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Black spruce extracts reveal antimicrobial and sprout suppressive potentials to prevent potato (*Solanum tuberosum* L.) losses during storage

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ABSTRACT

Canadian forest residues, such as bark, are an abundant and accessible biomass currently burned to produce energy, therefore neglecting their great potential for various applications owing to their multiple biological properties. Potato storage constitutes a challenge for potato producers because of disease propagation and potato sprouting. Barks appear to be promising candidates in the research of greener alternatives to synthetic chemicals presently used to limit these problems. Hence, this study aimed to develop a bio-based ingredient from bark residues to prevent diseases and sprouting of potatoes during storage. First, forest extracts were produced from the bark of black spruce (*Picea mariana* Mill.), balsam fir (*Abies balsamea* L. Mill.) and yellow birch (*Betula alleghaniensis* Britton) by three different methods: water extraction, ethyl acetate fractionation of the water extract, and acid-base extraction. Then, *in vitro* screening of extracts and commercial essential oils was performed to determine their ability to inhibit potato soft and dry rot and potato sprouting. More specifically, *Fusarium oxysporum*, *Fusarium graminearum*, *Fusarium sambucinum*, *Pectobacterium atrosepticum*, *Pectobacterium carotovorum*, and *Dickeya dianthicola* were selected for antimicrobial assays. Two black spruce extracts, ethyl acetate extract and essential oil, showed promising antimicrobial and anti-sprouting properties. The black spruce ethyl acetate extract inhibited microorganism growth with minimum concentrations ranging from 1.37×10^{-3} to 3.00% (w/w) depending on the strain. Black spruce essential oil completely prevented potato sprouting in Colomba cv. at a minimal concentration of 25% (w/w). Furthermore, when mixed, both properties were maintained, and even showed a synergistic effect. Indeed, in antimicrobial assays, the fractional inhibitory concentration index obtained was lower than 0.50. Therefore, these two black spruce extracts can be formulated into one product with broad properties aimed at controlling potato post-harvest losses due to rot and sprouting.

1. Introduction

More than 2 million metric tons of anhydrous bark residues are generated annually by forest industries in Quebec, Canada [1]. Their valorization is important for ecological and economic reasons. For decades, bark residues have been used to produce bioenergy [1–3]. However, this approach did not fully maximize the potential. Indeed, several studies have highlighted the interest of using forest residues as sources of bioactive molecules that possess numerous biological activities such as antimicrobial (fungal and bacterial), antiviral, anti-inflammatory, antioxidant, and anticancer properties [4–12].

The agriculture sector, specifically food storage and preservation, could benefit from these properties to reduce food waste. In fact, 10% of Canadian potato production is lost during storage [13]. These losses are even more significant for developing countries, varying from 20% to 50% [14]. Disease propagation, favored by the proximity of stored potatoes as well as early potato sprouting, is two main reasons for this percentage loss [15,16]. Storage conditions, such as cold temperatures and relative humidity, can be regulated to limit these challenges, but it is not sufficient to be completely effective [17]. Thus, the use of chemical products, such as fungicides and sprout suppressants, is necessary. However, the toxicity of these products (*e.g.,* chlorpropham or chlorothalonil) and the emergence of microbial resistance to antimicrobial products (*e.g.*, thiabendazole) are problematic [18–20].

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Research on greener, renewable, and sustainable alternatives, especially to replace chlorpropham (CIPC), began a few decades ago. Essential oils and the molecules they contain have been the subject of a lot of investigations. It has been reported that many of them inhibit potato sprouting, including essential oils from dill, caraway, coriander, citronella, English lavender, mint, sage, etc., and some have been commercialized, including Biox-C (clove oil), Biox-M (spearmint oil), and Talent (caraway oil) [21,22]. Some of them also exhibited antimicrobial properties. For instance, carvone detected in dill, caraway, and spearmint essential oils possess antimicrobial activity against phytopathogens (*Fusarium* sp. and *Helminthosporium solani*), and several human pathogens (either fungi or bacteria) [23]. However, one of the main drawbacks to the commercialization of sprout suppressants is their high production costs and the fact that many applications throughout the potato storage period are required to maintain effectiveness. Indeed, the mint essential oil cost 7.5 times higher than that of CIPC [24]. Consequently, they are currently no alternatives more environmentally friendly, sustainable, and affordable to replace CIPC.

This situation makes it interesting to study the potential of bark residues to prevent post-harvest losses. As sawmill residues, the raw material is bundled and readily available, which can drastically reduce costs. Using barks to extract either essential oil or any other specialized metabolites could be economically competitive, compared to the use of essential oils from cultivated plants. Although no research has investigated forest extracts as a sprout suppressant for potatoes, the diversity of bark biological properties observed so far supports the interest of studying its capacity to limit both sprouting and potato diseases [3,25]. Moreover, bioactive molecules found in sprout-suppressive essential oils, specifically monoterpenes (*e.g.*, carvone, pinene, limonene, and phelladrene), are also present in the bark residues of several Quebec trees. Altogether, this suggests that essential oils extracted from such bark residues may contain molecules with interesting antimicrobial and sprout suppressive activities.

In the present study, we evaluated the potential of forest residue extracts to limit post-harvest losses of potatoes due to the spread of diseases, mainly dry and soft rot, and early sprouting. To do so, different bark residues: particularly black spruce (*Picea mariana* Mill.), yellow birch (*Betula alleghaniensis* Britton), and balsam fir (*Abies balsamea* Mill.) were extracted differently (water extraction, ethyl acetate fractionation, and acid-base extraction) and their essential oils were obtained. Then, two screenings were performed to determine their antimicrobial and sprout suppressive potentials. Two black spruce extracts, the ethyl acetate fraction and the essential oil, successfully inhibited the development of microorganisms and potato sprouting. Therefore, by developing a bio-based ingredient with antimicrobial and sprout suppressive properties, it would be possible to substitute synthetic chemicals by a greener alternative upon while adding value to the forest industry by-products.

2. Material and methods

Different extracts of black spruce (BS), yellow birch (YB), and balsam fir (BF) bark were produced. In addition to these forest extracts, BoreA Canada, located in Chapais (Canada), supplied essential oils (EO) from BS twigs and needles, YB bark, and BF bark. All samples were tested for their antimicrobial activity against potato pathogens responsible for dry and soft rot (*Fusarium* spp., *Pectobacterium* spp., *Dickeya* sp.) and their capacity to inhibit potato sprouting.

2.1. Biomass conditioning

Forest extracts were produced from the bark of BS, YB, and BF, which were provided by the cogeneration plant of Greenleaf Power, Saint-Félicien (Canada). Each biomass was dried at room temperature to reach a minimal dryness of 85% (w/w), sifted using a William sieve to remove the <3 mm fraction containing inorganic matter and uniformly ground at 5 mm using a Wiley Mill crusher model 4 (Thomas Scientific, USA) [26]. The conditioned biomass was stored in closed containers at room temperature until utilization for extractions.

