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RESEARCH ARTICLE

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Compositional analysis of essential oil and solvent extracts of Norway spruce sprouts by ultrahigh-resolution mass spectrometry

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Abstract

Introduction: Coniferous trees, especially their needles and bark, are a rich source of bioactive compounds. The developing needles of Norway spruce (*Picea abies*), also known as spruce sprouts, are enriched with vitamin C and other antioxidants, and thus they are used as a dietary supplement and have been traditionally used to treat various inflammatory disorders such as rheumatism and gout. Their chemical composition is only limitedly known, however.

Objectives: The main objective of this work was to have a deeper understanding on the chemical composition of spruce sprouts to assess their full potential in different pharmaceutical, nutraceutical, or technochemical applications.

Materials and methods: Ultrahigh-resolution Fourier-transform ion cyclotron (FT-ICR) mass spectrometry, coupled to direct-infusion electrospray ionisation (ESI) or atmospheric pressure photoionisation (APPI) techniques, was used for in-depth compositional analysis of solvent extracts and essential oil of spruce sprouts.

Results: A combined use of ESI and APPI techniques offered a great complementary insight into the rich chemistry of different spruce sprout extracts, allowing detection of thousands of chemical constituents with over 200 secondary metabolites tentatively identified. These compounds belonged to different classes such as organic acids, terpenes, flavonoids, stilbenes, sterols, and nitrogen alkaloids.

Conclusion: Spruce sprouts have a complex metabolite profile that differs considerably from that of the old, developed needles.

KEYWORDS
essential oil, high-resolution mass spectrometry, Norway spruce, solvent extract, sprout

1 | INTRODUCTION

Coniferous trees synthesise a great amount of secondary metabolites, such as oleoresin terpenoids and phenolic compounds, which accumulate in high concentrations, especially in the bark, roots, and needles.[1–3] These secondary metabolites are constitutively present in plants, or they are induced by herbivore or pathogenic attacks and are responsible for plants' defense mechanisms against abiotic or biotic stresses.[4–6]

Norway spruce (*Picea abies*), belongs to the *Pinaceae* family and is one the most widespread coniferous trees in Finland, having a great economic and ecological importance.[7] Spruce trees are a rich source of bioactive compounds, such as terpenes, fatty acids, sterols, waxes, and phenolic compounds which could find use in different medicinal or technochemical applications. These compounds are present in varying quantities across different parts of the tree, and their amounts are also dependent on the tree age, provenance, and various environmental factors.[8] Each compound may possess its own bioactivity

characteristics, but different compounds may also act synergistically, thus enhancing the overall effect.[9]

Over the last three decades, a plethora of phenolic compounds have been isolated from spruce needles with their structures identified.[10–17] The needles are enriched with different phenolic compounds, e.g., acetophenones, neolignans, hydroxycinnamic acids, coumarins, stilbenes, tannins, and flavonoids, which possess a variety of biological, nutraceutical, and chemopreventive effects.[11] Terpene hydrocarbons and terpenoids are other abundant compound classes in spruce needles.[18–20] Essential oils, obtained from coniferous trees by hydrodistillation, are highly enriched with monoterpenes and are also primary constituents in the spruce needle essential oils, having different biological activities.[21]

Developing needles of spruce, also known as spruce sprouts or buds, are an interesting, yet limitedly available natural resource.[22] Spruce sprouts are highly enriched with vitamin C and other antioxidants,[23,24] and thus they are harvested during the late spring/early summer to be used as a dietary supplement or to make different culinary products.[24] The harvesting season of spruce sprouts is only a couple of weeks around mid-May to early June. Nevertheless, spruce sprouts offer a considerable business opportunity to the forest owners. An extensive review on the properties and the use of spruce sprouts has been recently published by Jyske *et al*.[24]

Despite the rich chemistry of spruce sprouts, only a very few studies on their chemical composition have been reported to date.[3,24–27] A deeper understanding on the chemical makeup of spruce sprouts is required to evaluate their potential in different pharmaceutical, nutraceutical or technochemical applications. In this study, an in-depth compositional analysis of essential oil and different solvent extracts of Norway spruce sprouts was performed with ultrahigh-resolution Fourier-transform ion cyclotron (FT-ICR) mass spectrometry, combined with atmospheric pressure photoionisation (APPI) and electrospray ionisation (ESI), providing a detailed view on the chemical composition of this limitedly available natural resource. Direct-infusion high-resolution mass spectrometry is an alternative means for non-targeted plant metabolomics and lipidomics studies with some clear advantages over the more traditional techniques.[28] We recently employed APPI/ESI FT-ICR mass spectrometry for a comprehensive compositional analysis of conifer needle extracts with hundreds of individual compounds detected in a single run.[29]

2 | EXPERIMENTAL SECTION

2.1 | Materials

Norway spruce (*Picea abies*) sprouts were harvested in May from the Ylä-Valtimo region, Eastern Finland (63° 42′ N, 28° 52′ E), and stored in a cold room to avoid loss of the volatile components. The solvents used in the solvent extractions were categorised into two groups: non-polar solvents (hexane, toluene, and dichloromethane) and polar solvents (methanol, ethanol, acetone, and water). All the solvents were obtained from VWR Chemicals (VWR International Oy, Helsinki, Finland) and they were of HPLC grade.

