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Green Chemistry Approaches for Processing of Coniferous Needles and Greenery to Implement Circular Bioeconomy Concepts in Forestry

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Abstract – **Replacing fossil-based materials with renewable biomass is crucial for addressing environmental health challenges and advancing the bioeconomy as a key element of sustainable development. Forestry is a significant biomass source, yet a substantial portion of its by-products, including coniferous greenery, remains underutilized. Maximizing the use of these side streams aligns with circular bioeconomy principles and can reduce dependence on fossil-derived materials. Coniferous needles and greenery are rich in biologically active compounds with potential applications in biopharmaceuticals, food and feed supplements, and material sciences. However, conventional extraction methods rely on toxic solvents, such as organochlorine and hydrocarbons, which pose environmental and health risks. This study uses environmentally friendly solvents to explore green chemistry approaches for extracting bioactive compounds from coniferous biomass. Various green solvents were tested, and conventional methods determined extraction yields. The obtained extracts were analysed using gas chromatography–mass spectrometry (GC-MS) to characterize their composition. Among the tested solvents, isopropanol, acetone, propyl acetate, and dimethyl carbonate demonstrated the highest extraction efficiencies while maintaining a favourable environmental and health profile. Dimethyl carbonate emerged as the most promising "green" alternative to hexane, offering improved sustainability, low toxicity, and a 50 % higher extraction yield than hexane for non-polar compounds. The findings support the integration of green solvents into biorefinery processes, enabling the sustainable utilization of forestry biomass while reducing reliance on hazardous chemicals.**

Keywords – **Analysis; extraction; gas chromatography; green solvents; *Pinus sylvestris* L.; *Picea abies*.**

1. INTRODUCTION

There is an evident need to replace limited fossil resources with renewable ones to address resource limitations and enhance environmental and workplace health. As a solution to the limitation of fossil resources, the bioeconomy can be considered a sustainable, biomass-based development, and it is one of the principal directions of the European Union in reaching sustainable development goals [1], [2]. European Union Forest strategy integrates sustainable

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forest wood and non-wood resource management concepts according to sustainable development principles [3]. To reach the aims of the bioeconomy, the progress of biomass processing technologies is needed, both concerning the selection of biomass sources as well as elaborating approaches of biomass processing to develop new products, reduce energy and material consumption as well and use toxic and dangerous chemicals thus replacing fossil material-based production [4]. A primary biomass source is from forests; however, significant amounts of forestry biomass side streams are formed besides timber, and from a circular bioeconomy perspective, full utilisation of valuable resources is needed [5]. Coniferous trees are a major tree species in the Northern regions of Eurasia and North America, covering significant parts of North European countries with a dominant position in the forestry business. In the case of coniferous tree greenery (a mixture of needles and small branches) can constitute up to half of the total tree mass (needles 27.9–32.3 %, branches 13.1–20.0 % of dry tree biomass) [6] and thus have a definite potential to serve as a significant source of biomass even if only a part from them is obtained after timber processing.

Coniferous needles can be considered a unique source of biomass as, during their evolution, defence mechanisms are developed, providing adaptation possibilities concerning predators, shock, infections, climate change, physical factors (UV radiation, etc.) [7]. Thus, besides the main components of coniferous needles and greenery (cellulose, hemicellulose, lignin), a high number of secondary metabolites have been found participating in cellular and plant regulation mechanisms and contributing to the chemical defence system of conifers [8]. A recent study demonstrated the presence of more than 3000 biologically active substances in coniferous needles. However, this number could be significantly higher [9]. Coniferous needles contain various lipophilic compounds such as resin acids, di-, tri-, and sesquiterpenes, as well as labdane-type alcohols, alkanes, fatty alcohols, acids (C_8 - C_{32}) and waxes have been identified [9]–[11]. Coniferous needles are a rich source of vitamins, essential oils, polyphenols, tannins [12], [13]. Coniferous needle and greenery components have demonstrated high biological activity and promising applications [14]–[16]. Thus, coniferous tree needles and greenery (presently an underutilised side stream of timber production) can serve as a rich source of different substances with direct application potential as a food supplement, for feed with application potential for the production of pharmaceuticals or as a source of building blocks, thus replacing fossil-based substances [7]. However, despite the highly promising composition of coniferous needles and greenery, few applications have been elaborated and presently can be found on the market.