2.2. Extraction process

Three extraction processes were carried out on each biomass: water extraction (W), ethyl acetate fractionation of the water extract (EAc), and acid-base extraction (AB). These extraction methods were chosen based on antimicrobial results of Blondeau et al., 2019 [27] and St-Pierre et al., 2018, 2019 [11,26]. They demonstrated that polar solvents were more efficient to retrieve the active molecules of barks, such as phenolic compounds. It should also be noted that nonpolar compounds, contained in the essential oils (EO) provided by BoreA, were included in the biological assays.

Once the fractions were collected, they were dried in an oven at 60 °C and weighed to calculate the extraction yield [26]. They were then stored at 4 °C until they were utilized for screening where they were dissolved at the desired concentration in MeOH–H_2O 20% (w/w). The extraction yield was calculated as follows:

$$\textit{Extraction yield}\left(\%\ w/_w\right) = \frac{w_{\textit{dry extract (g)}}}{w_{\textit{dry initial biomass (g)}}} \times 100$$

2.2.1. Water extraction

Molecules retrieved in extracts from biomass with W extraction are mainly polar molecules, *for example,* phenolic compounds [26]. The extraction was performed using a digester MK System 6 L (Massachusetts, USA), a laboratory equipment redirected from pulp and paper plants. A 100% cotton bag was filled with conditioned biomass and placed in a cooking vessel filled with water at a weight ratio of 1:20 biomass:water. The cooking profile was set to 40 min to reach 100 °C, 20 min at 100 °C, and 40 min to cool down at 25 °C, as suggested by an internal procedure. The resulting cooking liquor was either dried to be used as a water extract or partially evaporated for use in ethyl acetate fractionation.

2.2.2. Ethyl acetate fractionation

Based on the method of Diouf et al. [6], EAc fractionation enriches proanthocyanidin oligomers in the organic fraction (OF), whereas the aqueous fraction (AF) contains proanthocyanidin polymers. Following the water extraction, the cooking liquor obtained was evaporated to 2% of dried matter (w/w) in a ventilated oven at 60 °C. Then, liquid-liquid extraction was performed by washing five times the cooking liquor with ethyl acetate at a volume ratio of 1:1 in a 2 L separating funnel. Most of the ethyl acetate solvent in the OF was recovered using a Rotavapor device. Then, all fractions (OF and AF) were dried and stored until further use, as mentioned above.

2.2.3. Acid-base extraction

As proposed by St-Pierre et al. [28], AB extraction enriches the extract with alkaloid compounds. Biomass was macerated for 24 h in 80% (v/v) MeOH–H_2O with a pH adjusted to 4. Following filtration, the MeOH–H_2O solution was retrieved and used for a succession of liquid-liquid extractions. Two rinses with hexane were first realized in a volume ratio of 1:2 (filtrate:hexane) in a 2 L separating funnel. The aqueous fraction obtained was then alkalized with 1 N ammonium chloride and washed with chloroform under the same conditions as hexane. All fractions (OF and AF) were dried and stored until further use, as mentioned above.

2.3. Antimicrobial activity

Forest extracts and essential oils were tested for their antimicrobial potential against potato pathogens using a colorimetric method based on Eloff [29] in which the iodonitrotetrazolium chloride (INT) dye was

used to determine cell viability. Indeed, this tetrazolium salt can be reduced in red formazan, a purplish color, by the dehydrogenase present in live microbial cells.

2.3.1. MicroOrganisms strains

Microbial strains were chosen for their ability to cause serious storage diseases, particularly dry and soft rot. *Fusarium* spp. (fungi) are known to be causative agents of dry rot, whereas *Pectobacterium* spp. and *Dickeya* sp.(bacteria) cause soft rot. Therefore, *Fusarium oxysporum*, *Fusarium graminearum*, *Fusarium sambucinum*, *Pectobacterium atrosepticum*, *Pectobacterium carotovorum,* and *Dickeya dianthicola* strains were obtained from Ph.D. Richard Hogue from IRDA (Quebec City, Canada) and Ph.D. Yang Liu from IRBV (Montreal, Canada). Microbial strains were conserved on agar plates at 4 °C. The preserved isolates were cultured prior each antimicrobial test in order to reactivate the metabolism and the subsequent culture was used for the antimicrobial test. The bacterial strains were grown in tryptic soy broth (TSB) for 24 h at 26 °C, and fungal strains were grown in potato dextrose broth (PDB) for 48 h at 24 °C, as recommended by ATCC. Microbial cultures were adjusted to 0.5 McFarland by using a turbidimeter (Hach, 2100AN) to obtain a final inoculum estimated at 1.5×10^8 CFU/mL for bacteria and 1.5×10^4 CFU/mL for fungi.

2.3.2. Preliminary screening at fixed concentration

All extracts were prepared at 1.25% (w/w) in MeOH–H_2O 20% (w/w), except for essential oils that were used pure for this preliminary colorimetric screening adapted from Eloff [29]. The negative controls included in this test were MeOH–H_2O 20% (w/w) and H_2O. Fifty microliters of extracts, essential oils, or controls were added to 96 well microplates in triplicate. Then, 50 μL of the microbial culture was added to each well. Hence, the final concentration was 0.625% (w/w) for extracts and 50% (w/w) for essential oils. The microplates were incubated according to the microorganism optimal growth temperature described above during either 3 h for bacteria or 6 h for fungi. Then, 40 μL of INT (2.85 g/L) was added to two out of three wells per sample, and the third well served as an indicator of the initial color of the extract. The color revelation took 1 h and 16 h, more or less 15 min, for bacteria and fungi, respectively. A visual assessment was conducted to determine the coloration of the sample. The extract efficacy was rank as: "++'' for effective inhibition (no color change), "+'' for partial inhibition (a slight color change) or "-'' for no inhibition effect (a distinct color change). To facilitate the selection of promising extracts, antibacterial, antifungal, and antimicrobial potentials were established by calculating the mean of extract efficacies against all the bacteria, fungi, or the entirety of the microorganisms tested.

2.3.3. Broth microdilution method (MIC, MBC/MFC)

Adapted from the standardized method available in the M07-A9 document from the Clinical and Laboratory Standard Institute [30] and Eloff [29], this broth microdilution method allows the semi-quantification of antimicrobial potential by determining the minimal inhibitory concentration (MIC) and minimal bactericidal/fungicide concentration (MBC/MFC). This method was chosen because it only requires a small quantity of extract and because of its robustness, reproducibility and sensitivity that are 30 times higher than other methods [29,31]. Only the MIC and MBC (or MFC) of the three most promising extracts from the preliminary screening were determined. They were prepared at 5% (w/w) in MeOH–H_2O 20% (w/w). The included controls were MeOH–H_2O as a negative control and Emesto Silver (100 g/L penflufen and 18 g/L prothioconazole formulated as a suspension) or H_2O_2 30% (active ingredient of StorOx) as positive controls. These products are used to limit potato dry and soft rot, respectively [32]. As described by St-Pierre et al. [26], sterile broth culture media and treatments were added to a 96 well plate followed by serial dilution. Then, the inoculum was added, and plates were incubated before the addition of INT (2.85/L) and its color revelation, as previously described. MIC values were determined for each treatment as the lowest concentration inhibiting the proliferation of microorganisms, that is, the last well without color change. MIC values were repeated in duplicate. To determine MBC or MFC values, which correspond to the lowest concentration that kills all bacterial or fungal colonies (>99.9% reduction of the initial inoculum), no microbial colonies were grown on agar plates after 24 h of incubation. Therefore, once the MIC was determined, 100 μL of each well that showed microbial growth inhibition was subcultured onto agar plates and incubated. MIC and MBC/MFC values are given in % (w/w) and range from 3.00 to 1.52×10^{-4} for forest extracts (initial concentration of 5% w/w), from 60.02 to 3.05×10^{-3} for the BS extracts combination and BS-EO (initial concentration of 100% w/w), from 0.60 to 3.05×10^{-5} for BS-EAc_{OF} (initial concentration of 1% w/w), from 18.01 to 9.15×10^{-4} for H_2O_2 (initial concentration of 30%), from 60.02 to 3.05×10^{-3} for Emesto Silver (initial concentration of 100%) and from 12.00 to 6.10×10^{-4} for MeOH–H_2O (initial concentration of 20%).