2.2 | Hydrodistillation

The hydrodistillation apparatus was assembled using a Clevenger apparatus and a water cooled condenser. About 200 g of freshly harvested spruce sprouts were placed into a 1-L round bottom flask filled with 400 mL of distilled water and were subjected to hydrodistillation for 3 hours. The separated essential oil was then stored in the refrigerator for further analysis.

2.3 | Solvent extractions

The solvent extractions were carried out by using a continuous Soxhlet extraction system (Büchi B-811; Labortechnik AG, Flawil, Switzerland). About 50 g of spruce sprouts were placed into the sample tube which was screwed onto the condenser tube. Then 100 mL of each solvent was poured into the solvent beaker. Different extraction temperatures were used for different solvents depending on their boiling point. The extraction time was always 2 hours and 30 minutes. Following each extraction, the solvent was evaporated to dryness.

2.4 | Mass spectrometry

For MS, the sample stock solutions were prepared by diluting the obtained solvent-free extracts by 1:10 (*v/v*) with HPLC-grade methanol. The solubility was then visually checked. The stock solutions were further diluted by 1:500 (*v/v*) with methanol for the ESI experiments or 1:200 (*v/v*) with methanol/toluene mix (1:10, *v/v*) for the APPI experiments.

All the samples were analysed on a 12-T SolariX XR FT-ICR mass spectrometer (Bruker Daltonics GmbH, Bremen, Germany), equipped with a Paracell ICR cell and an Apollo-II ESI/APPI-II ion source. Both positive ion (+) and negative ion (−) modes for the ESI and APPI techniques were employed. The mass spectra were acquired within a mass range of *m/z* 98–1000, and 300 time-domain transients were coadded for each spectrum with a data set size of 8 MWord. The mass spectra were zero-filled once, prior to fast FT, and magnitude calculation. The samples were directly infused into the ion with a syringe pump, operating at a flow rate of 2 μL/min for ESI or 4 μL/min for APPI. Dry nitrogen was used as the drying and nebulising gas and the drying gas temperature was 220°C. The mass spectra were first calibrated externally using sodium trifluoroacetate clusters (for ESI) or a commercial APCI-L tuning mix (Agilent Technologies, Santa Clara, CA, USA) (for APPI). Before each sample run, a solvent blank was analysed using the same parameters to monitor possible carryover effects and to detect solvent-derived impurities. All such impurities were negligible in terms of the ion intensities, and therefore no blank filtering was needed in this case.

For the collision-induced dissociation tandem mass spectrometry (CID-MS/MS) experiments, the precursor ions were first isolated in the front-end quadrupole (isolation window was set to 10 *m/z* units) and subsequently activated in the hexapole ion trap with helium as collision gas. The collision energy was 10 V.

Compass ftmsControl software was used for the instrument control and data acquisition, and the mass spectra were further processed and analysed with DataAnalysis 5.0 software (Bruker Daltonik GmbH). To improve mass accuracy, the mass spectra were further internally recalibrated with selected analyte ions. For the peak picking, a signal-to-noise (*S/N*) ratio was set at ≥ 5.0 and the relative intensity threshold was 0.001%. For the molecular formula assignments, the main parameters were as follows: DBE = 0–80; H/C ratio ≤ 3; elemental formula: $^{12}C_{1-100}{}^{1}H_{1-200}{}^{14}N_{0-2}{}^{16}O_{0-25}{}^{32}S_{0-1}$; mass error < 1 ppm. The structure annotations were accomplished with a CompoundCrawler database search engine, implemented in DataAnalysis. Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and OriginPro 2018B (OriginLab Corporation, Northampton, MA, USA) were used for the data sorting and visualisation.

2.5 | Compound identifications

As many compounds as possible were tentatively identified (see Supporting Information Tables S1–S5) by comparing their molecular formulae to the compounds existing in several different databases as well as the compounds reported in the earlier studies. The databases used were PubChem, KNApSAcK, Lipid Maps, KEGGs, and Metabolomics Workbench. All the identifications can be considered "tentative identifications", consistent with the "confidence level 3" suggested earlier for untargeted metabolomics studies,[30,31] except those for which only a single molecular structure is retrieved upon the database search (i.e., a validated identification without a reference standard). In case of several isomeric structures, the most probable structure is given based on the previous reports or the known biochemical pathways.

3 | RESULTS AND DISCUSSION

3.1 | ESI/APPI FT-ICR analysis of spruce spout extracts

Figure 1 shows some selected negative-ion ESI FT-ICR mass spectra of spruce sprout extracts. There was a high degree of similarity between (−)ESI and (−)APPI spectra, while the spectra obtained with (+)APPI and (+)ESI were quite different (Supporting Information Figures S1–S4). While (−)ESI produced only deprotonated molecules $[M - H]^-$, (+)ESI produced both protonated molecules, $[M + H]^+$, and sodium/potassium adducts, $[M + Na/K]^+$. In contrast, (−)APPI produced both $[M - H]^-$ ions and radical anions, $M^{\bullet -}$, and (+)APPI produced $[M + H]^+$ ions as well as radical cations, $M^{\bullet +}$. Although a plethora of different ion types are observed with (±)ESI and (±)APPI, they often provide complementary compositional information.[29] After

