn, D., Lamy, M., & Deby, C. (2005). Resveratrol and curcumin reduce the respiratory burst of Chlamydia-Primed THP-1 cells. *Biochemical and Biophysical Research Communications, 333*(1), 21–27. https://doi.org/10.1016/j.bbrc.2005.05.073

Carmo, M. A. V. D., Fidelis, M., Pressete, C. G., Marques, M. J., Castro-Gamero, A. M., Myoda, T., … Azevedo, L. (2019). Hydroalcoholic *Myrciaria dubia* (Camu-Camu) seed extracts prevent chromosome damage and act as antioxidant and cytotoxic agents. *Food Research International, 125*, 108551. https://doi.org/10.1016/j.foodres.2019.108551

Đorđević, T., & Antov, M. (2018). The influence of hydrothermal extraction conditions on recovery and properties of hemicellulose from wheat chaff – A modelling approach. *Biomass and Bioenergy, 119*, 246–252. https://doi.org/10.1016/j.biombioe.2018.09.030

Escher, G. B., Borges, L. C. C., Santos, J. S., Cruz, T. M., Marques, M. B., do Carmo, M. A. V., Azevedo, L., Furtado, M. M., Sant'Ana, A. S., Wen, M., Zhang, L., Granato, D., 2019. From the Field to the Pot: Phytochemical and Functional Analyses of *Calendula officinalis* L. Flower for Incorporation in an Organic Yogurt. Antioxidants. 8 (11), 559. Doi: 10.3390/antiox8110559.

Ferreira-Santos, P., Genisheva, Z., Botelho, C., Santos, J., Ramos, C., Teixeira, J. A., & Rocha, C. M. R. (2020). Unravelling the biological potential of *Pinus pinaster* bark extracts. *Antioxidants., 9*(4), 334. https://doi.org/10.3390/antiox9040334

Fidelis, M., do Carmo, M. A. V., da Cruz, T. M., Azevedo, L., Myoda, T., Miranda Furtado, M., … Granato, D. (2020). Camu-Camu seed (*Myrciaria dubia*) - From side stream to an antioxidant, antihyperglycemic, antiproliferative, antimicrobial, antihemolytic, anti-inflammatory, and antihypertensive ingredient. *Food Chemistry, 310*, 125909. https://doi.org/10.1016/j.foodchem.2019.125909

de Mejia, E. G., Song, Y. S., Heck, C. I., & Ramirez-Mares, M. (2010). Yerba mate tea (*Ilex paraguariensis*): Phenolics, antioxidant capacity and *in vitro* inhibition of colon cancer cell proliferation. *Journal of Functional Foods, 2*(1), 23–34. https://doi.org/10.1016/j.jff.2009.12.003

Granato, D., Koot, A., Schnitzler, E., & van Ruth, S. M. (2015). Authentication of geographical origin and crop system of grape juices by phenolic compounds and antioxidant activity using chemometrics. *Journal of Food Science, 80*(3), C584–C593. https://doi.org/10.1111/jfds.2015.80.issue-310.1111/1750-3841.12794

Granato, D., Calado, V. M. A., & Jarvis, B. (2014). Observations on the use of statistical methods in food science and technology. *Food Research International, 55*, 137–149.

Harris, C. S., Lambert, J., Saleem, A., Coonishish, J., Martineau, L. C., Cuerrier, A., … Bennett, S. A. L. (2008). Antidiabetic activity of extracts from needle, bark, and cone of *Picea Glauca.*: Organ-specific protection from glucose toxicity and glucose deprivation. *Pharmaceutical Biology, 46*(1-2), 126–134. https://doi.org/10.1080/13880200701735080

Harris, I. S., & DeNicola, G. M. (2020). The complex interplay between antioxidants and ROS in cancer. *Trends in Cell Biology, 30*(6), 440–451. https://doi.org/10.1016/j.tcb.2020.03.002

Huang, J.-Q., Qi, R.-T., Pang, M.-R., Liu, C., Li, G.-y., & Zhang, Y. (2017). Isolation, chemical characterization, and immunomodulatory activity of naturally acetylated hemicelluloses from bamboo shavings. *J. Zhejiang Univ.-SCI.B, 18*(2), 138–151. https://doi.org/10.1631/jzus.B1500274

Hyöty, H. (2016). Viruses in Type 1 Diabetes. *Pediatric Diabetes., 17*(S22), 56–64. https://doi.org/10.1111/pedi.12370

Jiang, P., Xiong, J., Wang, F., Grace, M. H., Lila, M. A., & Xu, R. (2017). α -Amylase and α-Glucosidase Inhibitory Activities of Phenolic Extracts from Eucalyptus Grandis × E. Urophylla Bark. *Journal of Chemistry, 2017*, 1–7. https://doi.org/10.1155/2017/8516964

Johnson, M. H., Lucius, A., Meyer, T., & de Mejia, E. G. (2011). Cultivar evaluation and effect of fermentation on antioxidant capacity and *in vitro* inhibition of α-amylase and α-glucosidase by highbush blueberry (*Vaccinium corombosum*). *Journal of Agriculture and Food Chemistry, 59*(16), 8923–8930. https://doi.org/10.1021/jf201720z