2.3.4. Fractional inhibitory concentration (FIC)

To evaluate the presence of a synergistic antimicrobial activity within a combination of extracts, the fractional inhibitory concentration (FIC) was calculated according to the results of MIC and MBC/MFC previously obtained using the formula [33]: $FIC = \frac{MIC\ or\ MBC\ or\ MFC\ extract\ 1\ combined}{MIC\ or\ MBC\ or\ MFC\ extract\ 1\ alone} + \frac{MIC\ or\ MBC\ or\ MFC\ extract\ 2\ combined}{MIC\ or\ MBC\ or\ MFC\ extract\ 2\ alone}$

The interpretation of the FIC was based on the index proposed by Sopirala et al. [33] where synergy was defined as $\sum FIC \leq 0.5$; additivity as $0.5 < \sum FIC \leq 1$; indifference as $1 < \sum FIC \leq 4$; and antagonism as $\sum FIC > 4$.

2.4. Sprout suppressive activity

For these tests, Colomba potatoes were used because they are sold for fresh markets with a short dormancy period and which sprout aggressively. Potato samples were provided by Les Patates Dolbec (Saint-Ubalde, Quebec, Canada), and sprout suppressive tests were conducted when the endodormancy of tubers was completed.

2.4.1. Sprout suppressive tests

Sprout suppressive tests were based on the work of Vokou et al. [34] and Dai et al. [35]. First, all extracts were prepared at 5% (w/w) in MeOH–H_2O 20% (w/w), except for the pure essential oils. Controls included in this test were MeOH–H_2O 20% (w/w) and H_2O as negative controls and CIPC 1%, and l-carvone 99% as positive controls, where both are currently used as potato sprout suppressants (Table 1). The potatoes were washed according to the following process: removal of dirt under running water, inspection of tubers for any signs of diseases, and soaking for 30 s; 1) sodium hypochlorite 1% (v/v); 2) ethanol 70% (v/v); and 3) demineralized water. Once potatoes were dried at room temperature, each treatment was vaporized uniformly onto the surface of three potatoes before being placed in a closed bucket. After four weeks, different measures were taken for each potato: the longest sprout

Table 1
Extraction yield obtained from extraction of bark residues.

Extraction		Yield (g/100 g dried bark)		
Method	Fraction	BS	YB	BF
W	N.A.	9.35	6.75	3.27
EAc	OF	4.13	2.33	NA
	AF	2.79	5.13	NA
AB	OF	0.92	1.08	NA
	AF	3.63	4.40	NA

Abbreviations: BF, balsam fir; BS, black spruce; YB, yellow birch; W, water extraction; EAc, ethyl acetate fractionation; AB, acid-base extraction; AF, aqueous fraction; OF, organic fraction; NA, not applicable. The values are obtained from one replicate.

length, the dry sprout weight, and the notification of dry and soft rot signs. All measures coming from potatoes affected by illness were discarded for the analysis because the rottenness seemed to negatively affect the sprouting process and thus falsify the results. For each sprout suppressive test, an independent triplicate was performed; however, it must be noted that the physiological age of potato differed between them. Statistical analyses were done on means obtained from these three replicates by using JMP Pro 14.

2.4.2. Calculation of sprouting index and sprout growth inhibition

From the measures taken (initial potato weight and sprout height and weight), three variables were used to compare the antisprouting efficacy of forest extracts: sprouting index, industrial sprouting index, and sprout growth inhibition (Equations 1 to 3). Proposed by Demeulemeester [36], the sprout index was determined for each treatment and was defined by the length of the longest sprout. Developed by One Four Group [37], the industrial sprouting index allows verification of the marketing of potatoes. A result inferior to 10 signifies that potatoes can be sold for the processing sector and when below 5, potatoes may be intended for fresh markets. Based on Vokou et al. [34], the calculation of sprout growth inhibition as presented in Equation 3 allows the normalization of the sprout growth measure in relation to the initial potato weight because the potato size might influence sprouting. Moreover, sprout growth of the treated potatoes was compared with the mean sprout growth of control potatoes. This control varied depending on the nature of the treatments applied. Indeed, since extracts were diluted in MeOH–H_2O 20%, potatoes treated with MeOH–H_2O were used as the control, whereas the other treatments, such as EOs, used potatoes H_2O treated as the control. The standardization of sprout growth allows the replicas to be compared with each other; potato sprouting might vary according to its physiological age.

Index	1	2	3	4	5	6
Lengthiest sprout (mm)	No sprout	White point	<2	[2–5 [	[5–10]	>10

Equation 1. Sprouting index.

$$\text{Industrial sprouting index} = [(\%\,N \times 0) + (\%\,A \times 2) + (\%\,B \times 6) + (\%\,C \times 15) + (\%\,D \times 40)] \,/\, 100$$

Equation 2. Industrial sprouting index (scale of 0–40), where letters represent the height of the longest sprout per potato: N, no sprout; A, ≤2 mm; B,]2–10 mm]; C,]10–20 mm]; D, >20 mm.

$$Sprout\,growth\,inhinbition\left(\%\right) = 100 - \left[\left(\frac{\text{Treatment}\frac{sprouts\left(w_{dry}\right)}{potato(w_{initial})}}{Control\sum_i^n\left(\frac{sprouts\left(w_{dry}\right)}{potato(w_{initial})}\right)_i \Big/ n}\right) \times 100\right]$$

Equation 3. Sprout growth inhibition.

3. Results

3.1. Forest extract production

In order to select a promising natural preservative ingredient that could prevent the spread of diseases and the sprouting of potatoes during storage, various extractions were carried out on forest bark residues including YB, BS, and BF. These extraction methods allow the enrichment of W extracts with a variety of polar molecules, EAc_{OF} extracts with proanthocyanidin oligomers, and AB_{OF} extracts with alkaloids [6,26,27,36]. Table 1 shows the extraction yields for each extraction method.

Extraction yields ranged from 0.92 to 9.35 g/100 g, where BS-AB_{OF} gave the lowest yield and BS-W, the highest yield. It seems that the W extraction method resulted in the highest yields, followed by the EAc and AB methods. By calculating extraction yields, it is possible to distinguish two extracts with similar efficacies, since a higher yield is preferable for industrial production.