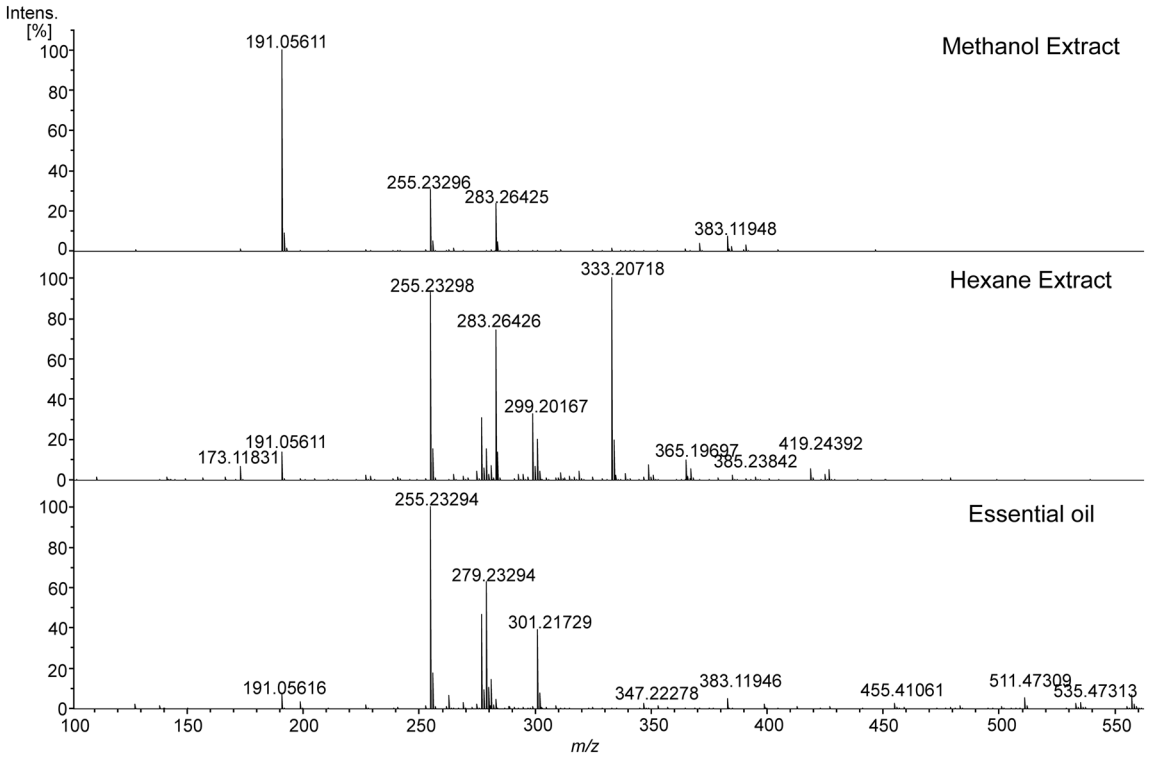

FIGURE 1 Negative-ion ESI FT-ICR mass spectra of some selected spruce sprout extracts

deisotoping and adduct removal, up to ~3200 monoisotopic molecular formulae were assigned to the peaks. The greatest amount of formula assignments was generally made with (+)APPI, followed by (+)ESI, (−)ESI, and (−)APPI (Figure 2).

The numbers of the assigned molecular formulae for each of the ionisation mode in Figure 2 are based on the non-duplicated formula lists, i.e., the peaks representing the same formula as radical, (de)protonated and/or adduct ions were treated as a single molecular formula. Across the 32 mass spectra measured (eight samples and four ionisation modes), a total of 14,030 unique molecular formulae were assigned. Even if a small proportion of the observed ions may result from the unintended in-source fragmentation reactions, ultrahigh-resolution mass spectrometry can resolve thousands of metabolites from a single sample, which is at least one order of a magnitude higher can be resolved by any conventional techniques.

For both (−)ESI and (−)APPI, a small number of high-intensity peaks dominated the mass spectra (Figures S1 and S3), with much of the further complexity of the samples represented by the low-intensity peaks. For all the extracts obtained with polar solvents, an intense peak at *m/z* 191.05605 ($[C_7H_{11}O_6]^-$, mass error +0.301 ppm) was observed, and was tentatively identified as quinic acid. This compound was further confirmed by CID-MS/MS experiments (Figure S5). This compound was also present as a non-covalent dimer (*m/z* 383), especially in water and acetone extracts. Quinic acid was also detected as a potassium adduct in (+)ESI (*m/z* 231.02657; $[C_7H_{11}O_6K]^+$, −0.093 ppm). In contrast, for the extracts obtained with non-polar solvents, the most abundant peak appeared at *m/z* 333.207042 ($[C_{20}H_{29}O_4]^-$, +0.273 ppm), which corresponds to the labdane-type triterpenoid (e.g., dehydropinifolic acid). The same compound was detected as a sodium adduct (*m/z* 357.20370; $[C_{20}H_{30}NaO_4]^+$, +0.193 ppm) with (+)ESI, and it was the highest peak in the mass spectra of hexane, dichloromethane, and toluene extracts (Figure S2). In the (+)APPI spectra of the polar extracts, the highest peak was observed at *m/z* 290.07862 ($[C_{15}H_{14}O_6]^{\bullet+}$, +0.025 ppm), which was identified as catechin, while the ion at *m/z* 302.22400 ($[C_{20}H_{30}O_2]^{\bullet+}$, 0.107 ppm) was more abundant within non-polar extracts, which corresponds to resin acid (e.g., abietic acid). Most ions in (+)APPI appeared as radical cations, $M^{\bullet+}$ (Figure S4).