The highly complex composition of coniferous needles and greenery allows obtaining a wide range of products from coniferous biomass using a biorefinery approach. Biorefinery can be described as: ‘the sustainable processing of biomass into a spectrum of marketable products and energy’ [17]. Biorefinery is an essential tool to achieve the bioeconomy aims, supporting incentives to replace fossil resource-based materials and substances with renewable ones and implement biorefinery concept. The biomass processing strategy requires during a stage of biomass processing to obtain as high as possible products for further refinery process [18].

To achieve progress in biomass transformation into products and materials required on the market, new approaches to biomass processing are needed, and, in this respect, promising can be considered Green Chemistry approaches. Green Chemistry can be described as: “design of chemical products and processes to reduce or eliminate the use and generation of hazardous substances” [19]. Green Chemistry follows several principles [20]. For applications in bioeconomy, the relevant key aspects are: 1) prevent wastes; 2) use renewable materials; 3) use biodegradable substances; 4) use non-toxic substances. Implementation of these principles is highly promising for processing of coniferous needles and greenery as, so far,

biologically active components, especially lipids, hydrocarbon or organochlorine solvents, have been used, despite their toxicity and hazardous nature [10], [16], [21]. Environmentally friendly "green solvents" can be highly promising for the processing of coniferous needles and greenery [22], [23].

The present study aims to develop possibilities to use green chemistry approaches for coniferous needle and greenery extraction and following biorefinery to obtain biologically active components as well as exploring the efficacy of green solvent impact on natural product extraction yield by substituting hydrocarbon-based and halogenated solvents with more sustainable solvents.

2. MATERIALS AND METHODS

2.1. Materials

Solvents: hexane, ethyl acetate, *t*-butyl methyl ether, isopropanol, acetone, butan-1-ol, isopropyl acetate, propyl acetate, dimethyl carbonate, ethyl propanoate, 3-pentanone, and dichloromethane (*Sigma-Aldrich*, Germany). Reagents and standards: N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA), pyridine (≥99.8) sclareol (≥98.0 %), D-(+)-glucose (≥99.5 %), palmitic acid (≥99.0 %)), ergosterol (≥99.0 %), 1-octadecanol (≥99.0 %), C_8-C_{20} alkane standard solution (40 mg/L), C_{21}-C_{40} alkane standard solution (40 mg/L) purchased from *Sigma-Aldrich*, Germany.

2.2. Biomass Samples and their Processing

Spruce (*Picea abies*) and pine (*Pinus sylvestris* L.) needles and greenery were sampled in August 2023 – January 2024 in the vicinity of Aglona train station (Spruce Lat. 56.166106, Long. 26.829501) and Celmraguciems (Lat. 56.794470, Long. 23.663303) respectively. After harvesting, spruce and pine greenery were transported to the laboratory and dried at 45 ℃ (48 h). After drying, the spruce and pine greenery was split manually into two parts: needles and branches. The resulting tree parts were grounded in a laboratory mill (*Fritsch*, Germany), separated by particle size (<2 mm) and stored at −20 ℃. Analyses were conducted within 5 months.

2.3. Extraction of Greenery Lipids

For the extraction of branch and needle lipids, 1 g of material was weighed in 20 mL chromatography headspace bottles with cap and mixed with 10 mL of solvent (solvents were selected according to GlaxoSmithKline solvent sustainability guide, and traditional solvents were used for comparison purposes, Table 1), then placed in a shaker for 24 h, 180 rpm (*Biosan PSU-20i*, Latvia). After extraction, all extracts were filtered through a cellulose filter paper and washed with 5 mL of fresh solvent, and extracts were dried at 40 ℃ under a stream of nitrogen. Dry extracts were weighed and stored at −20 ℃.