Table 2
Initial screen of the antimicrobial potential of forest extracts against potato storage pathogens using the INT colorimetric assay. The efficacy of the antimicrobial potential of an extract was rank as: "++" effective inhibition, "+" partial inhibition and "-" no inhibition effect.

Sample number	Origin	Extraction method	Antibacterial	Antifungal	Antimicrobial
1	Control	MeOH–H_2O	–	–	–
2	Control	H_2O	–	–	–
3	YB	W	++	–	+
4	YB	EAc_{OF}	–	++	–
5	YB	EAc_{AF}	–	–	–
6	YB	AB_{OF}	–	–	–
7	YB	AB_{AF}	–	–	–
8	YB	EO	–	–	–
9	BS	W	+	–	–
10	BS	EAc_{OF}	+	++	+
11	BS	EAc_{AF}	+	–	–
12	BS	AB_{OF}	+	+	+
13	BS	AB_{AF}	++	–	–
14	BS	EO	–	+	–
15	BF	EAc_{OF}	+	++	+
16	BF	EAc_{AF}	+	–	–
17	BF	EO	–	+	–

Abbreviations: BF, balsam fir; BS, black spruce; YB, yellow birch; W, water extraction; EAc, ethyl acetate fractionation; AB, acid-base extraction; EO, essential oil; AF, aqueous fraction; and OF, organic fraction. The values are obtained from two replicates.

3.2. Antimicrobial tests

To rapidly discriminate the antimicrobial efficacy of all 17 extracts, an initial screen was performed using the iodonitrotetrazolium chloride (INT) colorimetric assay on potato phytopathogens problematic in North American warehouses. More specifically, the antimicrobial activity of extracts was determined against causal agents of bacterial rot (*Pectobacterium* spp., *Dickeya* sp.) and fungal rot (*Fusarium* spp.) [38]. Table 2 shows the inhibition levels of the extracts against the selected potato pathogens. Detailed results are presented in the Appendix (Table A1).

The most antibacterial extracts were 3 (YB–W) and 13 (BS-AB_{AF}). All BS and BF extracts tested (9–13 and 15–16) displayed partial inhibition against bacterial rot, but interestingly, their corresponding essential oils (14 and 17) did not show any inhibition. The most antifungal extracts were YB, BS, and BF-EAc_{OF} (3, 7, 13), followed by BS-EO and BF-EO (14 and 17) with partial inhibition. Altogether, the three most promising extracts with a large spectrum of inhibition, that is, antibacterial and antifungal, are BF-EAc_{OF}, BS-EAc_{OF}, and BS-AB_{OF} (3, 7, 9). For industrial use, a treatment possessing the capacity to inhibit both bacterial and fungal rot becomes even more interesting. Hence, BF-EAc_{OF}, BS-EAc_{OF}, and BS-AB_{OF} extracts were selected for further investigation of their antimicrobial properties.

To evaluate the antimicrobial activities of the three most promising extracts (BF-EAc_{OF}, BS-EAc_{OF}, and BS-AB_{OF}), the minimal inhibitory concentrations (MIC) and minimal bactericidal and fungicidal concentrations (MBC and MFC) were established using the INT microdilution test. The MIC and MBC/MFC results are listed in Table 3.

As expected, the positive controls, H_2O_2 and Emesto Silver, were efficient against soft and dry rot, respectively, whereas the negative control, MeOH–H_2O, had no impact on microorganism growth. The most effective extract was the one with the lowest minimum concentrations, which corresponded to BS-EAc_{OF}, varying from 1.37×10^{-3} to 3.00% (w/w), depending on the microbial strain. Even though it is not as effective as H_2O_2 for controlling bacterial rot, MIC and MBC results of BS-EAc_{OF} support the possibility of preventing the development of soft rot with its application. As for its antifungal activity, it is more efficient

Table 3
Minimum antimicrobial concentration of most promising forest extracts against **(a)** bacterial and **(b)** fungi potato storage pathogens.

Treatment	(a) Soft rot (bacterial rot)					
	P. carotovorum		P. atrosepticum		D. dianthicola	
	MIC	MBC	MIC	MBC	MIC	MBC
Control; H_2O_2	8.23×10^{-3}	0.22	2.47×10^{-2}	0.22	9.15×10^{-4}	18.01
Control; MeOH–H_2O	*>12.00*	*>12.00*	*>12.00*	*>12.00*	*>12.00*	*>12.00*
BF-EAc_{OF}	3.00	3.00	3.00	3.00	3.00	3.00
BS-EAc_{OF}	0.60	3.00	0.60	3.00	0.60	3.00
BS-AB_{OF}	3.00	*>3.00*	*>3.00*	*>3.00*	*>3.00*	*>3.00*

Treatment	(b)Dry rot (fungal rot)			
	F. oxysporum		F. sambucinum	
	MIC	MFC	MIC	MFC
Control; Emesto Silver	3.05×10^{-3}	20.01	3.05×10^{-3}	20.01
Control; MeOH–H_2O	*>12.00*	*>12.00*	*>12.00*	*>12.00*
BF-EAc_{OF}	3.33×10^{-1}	3.33×10^{-1}	3.33×10^{-1}	3.33×10^{-1}
BS-EAc_{OF}	3.71×10^{-2}	1.11×10^{-1}	1.37×10^{-3}	4.12×10^{-3}
BS-AB_{OF}	3.00	3.00	3.00	3.00

Abbreviations: BF, balsam fir; BS, black spruce; EAc, ethyl acetate; AB, acid base; OF, organic fraction; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; MFC, minimum fungicidal concentration. Results are presented as the average of two replicates and is given in % (w/w). *>value* means that the treatment is not active at the maximal concentration tested, which is indicated by the value in italics.

than Emesto Silver because its MFC is much lower. Overall, it seems that forest extracts have a lower activity against bacteria causing soft rot compared to their efficacy in inhibiting the growth of fungi responsible for dry rot. Nevertheless, BS-EAc_{OF} showed promising MIC, MBC, and MFC, which would justify its application in potato storage to prevent the development of both soft and dry rots.