3.2 | Visualisation of mass spectrometry data

An upset plot is an effective means to visualise the intersections between multiple data sets.[32,33] It is similar to traditional Venn diagrams but deals better with more than three data sets. Figure 3 shows an upset plot generated for the distribution of the assigned formulae for the spruce sprout methanol extract. It shows the numbers of unique and common ions (intersections) detected with different ionisation techniques. The highest number of unique assignments was made with (+)APPI, followed by (+)ESI, (−)APPI, and (−)APPI. This trend follows the total numbers of assignments (black bars or Figure 2). Together, (+)ESI and (+)APPI had the highest number of common assignments, accounting for about 18.3% of the unique formulae. The upset plots for the rest of the extracts can be found in Figure S6.

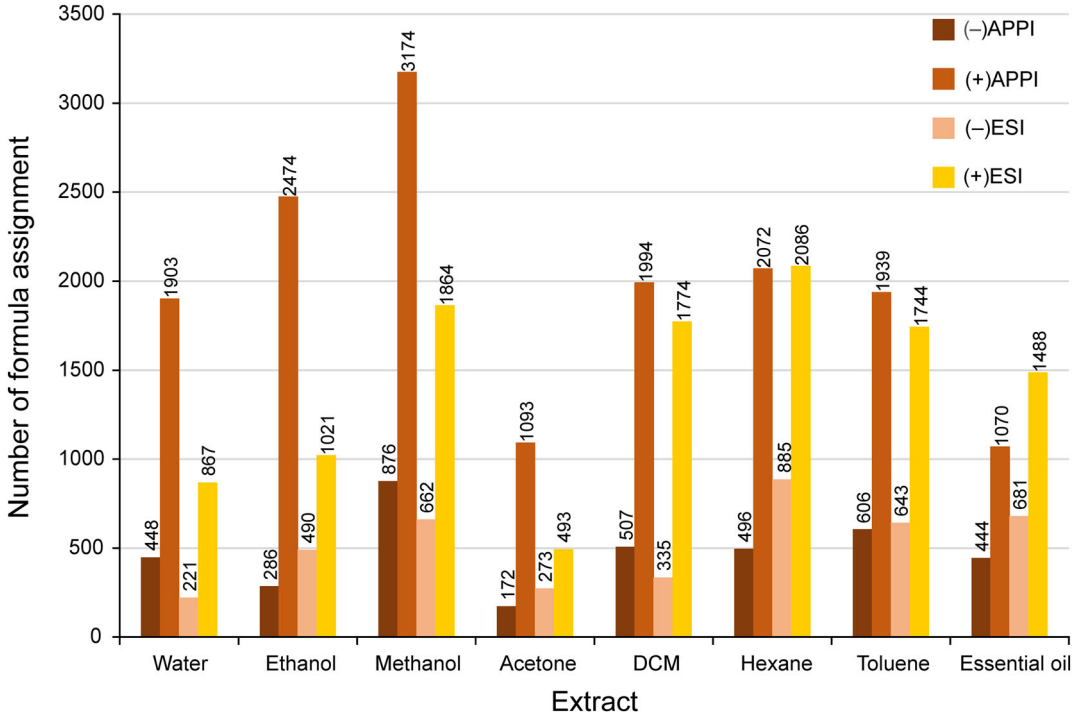

FIGURE 2 Number of assigned formulae with each ionisation technique used [Colour figure can be viewed at wileyonlinelibrary.com]

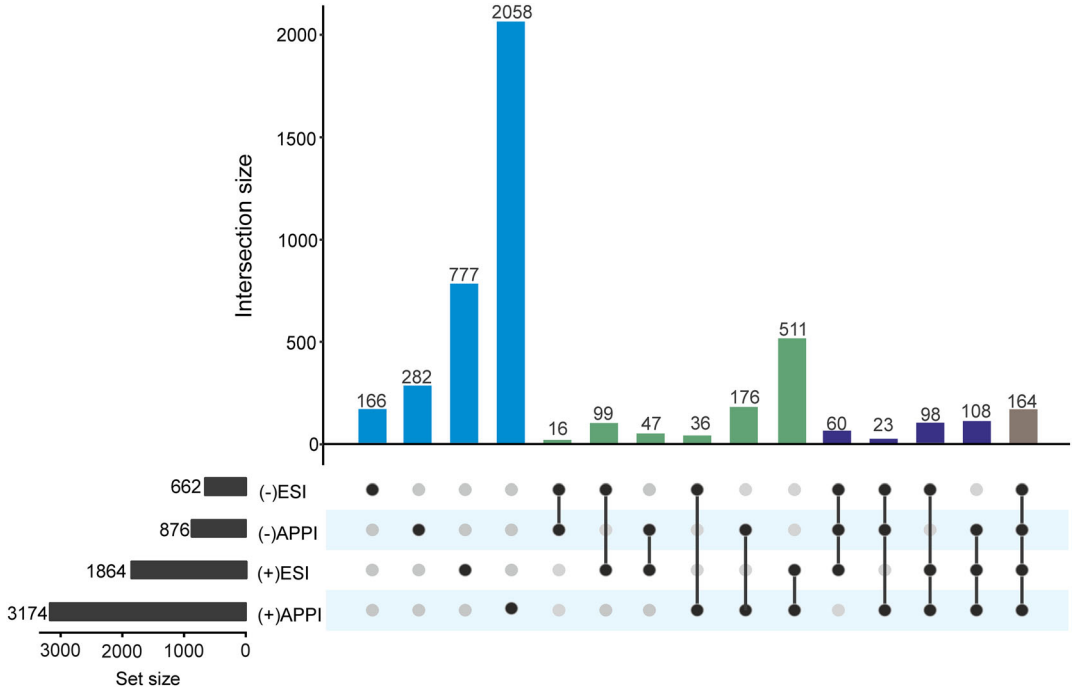

FIGURE 3 Upset plot showing the numbers of unique and common ions (intersections) detected in the spruce sprout methanol extract with different ionisation techniques [Colour figure can be viewed at wileyonlinelibrary.com]