TABLE 1. MODIFIED GLAXOSMITHKLINE SOLVENT SUSTAINABILITY GUIDE LIST OF SOLVENTS [24]

Solvent	Environmentally friendly	Health	Reactivity/ stability	Flammability and explosion	Life cycle score	Solvent recycling
Ethyl acetate	+++	+++	+++	+	++	+
Isopropanol	+++	+++	+++	++	+	–
Acetone	+++	+++	+++	+	++	–
Propyl acetate	++	+++	+++	++	+	+
3-pentanone	++	+++	++	++	+	+
Dimethyl carbonate	+++	++	+++	++	+++	+
Isopropyl acetate	++	++	+++	++	++	+
Butan-1-ol	++	+	+++	+++	+	+
Ethyl propanoate	++	+	++	++	+++	+
Dichloromethane	++	+	+++	++	++	–
t-Butyl methyl ether	+	+	+++	–	+++	+
Hexane	–	+	+++	–	++	+

Note: A "+" highlights the positive impact of different GSK parameters, and "–" highlights the harmful effects of different GSK parameters.

2.4. GC-MS Analysis

Extracted hexane, ethyl acetate, *t*-butyl methyl ether, isopropanol, acetone, 1-butanol, isopropyl acetate, propyl acetate, dimethyl carbonate, ethyl propanoate, 3-pentanone, and dichloromethane greenery lipids were weighed in a GC vial (approx. 2 mg) and dissolved in 1300 µL pyridine and 200 µL BSTFA derivatisation agent was added. Sample solutions were then heated for one hour at 60 °C. The prepared samples were analysed using gas chromatography mass spectrometry (GC-MS). Compounds were quantified using standard solutions of sclareol, D-(+)-glucose, palmitic acid, ergosterol and 1-octadecanol dissolved in pyridine at a concentration range of 3.2–400 µg/mL.

External standards corresponding to specific groups of chemical compounds were used to identify and quantify unknown compounds in pine and spruce needles and branches. This was done because compounds of different chemical groups have varying analytical signals in GC analysis and similar m/z fragmentation ions. D-(+)-glucose external standard, a hexose sugar, was used for quantification of all carbohydrates, cyclohexanols, cyclohexanecarboxylic acids, heterocyclic amines, alkyl phenyl ketones, alkaloids, benzofurans and phenanthrene; the palmitic acid standard was used for quantifying fatty acids; ergosterol was used as a standard for quantifying sterols, flavan-3-ols and polyphenols; 1-octadecnol was used for quantifying long alkyl chain alcohols, and sclareol was used for quantifying mono-, di- terpenes, terpenoids, sesquiterpenoids and unidentified substances. Compound retention indices were calculated by integrating known alkane standard solution signals using the GC-MS program LabSolutions 4.30 software (*Shimadzu*, Japan).

GC-MS analysis was performed using GC – 2010 plus, GC – MS QP – 2010 Ultra gas chromatograph and mass detector (*Shimadzu*, Japan). Rxi ® – 5MS (*Restek*, USA) column was used with a temperature range of 40–350 °C. Helium (5.0 *Linde gas*, Latvia) was used as a carrier gas with a flow rate of 16.0 mL/min, a column flow rate of 1.18 mL/min, a pressure

of 77.8 kPa, and a column purge flow rate of 3.0 mL/min. A split ratio of 1:10 and an injection temperature of 290 ℃ were used; temperature settings at the time of analysis: the initial temperature of the oven was 75.00 ℃ was held for 2.00 min, increased from 75 ℃ to 130 ℃ with a rate of 20 ℃/min, held for 10.00 min, increased from 130 ℃ to 310 ℃ with the rate of 4 ℃/min and held at 310 ℃ for 10 min. Total runtime – 70 min. Injection volume 1 µL. Mass analyser electron impact was set to 70 eV, detector gain of 0.98 kV + 0.40 kV, scan mode was used with event time 0.30 s, scan speed 2.500, from m/z 35.00 to m/z 650.00. LabSolutions 4.30 software (*Shimadzu*, Japan) and NIST19 (*NIST*, USA) Spectral Library were used to identify and quantify compounds. The analyses were performed in singlet [24].