3.3. Sprout suppressive tests

The sprout suppressive potential of all 17 forest extracts was assessed

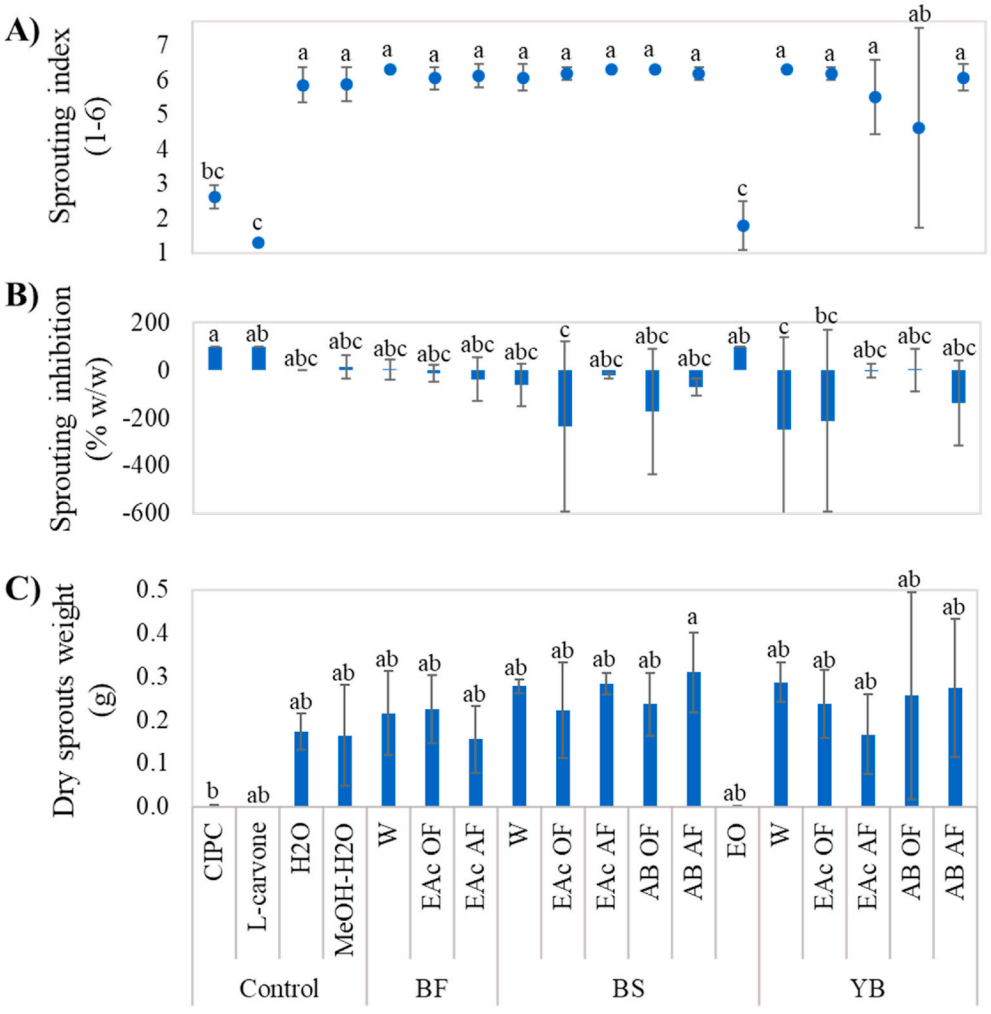

Fig. 1. Sprout suppressive potential of forest extracts according to A) Sprouting index, B) Sprouting inhibition and C) Dry sprout weight. Abbreviations: BF, balsam fir; BS, black spruce; YB, yellow birch; W, water extraction; EAc, ethyl acetate fractionation; AB, acid-base extraction; EO, essential oil; AF, aqueous fraction and OF, organic fraction. Except essential oils that were tested pure, extracts were applied at a concentration of 5% w/w. The values are presented as the average of the three replicates that included each three potato samples. Extracts having the same letter (a, b, c) present no significant differences ($p < 0.05$) according to HSD Tukey-Kramer statistical test. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

through the application of extracts on the surface of potatoes, and the results are presented in Fig. 1. Each extract was tested at a concentration of 5% (w/w), except for the pure EOs (100% w/w). The results are shown in Fig. 1.

An effective sprout suppressive potential is determined by three characteristics: (A) a low sprouting index, (B) a positive percentage of sprouting inhibition, and (C) a minimal dry sprout weight. In this regard, the positive controls, CIPC 1% and l-carvone 99%, were efficient in limiting sprouting because sprouts harvested from the potatoes treated weighed 0.0028 g and 0.0000 g, respectively. These results were expected because they are commercialized as potato sprout inhibitors [25]. The negative controls H_2O and MeOH–H_2O 20% did not have anti-sprouting activity. Of all the forest extracts, only BS-EO showed significant ($p < 0.05$) sprout suppressive activity with a sprouting inhibition of 99.6% (w/w). It inhibited sprouting as efficiently as CIPC, the most applied sprout suppressive product during storage. However, signs of phytotoxicity on potato skin treated with BS-EO were noted. This phytotoxicity was also observed in BF-EO and YB-EO. It affected the potatoes treated so severely that it disintegrated them, invalidating the sprout suppressant results of BF-EO and YB-EO. Furthermore, some extracts, such as BS-EAc_{OF} and YB-W, seem to stimulate sprout development according to the negative percentages of sprouting inhibition of −235.3 and 249.5% (w/w). By showing efficacy similar to that of CIPC, BS-EO was selected to evaluate its anti-sprouting property more accurately.

To further investigate the sprout suppressive activity and to diminish the phytotoxicity of the BS-EO sample, various concentrations were tested, and the results are shown in Fig. 2.

BS-EO 10% (w/w) inhibited sprouting but was as effective as the CIPC, with a BS-EO concentration of 25% (w/w). This is also the maximum concentration before the appearance of signs of phytotoxicity on the potato periderm, that is, mild necrosis that may promote rotting. Necrosis of emergent sprouts can be observed following treatment with BS-EO, confirming that the EO acts as a curative treatment by burning the bud meristematic cells like the others sprout EOs commercialized under Sprout Torch, Biox-M, and Talent [22,25,39]. Therefore, BS-EO could be a promising treatment as a sprout inhibitor that could replace CIPC.

3.4. *Promising potato post-harvest product based on black spruce extracts combination*

BS-EAc_{OF} and BS-EO showed promising antimicrobial and sprout-suppressive activities, respectively. By combining the antimicrobial extract and the sprout suppressive one, BS-EAc_{OF} and BS-EO, an ideal potato post-harvest product could be obtained. Therefore, BS-EAc_{OF} and BS-EO were mixed at a minimal concentration to ensure antimicrobial and sprout suppressive activities, that is, 25% EO and 1% EAc_{OF}. Tests were carried out to confirm that the BS extract mix retained both biological properties. For antimicrobial activity, minimum concentrations of BS mix were determined using the INT microdilution assay. The results are listed in Table 4.