Additionally, compound class distributions (i.e., compounds with a varying number of hydrogen and carbon atoms and a fixed number of heteroatoms) were also used to visualise differences in the sample compositions (Figure S7). The oxygen (O_x) classes were assigned up to O_{25} because the relative abundances beyond this class were negligible. Figure S7 shows that (+)APPI preferentially ionised compounds belonging to the lower O_x classes and hydrocarbon (HC) class as compared to the other methods, consistent with its ability to ionise non-polar and semi-polar molecules. In contrast, (+)ESI targeted the most polar constituents and ionised the greatest number of compounds in general.

Figure 4 shows van Krevelen diagrams for the selected spruce sprout extracts (for the others, see Figure S8). A van Krevelen diagram is an effective visual means for the overall sample composition visualisation. In general, there were marked differences between different samples (solvent polarities) and the ionisation methods used. Although (+)APPI did not cover as much chemical space as the others, it preferentially targeted the compounds at low O/C ratios, consistent with the heteroatom class distributions (Figure S7). The chemical species were concentrated around the region H/C ≈ 1–2 and O/C ≈ 0–0.5, corresponding to terpenoids, acids, and esters. For the essential oil, the most abundant species were observed at O/C = 0, representing terpene hydrocarbons, i.e., monoterpenes, sesquiterpenes and triterpenes.[21] Some abundant species were also seen at O/C ≈ 0.1–0.2 which is a typical region for terpenoids. Another abundant class of species were lipids (e.g., fatty and resin acids) observed around H/C ≈ 1.5–2 and O/C ≈ 0.1–0.25. In contrast, the van Krevelen diagrams for (+) SI showed a different appearance; they covered a wider chemical space, spreading across of H/C ≈ 0–2.5 and O/C ≈ 0–1. In the polar solvent extracts (i.e., water, methanol, ethanol, and acetone), highly abundant compounds were observed at H/C ≈ 1.5–2 and O/C ≈ 0.7–1, comprising a variety of organic acids and sugars or other polyols. In addition, a few species were found around H/C ≈ 0.8–1.2 and O/C ≈ 0.2–0.6, a typical region for phenolic compounds. These compounds were not present in the essential oil and only a few of them were present in the non-polar solvent extracts. In the water (Figure S8) and acetone (Figure 4) extracts, a lower content of terpenes and fatty/resin acids was found. Both (−)ESI and (−)APPI efficiently ionised free fatty acids and other lipophilic compounds, appearing at H/C ≈ 1.2–2 and O/C ≈ 0–0.3.

Additional van Krevelen diagrams were plotted for the nitrogen-containing compounds detected with (+)ESI (Figure S9). These compounds comprised a variety of piperidine alkaloids, amino acids, and indoles, and they were enriched in the polar solvent extracts.

3.3 | Compound identifications

In recent years, FT-ICR mass spectrometry has been extensively applied in the analysis of plant metabolomics and lipidomics.[28] In many cases, it is also possible to annotate compounds solely based on the accurate mass data. In most cases, though, the identifications should be regarded as "tentative identifications", especially when multiple constitutional and/or stereoisomers occur. Tables S1–S4 provide the lists of tentatively identified compounds in the spruce sprout solvent extracts. More than 200 compounds, belonging to different chemical classes, were identified. The chemical structures of some representative compounds are shown in Figure 5.