3. Results and Discussion

3.1. Biomass Samples and their Processing

Sample drying is essential to ensure that no biological processes occur during storage for a prolonged period of time. The drying temperature was chosen to be 45 ℃ because biologically active polyphenols start to decompose at 50 ℃ and to ensure no volatile substances evaporate during drying. Sample milling is necessary to ensure that all material particle sizes are constant, thus ensuring that extraction is replicable and increasing the extraction active surface area to obtain higher yields. Pine and spruce greenery do not differ significantly, as they contain similar amounts of needles (79 %) and branches (21 %) in the air-dry sample. The most significant differences between the greenery and their fractions are observed in the moisture content, which can reach 51.4–63.3 % in spruce and 41.2–46.6 % in pine (Table 2).

Table 2. Moisture Composition of Spruce and Pine Greenery

Material	Water, %	Fraction in greenery (wet material), %	Fraction in greenery (dry material), %	Moisture in the air-dry fraction, %
Spruce needles	51.4±1.5	74±3	79±3	4.0±0.3
Spruce branches	63.3±1.9	26.4±1.1	21.3±0.8	6.3±0.2
Pine needles	41.2±1.2	77±3	79±3	4.2±0.2
Pine branches	46.6±1.4	22.5±0.9	20.9±1.5	4.0±0.3

3.2. Evaluation of Green Solvent Application Potential

Traditional and prospective green solvents have different environmental impact and operational characteristics, as depicted in Table 1. Solvents' most damaging effect (triple "–") is observed in their recycling and flammability/explodability. Solvents like isopropanol, dichloromethane and acetone exhibit negative impacts on waste recycling associated with their use. Similarly, solvents such as MBTE and hexane pose significant risks in terms of flammability and explosion hazards, which can have severe consequences concerning working safety [24].

Still, several solvents demonstrate relatively neutral impacts (+) concerning the possibilities of their waste recycling and environmental impact. Many solvents, including ethyl acetate and dimethyl carbonate, show positive scores for waste recycling (+++), suggesting their potential for reuse or recycling processes. Moreover, several solvents like ethyl acetate and isopropanol exhibit positive environmental impacts, implying lower adverse effects on the environment compared to other factors [26].

The classical organic solvents used to process coniferous needles/greenery are hexane and dichloromethane, most commonly used to extract lipophilic compounds [27], [28]. The ability of a solvent to extract lipophilic compounds is influenced by the solvent's polarity (characterised by dipole moment and dielectric constant). Solvents with a dielectric constant of less than 5 indicate non-polar solvents, while those with a higher dielectric constant indicate polar solvents. Polar solvents can be divided into two groups: 1) protic solvents (hydrogen bound to oxygen, like water, methanol, etc.) and 2) aprotic solvents (dichloromethane, ethyl acetate, etc.). Table 3 distinguishes between protic and aprotic solvents and can guide the selection of the most appropriate solvents for biomass processing. The distinguishing feature of the solvents in the table is the presence of an -OH group, the most common characteristic of a protic solvent [29].