The minimum concentrations of the BS mix ranged from 20.01% to >60.02% (w/w) and from 6.67 to 20.01% (w/w) against soft rot and dry rot causal agents, respectively. These values are lower than those of BS-EO alone, with an overall range of 15.01 to >60.02% (w/w). Altogether, the combination of BS-EAc_{OF} and BS-EO has an antimicrobial synergistic effect, as confirmed by the FIC values lower than 0.5. Even though the efficacy was increased, it still did not reach the H_2O_2 antibacterial activity (Table 3). As for the antifungal activity, BS-EAc_{OF} alone was already comparable to Emesto Silver (Table 3); hence, the efficiency of the BS mix is further interesting. However, it is possible to observe that the results for dry rot differ slightly for BS-EAc_{OF} from previously, where the minimal concentrations generally increased from 1.37×10^{-3} to 1.11×10^{-1}% (w/w) (Table 3b) to 0.20–0.60% (w/w) (Table 4b). These results confirmed that the antimicrobial activity of the BS extract mix

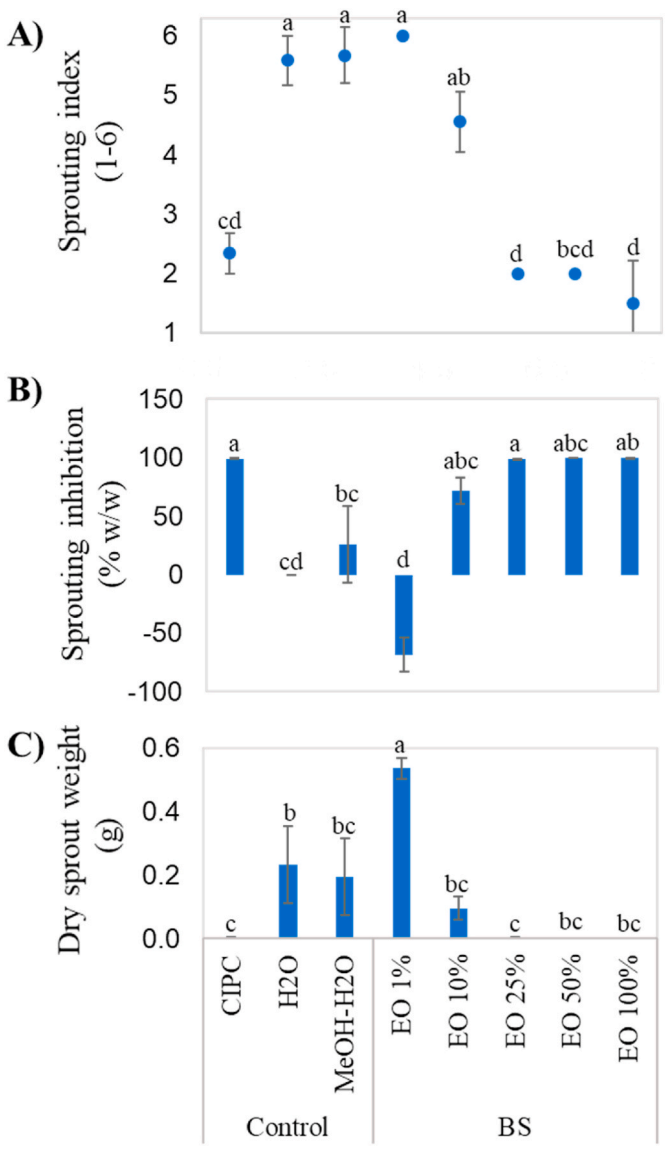

Fig. 2. Minimum sprout suppressive concentration of black spruce essential oil indicated by A) Sprouting index, B) Sprouting inhibition and C) Dry sprout weight. Abbreviations: BS, black spruce; EO, essential oil. The values are presented as the average of the three replicates that included each three potato samples. Treatments having the same letter (a, b, c, d) present no significant differences ($p < 0.05$) according to HSD Tukey-Kramer statistical test.

was higher than that of its components alone.

With such positive antimicrobial results, the same BS mix was tested for its sprout inhibition potential on Colomba potatoes and the results are presented in Fig. 3.

Although BS-EAc_{OF} alone did not inhibit sprouting, and even stimulated it with a result of 235.28% w/w, the BS mix, with a sprouting inhibition of 95.71% w/w, maintained the capacity to inhibit sprout growth of BS-EO, which was as effective as CIPC (99.12% w/w).

To confirm if the BS mix allows potatoes to be sold to markets, it is possible to calculate the industrial sprouting index. Since markets have different degrees of potato sprouting acceptance, this index indicates the markets where potatoes are suitable for sale. For instance, fresh packaging, such as shipping to supermarkets, requires an industrial sprouting index of less than 5, whereas for the processing sector, it must be less than 10. The results are listed in Table 5.

With an industrial sprout index of 0.7 ± 1.2, it would be acceptable to sell potatoes treated with BS mix to all the different markets, even the

Table 4
Minimum antimicrobial concentration and fractional inhibitory concentration (FIC) of the BS extracts combination (25% EO, 1% EAc_{OF}) against **(a)** bacterial and **(b)** fungi potato storage pathogens.

Treatment	(a) Soft rot (bacterial rot)					
	P. carotovorum		P. atrosepticum		D. dianthicola	
	MIC	MBC	MIC	MBC	MIC	MBC
BS-EAc_{OF}	0.60	3.00	0.60	3.00	0.60	3.00
BS-EO	60.02	>60.02	>60.02	>60.02	>60.02	>60.02
BS mix	20.01	60.02	20.01	>60.02	20.01	>60.02
$FIC_{BS\ mix}$	0.42	<0.45	<0.42	N.A.	0.42	N.A.

Treatment	(b)Dry rot (fungal rot)			
	F. oxysporum		F. sambucinum	
	MIC	MFC	MIC	MFC
BS-EAc_{OF}	0.20	0.20	0.20	0.60
BS-EO	15.01	15.01	15.01	15.01
BS mix	6.67	6.67	6.67	20.01
$FIC_{BS\ mix}$	0.45	0.45	0.45	0.67

Abbreviations: BS, black spruce; EO, essential oil; EAcOF, organic fraction of ethyl acetate fractionation; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; MFC, minimum fungicidal concentration; FIC, fractional inhibitory concentration. Results are presented as the average of two replicates and is given in % (w/w). >MIC, MBC, or MFC values indicated that the treatment was not active at the maximal concentration tested, whereas > or < FIC values meant that the FIC was greater or lower than the mentioned value, respectively.

most demanding one like fresh packaging.

4. Discussion

4.1. Forest extract production

The extraction yields obtained are consistent with the literature that reports a general variation between 2% and 20% for bark extractions [3], but they were inferior to the yields obtained by St-Pierre et al. [26]. This information is important to calculate because it can be decisive for commercialization; indeed, a lower yield tends to lead to a higher production cost. However, a higher yield does not directly imply a higher activity. Many factors influence both characteristics: extraction method, tree species, tissue used for extraction (bark, wood, leaves/needles), harvest site and time, storage period before extraction, *etc.* [3,34,35]. In fact, AB extraction was expected to give the lowest yields (approximately 1 g/100 g) because alkaloids are present only at low concentrations in plants. Nevertheless, from the perspective of industrial production, the properties of AB extracts could overcome the low yields obtained because alkaloids are known to possess strong biological properties at a minimal concentration [40,41]. Data on the extraction yields of the commercial EOs used herein were not available; however, according to the literature, EOs obtained by hydro-distillation have yields close to 1% (w/w) [42].