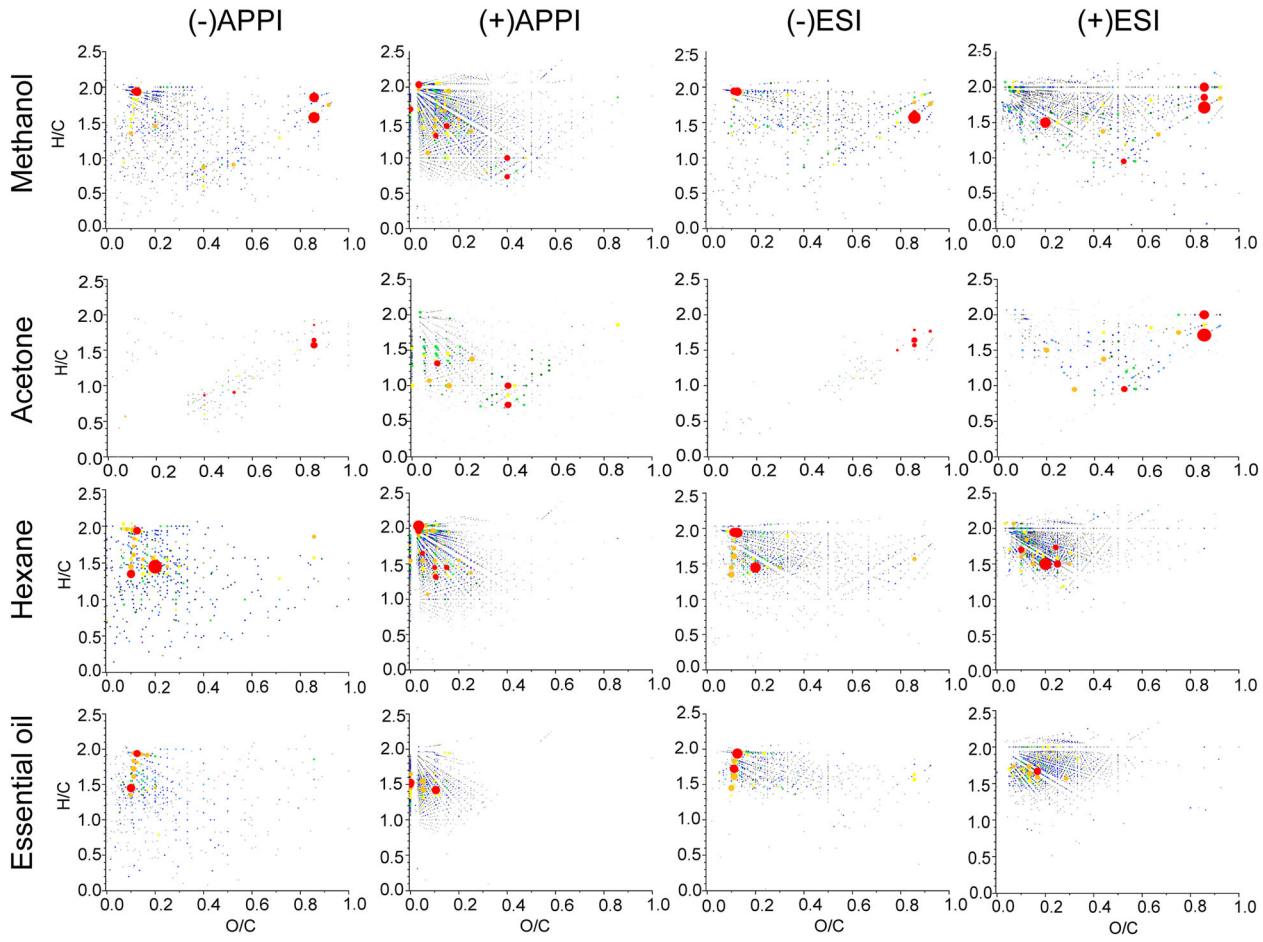

FIGURE 4 Van Krevelen diagrams for each ionisation method for some of the extracts [Colour figure can be viewed at wileyonlinelibrary.com]

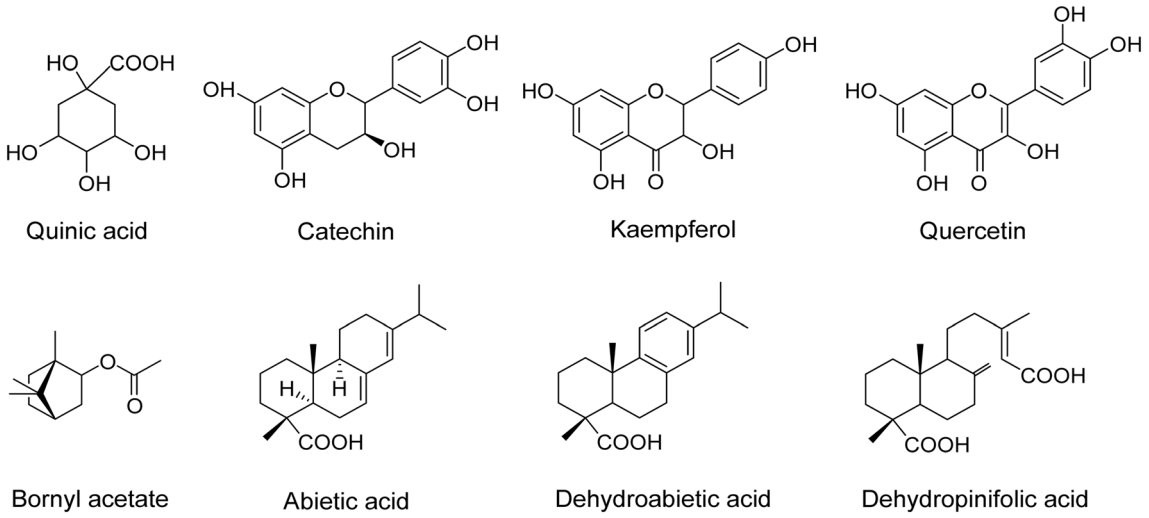

FIGURE 5 Structures of some of the most abundant compounds found in the spruce sprout extracts

3.3.1 | Organic acids and their derivatives

Both ESI and APPI effectively ionised organic acids. Quinic acid (Figure 5) had the highest abundance in the polar solvent extracts and was also relatively high in the non-polar solvent extracts. In contrast, shikimic acid, a close structural relative to quinic acid, was present at very low abundance. This agrees well with the results of Luethy-Krause *et al*.[34] that quinic acid is highly abundant in the freshly sprouted spruce needles and that its concentration lowers quickly upon the needle growth with a concomitant increase in the concentration of shikimic acid. Quinic acid itself is an interesting compound since it can be used as a chiral starting material in the synthesis of oseltamivir, an antiviral drug. In addition, malic and citric acids were also present in measurable amounts but were detected only by (−)ESI. Benzoic acid derivatives, like gallic acid, protocatechuic acid, salicyclic acid, and vanillic acid, were found in minor quantities. Another class that was present and detected at varying concentrations was cinnamic acids (sinapic, ferulic, caffeic, coumaric, and cinnamic acids). Also, hydroxyferulic acid and feruloyl glucoside, were tentatively identified as well.