TABLE 3. PERSPECTIVE PHYSIO-CHEMICAL SOLVENT PARAMETERS FOR USE IN EXTRACTION

Solvent	Auto ignition, °C	Boiling point, °C	Dipole moment, D	Dielectric constant	Solvent polarity classification	Solvent recommendation
Isopropyl acetate	425	89	1.75	6.3	Polar aprotic	Recommended
Propyl acetate	455	102	1.86	6.3	Polar aprotic	Recommended
Isopropanol	399	82	1.63	19.92	Polar protic	Recommended
Butan-1-ol	345	118	1.75	17.8	Polar protic	Recommended
Dimethyl carbonate	458	90	0.18	3.09	Nonpolar	Recommended
t-Butyl methyl ether	375	55	1.32	4.5	Nonpolar	Recommended
Acetone	465	56	2.69	20.7	Polar aprotic	Limited use
Ethyl acetate	426	77	1.88	6.02	Polar aprotic	Limited use
Ethyl propionate	440	99	1.74	5.7	Polar aprotic	Limited use
3-Pentanone	452	102	2.70	17.3	Polar aprotic	Limited use
Dichloromethane	556	40	1.14	8.93	Polar aprotic	Not recommended
Hexane	224	69	0.08	1.88	Nonpolar	Not recommended

3.3. Coniferous Needle/Greenery Extraction Yield Depending on Solvents Used in the Extraction

Lipid extraction was performed using milled (<2 mm) and oven-dried pine and spruce biomass (needles and branches). The observed colour of extracts was significantly different between branches and needles: the resulting solutions of branch extracts were yellow but became brown after drying, which could indicate a large share of tannins and terpenes, while the needle extracts were green, indicating the presence of chlorophyll. The obtained extraction yields significantly vary: 1.59–11.24 g of extract/100 g of dried plant material (equal to the % concentration) (Fig. 1., Table S1 in Annex). The extraction efficiency of solvents varies significantly across different plant materials. For instance, isopropanol yields the highest spruce needle extractive yield (8.77 g/100 g), while acetone is the most efficient for extracting spruce branches (8.57 g/100 g). For pine needles, acetone yields the highest amount of total extractives for needles (10.03 g/100 g) and ethyl acetate for total extractive yield for branches (11.24 g/100 g).

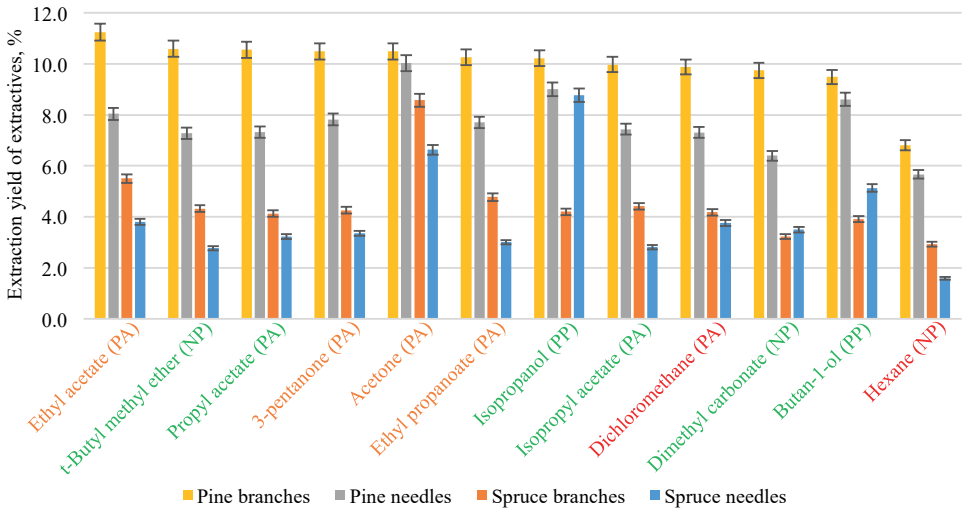

Fig. 1. Effect of organic solvents on the yield of extractives of spruce and pine greenery by conventional extraction. Green – recommended solvents; yellow – limited use solvents; red - not recommended solvents; PA – polar aprotic; PP – polar protic; NP – nonpolar.

Pine biomass, including needles and branches, generally yields more extractable compounds than spruce. Among all tested solvents, branches consistently produced higher extraction yields than needles, except for isopropanol with spruce, where needle extracts surpassed branch extracts. This suggests that pine biomass, particularly branches, may contain a greater concentration of extractable compounds or that these compounds exhibit higher solubility in the tested solvents.