4.2. Antimicrobial tests

BS-EAc_{OF} showed an antimicrobial activity against *Pectobacterium* spp. and *Dianthicola* sp. and a greater one against *Fusarium* spp. However, these results should be gauged in the context of the dominant pathogens to which potatoes are exposed to. The antimicrobial efficacy of BS-EAc_{OF} can be partially explained by its chemical nature, which contains phenolic compounds such as taxifolin, epicatechin, quercetin, and resveratrol [26]. Although studies have reported antimicrobial activities of BS extracts [8,26], none has identified precisely which metabolites are responsible for it and how they interact. However, phenolic compounds, particularly flavonoids and terpenoids, are known to have multiple mechanisms involving many sites of action [43]. Indeed, these

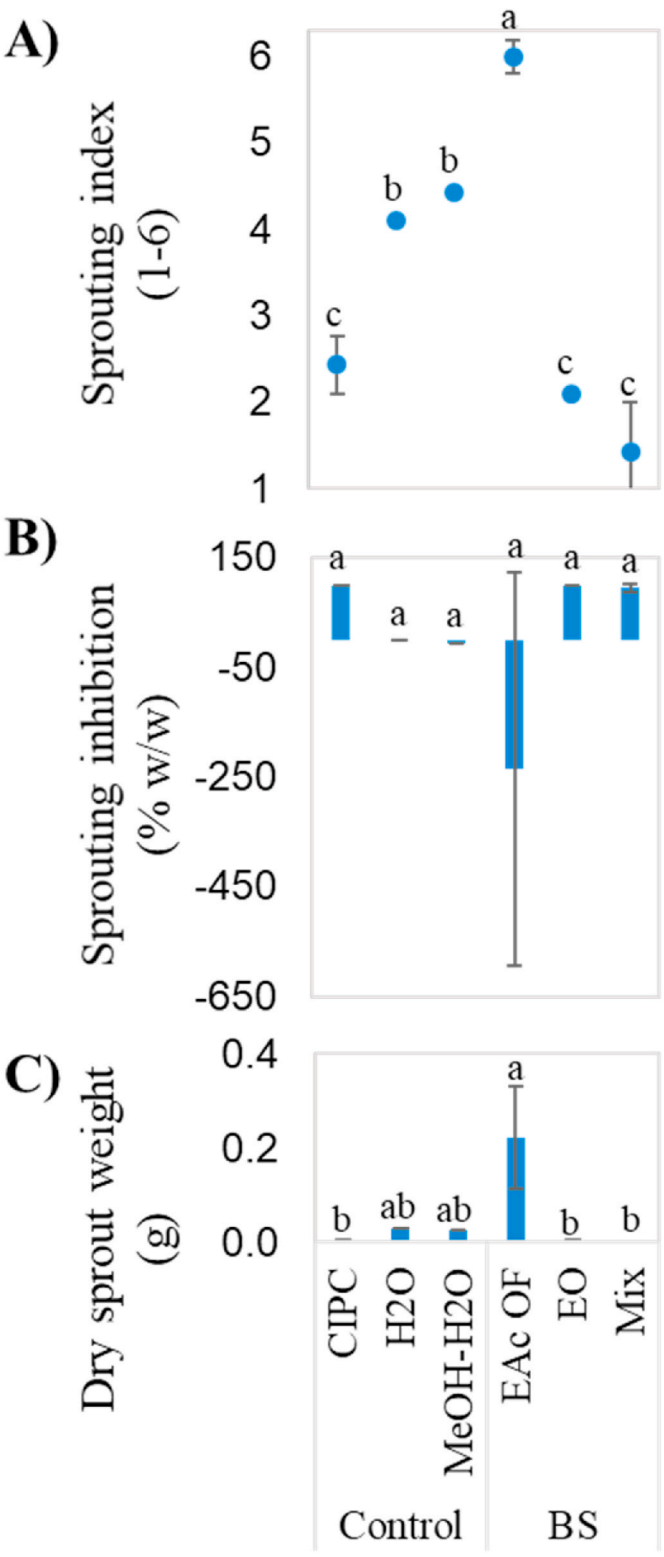

Fig. 3. Sprout suppressive activity of black spruce extracts combination (25% EO, 1% EAc_{OF} w/w) indicated by A) Sprouting index, B) Sprouting inhibition and C) Dry sprout weight. Abbreviations: BS, black spruce; EAc_{OF}, organic fraction of ethyl acetate fractionation and EO, essential oil. The values are presented as the average of the three replicates that includes each three potato samples. Treatments having the same letter (a, b, c) present no significant differences ($p < 0.05$) according to HSD Tukey-Kramer statistical test.

Table 5
Potatoes market acceptability according to industrial sprouting index.

Treatment	Industrial sprouting index (scale of 0–40)			Market	
				Fresh packaging (<5)	Processing (<10)
CIPC	0.7	±	0.7	Yes	Yes
H_2O	10.5	±	3.9	No	No
MeOH–H_2O	18.7	±	13.6	No	No
BS-EO	0.0	±	0.0	Yes	Yes
BS-EAc_{OF}	23.3	±	14.4	No	No
BS-Mix	0.7	±	1.2	Yes	Yes

Abbreviations: BS, black spruce; EO, essential oil; EAcOF, organic fraction of ethyl acetate fractionation. Values are presented as the mean ± standard deviation of three replicates containing three potato samples.

molecules can interact with cell membranes and proteins, inducing structural changes or causing ion leakage, cell disruption, and even agglutination of cells [43–46]. More specifically, the components found in BS-EAc_{OF}, such as the standard compound of taxifolin, have been proven to inhibit some enzymes essential to the production of precursor components of the cell membrane [47]. Resveratrol has exhibited inhibitory activity against efflux pumps present in microbial cells [47]. Therefore, although no study has elucidated the antimicrobial mode of action of BS extracts, phenolic compounds are thought to be responsible for it, and interestingly, their mechanisms generally differ from usual antibiotics [43,48].

In addition, it is worth noting that BS-EAc_{OF} extract is known to have antioxidant and anti-inflammatory properties [6,8,49]. Besides its antimicrobial activity, these properties could help to prevent the infection of *Fusarium* spp. by promoting the healing of the potato skin because the success of the infection relies on the presence of wounds [6,8,38,49,50]. Hence, to determine if this is another mechanism that could prevent the development of dry rot, it would be pertinent to evaluate the suberization by characterizing the suberin level as proposed by Peck [51] in a future experiment.

Furthermore, EOs are known to inhibit the growth of a large variety of microorganisms and even phytopathogens responsible for dry and soft rot, such as *Fusarium* spp. and *Pectobacterium* spp [52–54]. However, the EOs of BS, YB, and BF tested herein only showed weak or no antimicrobial activity, especially against *Fusarium* spp. These results may be explained by two possible reasons. First, EOs are generally less active against gram-negative bacteria, such as *Pectobacterium* spp. and *D. dianthicola*, due to their outer membrane that prevents hydrophobic compounds from disrupting the cytoplasmic membrane [52]. Second, the results may underestimate the antimicrobial potential of EOs because of the impossibility of diluting them homogeneously. Therefore, to more accurately evaluate the antimicrobial potential of EOs, it may be more appropriate to use the antibacterial hydrophobic assay developed by Côté [55] or formulate EOs by adding an emulsifying agent, such as Tween.

4.3. Sprout suppressive tests

The signs of phytotoxicity present on potato skin could be explained by the phytotoxic effect of EOs against potato skin, which promoted the development of rottenness. A higher volatility, such as the high concentration of pinenes (α and β) and limonene present in BF-EO, in addition to the presence of oxygen functions such as methyl salicylate in the YB-EO, could be responsible for this phytotoxicity [56–59]. Therefore, it is likely that with a lower concentration of BF and YB-EOs, it would be possible to obtain conclusive and promising results about their sprout suppressive activity because some of their molecules have already demonstrated this property [25,60–63].