3.3.2 | Coumarins

Coumarins were present as their free aglycones and the corresponding glucosides. The main coumarins present were aesculetin, aesculin (aesculetin-6-*O*-glucoside), umbelliferone and skimmin (umbelliferone-7-*O*-glucoside). Skimmin has been previously isolated from the needles of Norway spruce.[11]

3.3.3 | Amino acids and peptides

Amino acids are widespread in plants and they have shown various health promoting effects. Three amino acids (proline, threonine, and tryptophan) and their derivatives were tentatively identified in the spruce sprout extracts. These compounds have also been identified in spruce buds.[35] The earlier studies have shown that the concentration of nitrogen-containing compounds in tree needles varies considerably. This could be due to weather acclimation,[36] pollutant exposure[37], soil type, or even the use of nitrogen fertilisers.[38] It was also observed that (+)ESI ionised more nitrogen-containing compounds compared to the other ionisation methods.

3.3.4 | Simple phenols and derivatives

Piceol (4-hydroxyacetophenone) and its glucoside picein are the major phenolics found in the needles of Norway spruce.[39] These compounds were preferentially ionised by both polarities of APPI. Both compounds have been previously reported to be present in spruce sprouts and it has been suggested that their concentration ratio can be used as an indicator of the plant stress.[39] However, several authors have questioned this idea, and Løkke reported that their concentration is not only influenced by the stress factor but can be highly dependent on the provenance and ozone levels, too.[40] Another example of phenolic acid present is chlorogenic acid (3-*O*-caffeoylquinic acid) which was detected in all the polar solvent extracts. Slimestad and Hostettmann were the first to report the occurrence of chlorogenic acid in the mature needles of Norway spruce.[13] Chlorogenic acid was ionised by all the ionisation methods, except (−)APPI. The other simple phenolics present were tyrosol (4-hydroxyethyl phenol) and hydroxytyrosol.

3.3.5 | Flavonoids

Flavonoids are widespread in many plants and they are the largest phenolics group in nature.[8] Their structural diversity gives the possibility to generate different subgroups and they can be present in their aglycone or glucoside forms. The sprouting needles were characterised by high concentration of flavonoids and their glucosides. Six types of flavonoids (i.e., flavones, flavanones, flavonols, dihydroflavonols, flavan-3-ols, and anthocyanidins) have been reported to be present in Norway spruce.[8] Naringerin (flavanone) and its glucoside form were detected by APPI. The reports have shown that naringenin possesses microbial properties.[8] Apigenin (a flavone) was detected in the polar extracts and it was present in its aglycone, glucoside, and acetyl-glucoside forms. Kaempferol (Figure 5) was the most abundant flavonol present in the polar extracts and it was efficiently ionised by (+)APPI as a radical cation (*m/z* 286.047181) although it also formed a protonated molecule. In the methanol extract, kaempferol glycoside, which was present as a sodium adduct ($[M + Na]^+$; *m/z* 471.08983), was observed at high intensity which supports the earlier reports that kaempferol 3-*O*-glucoside dominates in young spruce needles.[3,11] The other flavonols present were quercetin, isorhamnetin, myrincetin, laricitrin, syringetin and their derivatives. The previous reports have shown that as the sprouting needles develop, there is a change in the accumulation pattern of certain phenolic compounds.[10] Acylated flavonols and their glucosides and rutinosides have been identified in the young needles of Norway spruce, and these compounds were also detected in this study. A few dihydroflavonols, aromadendrin, taxifolin, and ampelopsin, were also detected but they were present in minor amounts. Taxifolin is a strong antioxidant with antiradical activity.[8]

Another subclass of flavonoids present in the extracts was flavan-3-ols. Especially, catechin (Figure 5) was observed at high intensity, observed both as a radical as well as a protonated molecule in all the polar solvent extracts. It was ionised efficiently by (+)APPI, but to the lesser extent with the other methods. Catechin has been identified as one of the most abundant flavonoids in the sprouting spruce needles.[41] Its concentration can be influenced, e.g., by the province or the location of the tree in the forest vegetation, or by the position of the needles in the canopy. Vrchotová *et al*. reported that the needles exposed more to the sunlight had an increased level of catechin.[42] Other identified flavan-3-ols were gallocatechol and

methylgallocatechol while the glycosides of these compounds were not present, although the reports have indicated their presence in the Norway spruce needles.[14] The last subclass, anthocyanidins, was not present, albeit they have been observed in the spruce needles as well.[14]

3.3.6 | Stilbenes

Stilbenes or stilbenoids are natural compounds with various biological activities. They are the most studied compounds in the bark of Norway spruce, and they occur both as free aglycones or the corresponding glucosides.[43] Stilbenes, like astringin, piceid, isorhapontin, and piceatannol, which have been previously reported,[10] were also identified in this work. They were detected by all the ionisation methods except (−)ESI.

3.3.7 | Lignans

Lignans are the dimers of coniferyl alcohols, and they are abundant in the knots of Norway spruce.[8] The major lignans present were conidedendrin, pinoresinol, hydroxymatairesinol, and secoisolariciresinol, and they have been widely described in the literature as well.