Analysis of Table 3 and Fig. 1 highlights significant differences in extraction efficiency across solvent classes, emphasizing the role of solvent polarity in bioactive compound recovery. Among polar aprotic solvents, ethyl acetate achieved the highest extraction yield, though its advantage over other recommended solvents – propyl acetate and isopropyl acetate – was marginal (1–2 %). The relatively low boiling point of ethyl acetate (boiling point: 77 °C) facilitates controlled evaporation, reducing solvent loss in large-scale processing under non-hermetic conditions. However, propyl acetate (102 °C) and isopropyl acetate (89 °C) present viable alternatives for environmentally and health-conscious extraction approaches, albeit with higher energy requirements for solvent recovery. Propyl acetate exhibited the second-highest extraction efficiency, while isopropyl acetate ranked sixth among polar aprotic solvents.

For polar protic solvents, isopropanol and butan-1-ol were selected. Isopropanol, a well-established solvent in extraction processes, demonstrated superior industrial applicability due to its relatively low boiling point (82 °C). In contrast, butan-1-ol (boiling point: 118 °C) requires higher energy input for solvent recovery, which limits its large-scale adoption despite its extraction efficiency.

Solvents with different polarities exhibited varied extraction efficiencies, underscoring the diverse polarities of the extracted compounds. Polar solvents such as isopropanol and acetone provided higher extraction yields, indicating the prevalence of polar compounds such as polyphenols, carbohydrates, and phenolic acids in the biomass. Comparing hexane with

acetone and isopropanol, substantial differences in extraction yields were observed, particularly between different plant components. The most pronounced variations were noted in spruce twigs and conifer extracts, where acetone increased extraction yields by 2.9–4.2 times and isopropanol by 1.4–5.5 times. Conversely, differences in extraction yields for pine needles and branches were relatively minor, with only a 1.5–1.7-fold increase when using acetone and isopropanol, suggesting a lower concentration of polar compounds in pine biomass.

Spruce needles contain a substantial fraction of non-polar compounds, necessitating using nonpolar solvents for effective extraction. To evaluate sustainable alternatives to hexane, *t*-butyl methyl ether and dimethyl carbonate were investigated. While *t*-butyl methyl ether exhibited high extraction efficiency, its instability at elevated temperatures and pressures raises concerns due to peroxide formation, which poses a potential explosion risk. Although stabilizers can mitigate this hazard, they may introduce impurities into the final extract during concentration steps. In contrast, while slightly less efficient than *t*-butyl methyl ether, dimethyl carbonate demonstrated a 50 % higher extraction yield than hexane. Its higher boiling point further minimizes solvent loss during transport and storage, making it a safer and more environmentally friendly alternative. However, industrial adoption of dimethyl carbonate must account for increased production costs due to higher energy demands for solvent recovery. Nonetheless, reduced solvent losses could partially offset these costs, balancing overall extraction efficiency and economic feasibility.

The target bioactive compounds should guide the selection of an optimal extraction solvent. For highly polar compounds, solvents such as isopropanol and acetone are preferable. In contrast, for less polar compounds, green solvents such as dimethyl carbonate, ethyl acetate, and isopropyl acetate provide viable alternatives to traditional solvents like hexane and dichloromethane, which pose significant environmental and health risks due to their toxicity and persistence. The transition to greener solvents aligns with sustainable extraction practices, promoting both environmental responsibility and industrial feasibility [24], [30].

3.4. GC-MS Analysis of the Extracts Obtained

Pine and spruce, although both coniferous trees contain different types of compounds in their needles and branches. These differences are attributed to their unique phytochemical compositions, which influence the types of volatile and non-volatile compounds extracted during the extraction process. In the present study, 14 different groups of substances were identified in the extracts obtained by gas chromatography-mass spectrometry (see Fig. 2). Terpenes, carbohydrates, fatty acids, sterols and unidentified compound groups were found in the highest concentrations of these groups. Terpenes are found in all spruce and pine extracts at significantly variable amounts of 1.38–23.65 %. Still, pine branches exhibit the highest concentration of terpenes compared to other materials, ranging from 12.84 % to 23.65 %. Carbohydrates are present in all extractable materials but can be extracted in higher concentrations (3.13–12.83 %) with certain more polar solvents: acetone, isopropanol, and butan-1-ol.