The ability of some extracts to stimulate sprouting could be of interest for pre-sprouting seed potatoes, which is important for early tuber harvest and organic potato production [64,65]. Indeed, better plant growth would shorten the growing period and therefore minimize the risk of disease development, such as late blight [64]. Moreover, plant extracts have been demonstrated to induce the production of phytoalexins, meaning that in addition to stimulating plant growth, the plant may be more resistant to further infections in the field [66,67].

The efficiency of BS-EO is not surprising considering that some of its compounds, such as α-pinene, d-limonene, and linalool, have been proven to inhibit sprouting [25,61–63]. The mode of action for the sprout-suppressive activity of BS-EO may be due to its monoterpenes. The cytotoxic effects of these compounds reduce mitochondria and Golgi bodies, which interfere with cellular respiration and photosynthesis [68]. They are also known to compromise the permeability and fluidity of the cell membrane [52,68,69]. Overall, the volatility and presence of oxygenated functions of the monoterpenes contained in BS-EO appear to be important for sprouting inhibition [57].

4.4. Promising potato post-harvest product based on black spruce extracts combination

The variation in the MIC and MFC of BS-EAc_{OF} against fungal rot may partially be explained by the batch difference between each bark extraction, whereas the origin of BS-EAc_{OF} differed for these two antimicrobial tests. This observation corroborates the need of studies and traceability markers to evaluate the variability to standardize and regulate the efficacy of the final product as the chemical composition of the bark originally varies depending on the harvest season and site, the age of the tree and the conditioning of the biomass after harvesting [3,42,68].

The effectiveness of the antimicrobial and sprout suppressive results demonstrated a synergy between the molecules of BS-EAc_{OF} and BS-EO. In reviewing the literature, many authors have reported this type of observation using plant extracts and EOs [53,70–72]. This synergy not only reduces the active dose, but also limits the risk of developing resistance by multiplying the mechanisms of action, either by directly affecting the cellular components or the resistance mechanisms [68,73]. It is interesting to note that synergistic effects can occur as well through physicochemical interactions between the different molecules of the two extracts [74]. To better understand this phenomenon, it would be necessary to test if it is the lipophilic nature of the EO that is responsible for this synergy or if it is due to the activity of its molecules.

However, formulation work is required because the BS mix is heterogeneous in MeOH–H_2O 20%. Therefore, particular attention should be paid to the formulation base because various factors, such as pH and salinity, can seriously affect the biological properties of the molecules in synergy [68].

In addition to antimicrobial and sprout suppressant activities, the use of plant extracts, including EO, has numerous advantages, such as being generally recognized as safe (GRAS), being rapidly degraded in the environment and being accepted in organic culture [53,67]. However, some molecules, such as d-limonene, α-pinene, and linalool, may irritate, particularly in the case of degraded products due to oxidation [68]. Tripathi et al. [68] also reported that the oxidation of these compounds can generate a range of secondary organic pollutants. Application in industrial storage represents another obstacle. Indeed, these molecules are easily denatured at a higher temperature, so a cold fumigation application is necessary [68]. Nevertheless, it is worth mentioning that since potatoes are eaten, EOs are already used in animal diets and their accumulation in the system is unlikely because of their fast transformation and elimination [55]. Even though BS-EAc_{OF} and BS-EO exhibit a strong fragrance, it does not necessarily mean that it is going to leave an aftertaste to the treated potatoes, but it would have to be confirmed [73].

Moreover, to further reduce the cost of production, it would be interesting to assess the possibility of successive extractions on the same biomass. For instance, it would be possible to obtain both the EAc extract and the EO by first producing the EO by hydro-distillation of BS

barks, followed by EAc extraction on the residual biomass.

In conclusion, the importance of this work is reflected in the advancement of knowledge in post-harvest potato management to replace harmful synthetic chemical compounds, leading to a bio-sourced product capable of improving potato conservation. Among the 15 forest extracts derived from BS, YB, and BF, BS-EAc_{OF} was the most active extract, according to MIC and MBC values, against the microbial development of dry rot (*Fusarium* spp.) and soft rot (*Pectobacterium* spp., *Dickeya* sp.). BS-EO, with a minimal concentration of 25% (w/w), prevented the sprouting of Colomba potatoes at 100% (w/w) and was as efficient as CIPC. Furthermore, when BS-EAc_{OF} and BS-EO are mixed, antimicrobial and sprout suppressive properties are maintained, allowing the formulation of a single product possessing broad properties to limit post-harvest losses. The development of a natural antimicrobial and sprout suppressive ingredient will thus support the potato industry, help to reduce food waste, and promote the use of forest residues.

CRediT authorship contribution statement

Michelle Boivin: Conceptualization, Methodology, Investigation, Formal analysis, Validation, Visualization, Writing – original draft. **Nathalie Bourdeau:** Conceptualization, Methodology, Project administration, Resources, Funding acquisition, Writing – review & editing. **Simon Barnabé:** Supervision, Writing – review & editing. **Isabel Desgagné-Penix:** Conceptualization, Methodology, Resources, Funding acquisition, Supervision, Writing – review & editing.

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

Table A1

Detailed results on the initial screens of the antimicrobial potential of forest extracts against potato storage pathogens using the INT colorimetric assay. The efficacy of the antimicrobial potential of an extract was rank as: "++'' effective inhibition, "+'' partial inhibition and "-'' no inhibition effect.

Sample number	Origin	Extraction method	Bacteria			Fungi		
			P. carotovorum	P. atrosepticum	D. dianthicola	F. oxysporum	F. graminearum	F. sambucinum
1	Control	MeOH–H_2O	–	–	–	–	–	–
2	Control	H_2O	–	–	–	–	–	N.A.
3	YB	W	++	++	+	++	–	–
4	YB	EAc_{OF}	–	–	–	N.A.	++	N.A.
5	YB	EAc_{AF}	+	+	–	–	–	–
6	YB	AB_{OF}	+	+	–	–	+	–
7	YB	AB_{AF}	+	N.A.	–	–	–	–
8	YB	EO	+	–	–	+	–	N.A.
9	BS	W	N.A.	+	N.A.	–	–	–
10	BS	EAc_{OF}	+	+	N.A.	N.A.	++	++
11	BS	EAc_{AF}	+	++	+	–	–	–
12	BS	AB_{OF}	++	+	–	+	+	++
13	BS	AB_{AF}	N.A.	++	N.A.	–	–	–
14	BS	EO	+	–	–	+	+	N.A.
15	BF	EAc_{OF}	+	+	N.A.	N.A.	++	N.A.
16	BF	EAc_{AF}	++	+	+	–	–	+
17	BF	EO	+	–	–	+	++	N.A.

Abbreviations: YB, yellow birch; BS, black spruce; BF, balsam fir; water, W; ethyl acetate, EAc; acid base, AB; essential oil, EO; AF, aqueous fraction; OF, organic fraction.

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