3.3.8 | Carbohydrates

Carbohydrates serve as the carbon energy source for the growth of plants. In this work, carbohydrate like polyols, such as pinitol, some monosaccharides (glucose, fructose), and a few disaccharides (cellobiose, galactinol) were observed at high abundance. In addition, raffinose and sorbitol were also present. These compounds have been identified in a recent study on the metabolite changes occurring during cold acclimation.[36]

3.3.9 | Tocopherols and sterols

Tocopherols – a natural source of vitamin E, which is present in almost all plant leaves – were identified as well, especially stigmasterol, campesterol, campesterol, and sitosterol. The other phytosterol derivatives, like cycloartenol, methylenecycloartan-3-one, and stigmastan-3,5-diene were also found in the extracts. These compounds have been detected in the wax extract of Norway spruce needles.[44]

3.3.10 | Fatty acids and their derivatives

Some of the most common fatty acids found in coniferouis trees were detected. A homologous series of fatty acids, ranging from C9:0 (nonanoic acid) to C22:0 (behenic acid) were identified. Also, unsaturated fatty acids (oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3) and palmitoleic acid (C16:1)) were detected by all the ionisation methods used. In addition, some hydroxy and dihydroxy fatty acids were also detected. A few fatty alcohols (e.g., nonacosanol and nonacosanediol) were present in considerable amounts but they were ionised only by (+)ESI. Nonacosanol is the most abundant fatty alcohol in the wax extract of the spruce needles. They can be utilised, e.g., in the preparation of superhydrophobic coatings due to their hydrophobic nature.[44]

3.3.11 | Terpenes and terpenoids

The spruce sprout essential oil, obtained in this work, was highly enriched with monoterpenes and sesquiterpenes while the lipophilic extracts were dominated with diterpenes and resin acids. Monoterpenes are the primary constituents of plant essential oils, but they were not efficiently ionised by all of the ionisation methods. The dominating compound in the essential oil by (+)ESI was bornyl acetate ($[M + Na]^+$; *m/z* 219.135538) (Figure 5). This agrees with a previous study by Radulescu *et al.*[22] on the chemical composition of spruce sprout essential oil. That study also reported high amounts of bornyl acetate (monoterpene ester), cadinol (sesquiterpene alcohol), and manool (diterpene alcohol). These compounds were also present at high abundance in the spruce essential oil obtained in this work and also at low abundance in the lipophilic extracts. The compound with the highest intensity, ionised by (+)APPI, was ferruginol (*m/z* 286.229167 for $M^{\bullet+}$), a sesquiterpenol. Terpene hydrocarbons (sesquiterpenes and diterpenes) were efficiently ionised by (+)APPI. Monoterpenes and sesquiterpenes were also present in the solvent extracts but in small quantities. Diterpenes were more efficiently ionised as compared to the other terpenes. Resin acids dominated the solvent extracts. The most abundant compound in the lipophilic extracts was dehydropinifolic acid, a labdane-type resin acid, and it was consistent with all the ionisation methods used. Abietic acid (an abietane-type resin acid) was also found at very high intensity. The other resin acids, reported by several authors, included dehydroabietic acid and its methyl ester as well as hydroxyabietic, neoabietic, pimaric, imbricatolic, and pinifolic acids. The earlier studies have also reported their antimicrobial activities, antifungal activities and wound healing properties.

3.3.12 | Piperidine alkaloids

In addition to phenolics and terpenoids, another class of compounds that was identified, piperidine alkaloids, which are minor secondary metabolites in the *Pinaceae* species and are proposed to play a crucial role in the defensive chemistry of the trees.[26] The earlier studies have reported piperidine alkaloids to be abundant especially in the new shoots of spruce.[25,26] The piperidine alkaloids were efficiently ionised by both (+)ESI and (+)APPI. The identified piperidine alkaloids included pinidinone, 1,6-hydropinidine, pinidinol,

and dehydropinidinone. These compounds were quite abundant in the water extract.

3.4 | Overview on the compounds detected

Norway spruce sprouts contain thousands of secondary metabolites, such organic acids, flavonoids, stilbenes, and other phenolic compounds, which all contribute to their beneficial health promoting properties. Their chemical makeup also differs considerably from that of the old, developed needles. The traditional syrup making is an example of utilisation of this limitedly available natural resource. Some of the compounds present in spruce sprouts may also be potential starting materials for the synthesis of biopharmaceuticals. Spruce sprout harvesting is therefore a considerable business opportunity to the forest owners. Despite the potential applications of spruce sprouts, their chemical composition has been very limitedly studied to date. In this work, we used different polar and non-polar solvents for the compound extraction from spruce sprouts and analysed the products with high-resolution mass spectrometry, which allows a non-targeted chemical fingerprinting of complex organic mixtures. The use of the two different ionisation methods, ESI and APPI, allowed the detection of both the polar and non-polar analytes, directly from these mixtures without their chromatographic separation. This resulted in the assignment of thousands of individual molecular formulae, and further identification of more than 200 secondary metabolites. The polar solvents gave rise to hydrophilic extracts, while the non-polar solvents resulted in more lipophilic extracts, as expected. These results can be taken into account when designing selective extraction methods for specific compound (or compound type) recovery from spruce sprouts.

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DATA AVAILABILITY STATEMENT

The numerical mass spectrometry data (the accurate masses and the assigned atomic formulae) are provided as a supplementary Excel file. The raw mass spectrometry data are available from the corresponding author by a reasonable written request.

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