Fatty acids and terpenes have been found in almost all extracts. High concentrations of fatty acids were found in extracts from spruce branches (6.68–11.11 %) and pine branches (2.29–5.20 %). The fatty acid concentration in spruce needles is lower than 2.64 %, except in hexane extract, where the yield reaches 6.56 %. Sterols are present in all extracts obtained in low concentrations (0.85–3.93 %). The concentration of unidentified compounds in the extracts varied depending on the material used. The extracts of spruce and pine needles have a higher concentration of unidentified compounds (3.99–15.28 %) than the extracts of branches (0.15–

4.54 %), indicating the need to fractionate these extracts to improve the possibility of identifying the compounds. The acetone, isopropanol and butan-1-ol extracts of spruce needles are capable of selectively extracting cyclohexane carboxylic acid (2.20–5.38 %) and cyclohexanol compounds (2.20–5.30 %) such as shikimic acid and D-pinitol (11.15–18.40 %) (Fig. 3, Table S2 in Annex).

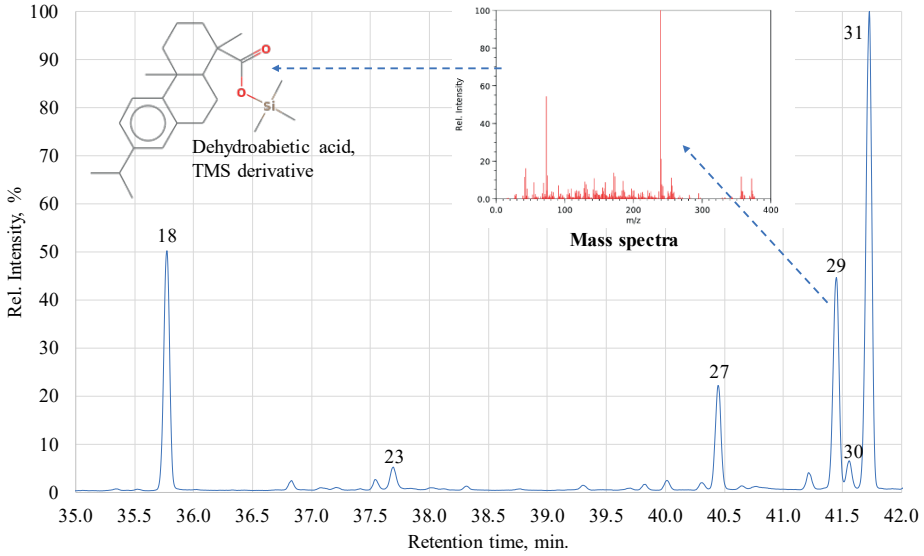

Fig. 2. Example of compound identification in spruce needle dimethyl carbonate extract – original chromatogram, compound 29 mass spectra and identified compound structure.