Kilpeläinen, P. O., Hautala, S. S., Byman, O. O., Tanner, L. J., Korpinen, R. I., Lillandt, M.-K.-J., … Ilvesniemi, H. S. (2014). Pressurized hot water flow-through extraction system scale up from the laboratory to the pilot scale. *Greem Chemistry, 16*(6), 3186–3194. https://doi.org/10.1039/C4GC00274A

Kim, K. J., Hwang, E.-S., Kim, M.-J., Park, J.-H., & Kim, D.-O. (2020). Antihypertensive effects of polyphenolic extract from korean red pine (*Pinus densiflora* Sieb. et Zucc.) bark in spontaneously hypertensive rats. *Antioxidants., 9*(4), 333. https://doi.org/10.3390/antiox9040333

Lahtinen, M. H., Valoppi, F., Juntti, V., Heikkinen, S., Kilpeläinen, P. O., Maina, N. H., & Mikkonen, K. S. (2019). Lignin-rich PHWE hemicellulose extracts responsible for extended emulsion stabilization. *Frontiers in Chemistry, 7*, 1–18. https://doi.org/10.3389/fchem.2019.00871

Li, K.e., Li, S., Wang, D.i., Li, X., Wu, X., Liu, X., … Du, Y. (2019). Extraction, characterization, antitumor and immunological activities of hemicellulose polysaccharide from *Astragalus radix* herb residue. *Molecules, 24*(20), 3644. https://doi.org/10.3390/molecules24203644

Li, W., Li, D., Kuang, H., Feng, X., Ai, W., Wang, Y., … Fan, R. (2020). Berberine Increases Glucose Uptake and Intracellular ROS Levels by Promoting Sirtuin 3 Ubiquitination. *Biomedicine & Pharmacotherapy, 121*, 109563. https://doi.org/10.1016/j.biopha.2019.109563

Marjomäki, V., Turkki, P., & Huttunen, M. (2015). Infectious Entry Pathway of Enterovirus B Species. *Viruses, 7*, 6387–6399. https://doi.org/10.3390/v7122945

Metsämuuronen, S., & Sirén, H. (2019). Bioactive Phenolic Compounds, Metabolism and Properties: A Review on Valuable Chemical Compounds in Scots Pine and Norway Spruce. *Phytochemistry Reviews, 18*(3), 623–664. https://doi.org/10.1007/s11101-019-09630-2

Mikkonen, K. S., Kirjoranta, S., Xu, C., Hemming, J., Pranovich, A., Bhattarai, M., … Willför, S. (2019). Environmentally-Compatible Alkyd Paints Stabilized by Wood Hemicelluloses. *Industrial Crops and Products, 133*, 212–220. https://doi.org/10.1016/j.indcrop.2019.03.017

Neiva, D. M., Araújo, S., Gominho, J., Carneiro, A.d. C., Pereira, H., & Lightfoot, D. A. (2018). An Integrated Characterization of *Picea abies* Industrial Bark Regarding Chemical Composition, Thermal Properties and Polar Extracts Activity. *PLoS ONE, 13*(11), e0208270. https://doi.org/10.1371/journal.pone.0208270

Olchowik, E., Lotkowski, K., Mavlyanov, S., Abdullajanova, N., Ionov, M., Bryszewska, M., & Zamaraeva, M. (2012). Stabilization of Erythrocytes against Oxidative and Hypotonic Stress by Tannins Isolated from Sumac Leaves *(Rhus typhina* L.) and Grape Seeds (*Vitis vinifera* L.). *Cellular & Molecular Biology Letters, 17* (3), 333–348. https://doi.org/10.2478/s11658-012-0014-7

Pap, N., Reshamwala, D., Korpinen, R., Kilpeläinen, P., Fidelis, M., Furtado, M. M., … Granato, D. (2021). Toxicological and bioactivity evaluation of blackcurrant press cake, sea buckthorn leaves and bark from Scots pine and Norway spruce extracts under a green integrated approach. *Food and Chemical Toxicology, 153*, 112284. https://doi.org/10.1016/j.fct.2021.112284

Pitkänen, L., Heinonen, M., & Mikkonen, K. S. (2018). Safety considerations of plant polysaccharides for food use: A case study on phenolic-rich softwood galactoglucomannan extract. *Food & Function, 9*(4), 1931–1943. https://doi.org/10.1039/C7FO01425B

Ragab, T. I. M., Amer, H., Mossa, A. T., Emam, M., Hasaballah, A. A., & Helmy, W. A. (2018). Anticoagulation, Fibrinolytic and the Cytotoxic Activities of Sulfated Hemicellulose Extracted from Rice Straw and Husk. *Biocatalysis and Agricultural Biotechnology, 15*, 86–91. https://doi.org/10.1016/j.bcab.2018.05.010

Rasi, S., Kilpeläinen, P., Rasa, K., Korpinen, R., Raitanen, J.-E., Vainio, M., … Jyske, T. (2019). Cascade Processing of Softwood Bark with Hot Water Extraction, Pyrolysis and Anaerobic Digestion. *Bioresource Technology, 292*, 121893. https://doi.org/10.1016/j.biortech.2019.121893

Rasouli, H., Hosseini-Ghazvini, S.-M.-B., Adibi, H., & Khodarahmi, R. (2017). Differential α-Amylase/α-Glucosidase Inhibitory Activities of Plant-Derived Phenolic Compounds: A Virtual Screening Perspective for the Treatment of Obesity and Diabetes. *Food & Function, 8*(5), 1942–1954. https://doi.org/10.1039/c7fo00220c

Ruokolainen, V., Domanska, A., Laajala, M., Pelliccia, M., Butcher, S. J., Marjomäki, V., & López, S. (2019). Extracellular Albumin and Endosomal Ions Prime Enterovirus Particles for Uncoating That Can Be Prevented by Fatty Acid Saturation. *Journal of Virology, 93*(17). https://doi.org/10.1128/JVI.00599-19

Santos, J. S., Deolindo, C. T. P., Hoffmann, J. F., Chaves, F. C., do Prado-Silva, L., Sant'Ana, A. S., … Granato, D. (2018). Optimized *Camellia sinensis* var. *sinensis*, *Ilex paraguariensis*, and *Aspalathus linearis* blend presents high antioxidant and antiproliferative activities in a beverage model. *Food Chemistry, 254*, 348–358. https://doi.org/10.1016/j.foodchem.2018.02.021

Schmidtke, M., Schnittler, U., Jahn, B., Dahse, H.-M., & Stelzner, A. (2001). A Rapid Assay for Evaluation of Antiviral Activity against Coxsackie Virus B3, Influenza Virus A, and Herpes Simplex Virus Type 1. *Journal of Virological Methods, 95*(1), 133–143. https://doi.org/10.1016/S0166-0934(01)00305-6

Spinelli, S., Costa, C., Conte, A., La Porta, N., Padalino, L., & Nobile, M. A. D. (2019). Bioactive Compounds from Norway Spruce Bark: Comparison Among Sustainable Extraction Techniques for Potential Food Applications. *Foods, 8*, 524. https://doi.org/10.3390/foods8110524

Teo, C. C., Tan, S. N., Yong, J. W. H., Hew, C. S., & Ong, E. S. (2010). Pressurized hot water extraction (PHWE). *Journal of Chromatography A, 1217*(16), 2484–2494. https://doi.org/10.1016/j.chroma.2009.12.050

Vadivel, V., & Biesalski, H. K. (2011). Contribution of phenolic compounds to the antioxidant potential and type II diabetes related enzyme inhibition properties of *Pongamia pinnata l.* Pierre seeds. *Process Biochemistry, 46*(10), 1973–1980. https://doi.org/10.1016/j.procbio.2011.07.007

Valoppi, F., Lahtinen, M. H., Bhattarai, M., Kirjoranta, S. J., Juntti, V. K., Peltonen, L. J., … Mikkonen, K. S. (2019). Centrifugal fractionation of softwood extracts improves the biorefinery workflow and yields functional emulsifiers. *Greem Chemistry, 21*(17), 4691–4705. https://doi.org/10.1039/C9GC02007A

Varila, T., Brännström, H., Kilpeläinen, P., Hellström, J., Romar, H., Nurmi, J., & Lassi, U. (2020). From Norway Spruce Bark to Carbon Foams: Characterization, and Applications. *Bioresources, 15*(2), 3651–3666.

do Carmo, M. A. V., Pressete, C. G., Marques, M. J., Granato, D., & Azevedo, L. (2018). Polyphenols as Potential Antiproliferative Agents: Scientific Trends. *Current Opinion in Food Science, 24*, 26–35. https://doi.org/10.1016/j.cofs.2018.10.013

Weber-Lotfi, F., Guillemaut, P., Poirey, R., Schmitz, M., & Dietrich, A. (2002). Biochemical and Molecular Studies on Declining and Decline-Resistant Spruce in the North-East of France. *Environmental Science and Pollution Research, 9*(2), 122–129. https://doi.org/10.1007/BF02987459

Wu, F., Jia, X., Yin, L., Cheng, Y., Miao, Y., & Zhang, X. (2019). The effect of hemicellulose and lignin on properties of polysaccharides in lentinus edodes and their antioxidant evaluation. *Molecules, 24*, 1834. https://doi.org/10.3390/molecules24091834

Zhang, L., Santos, J. S., Cruz, T. M., Marques, M. B., do Carmo, M. A. V., Azevedo, L., … Granato, D. (2019). Multivariate effects of Chinese keemun black tea grades (Camellia sinensis var. sinensis) on the phenolic composition, antioxidant, antihemolytic and cytotoxic/cytoprotection activities. *Food Research International, 125*, 108516. https://doi.org/10.1016/j.foodres.2019.108516