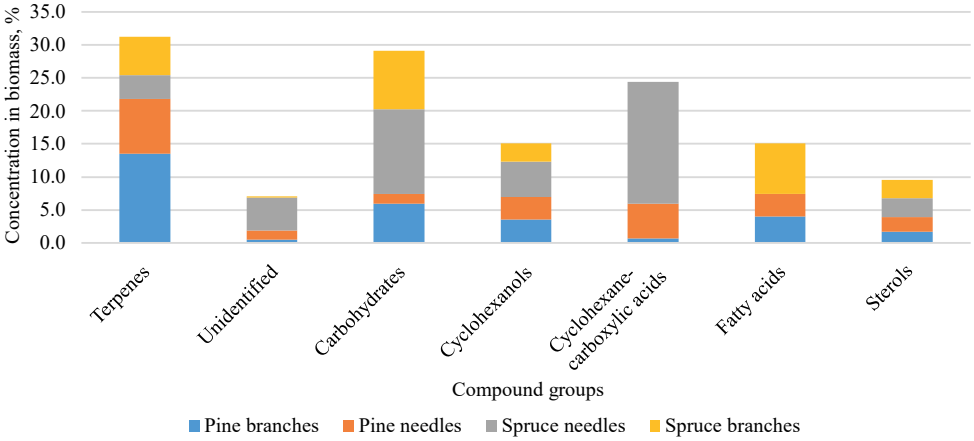

Fig. 3. Major compound groups detected in different biomass acetone extracts.

D-Pinitol has well-known antioxidant, antimicrobial and anticancer effects. Recent studies indicate that the compound can reduce inflammation more effectively by evaluating the molecular interactions with cyclooxygenase-2 (COX-2) compared to the synthetic pharmaceuticals celecoxib and ketoprofen [31].

Gas chromatography of spruce needle extracts revealed 32 compounds with an area greater than 1 %, of which nine could not be identified by the method used. One of the primary individual compounds identified in the spruce needle extracts is shikimic acid, which can be extracted mainly using acetone (18.40 %) and isopropanol (13.26 %). Terpene compounds such as labda-8(20),14-dien-13-ol, (13S)-(1.56–13.88 %), isopimaric acid (0.32–4.65 %) and dehydroabietic acid (1.34–11.44 %) were found in high concentrations in spruce needles. These before-mentioned compounds are found in the highest concentrations in hexane, ethyl propanoate and dimethyl carbonate extracts. Three fatty acids (C-16:0, C-18:1 and C-22:0) were identified in the extracts, accounting for approximately 0.54–2.47 %. All three fatty acids were found in higher concentrations in the hexane (1.94–2.47 %) and dimethyl carbonate (0.57–0.78 %) extracts. In contrast, no fatty acids were identified in the isopropanol, 3-pentanone, acetone, butan-1-ol, ethyl propanoate and isopropyl acetate extracts. Sterols were identified as one of the groups of biologically active compounds in needle extracts. These extracts contain only one identified sterol, stigmast-5-ene, 3β-(24S), found in all extracts obtained (1.17–7.56 %). The highest concentrations of the sterol are found in the extracts of ethyl propanoate (7.65 %), isopropyl acetate (3.69 %), ethyl acetate (3.63 %) and dimethyl carbonate (3.44 %). In comparison, the lowest observed concentration was found in hexane (1.17 %) and isopropanol (1.58 %) extracts (see Table 4, S3).

The chemical composition of spruce branches differs from that of spruce needles, as the extracts obtained do not contain high concentrations of distinct compounds. The highest concentration of an individual compound does not exceed 5.32 % by weight of the total extract. In spruce branches, 33 individual compounds with an area greater than 1 % on the gas chromatogram were found, of which eight compounds could not be identified. In all extracts obtained, five compounds were found in the highest concentrations: isopimaric acid (2.35–5.32 %), C-22:0 acid (2.45–4.31 %), dehydroabietic acid (1.99–4.28 %), stigmast-5-ene, 3β-(24S) (2.48–3.93 %) and abietic acid (1.15–3.14 %). Spruce twig extracts are more prosperous in fatty acid diversity than spruce needles, with six fatty acids: C-16:0 (0.77–1.71 %), C-18:1 (1.12–1.68 %), C-18:2 (1.13–1.92 %), C-20:0 (0.62–0.96 %), C-22:0 and C-24:0 (0.90–1.30 %) found in the extracts. Acetone, isopropanol, and butan-1-ol extracts are the only ones in which different carbohydrates (pentoses and hexoses) could be detected at higher concentrations (0.42–3.37 %). The concentration of these extracts in the aqueous phase could be used in future studies to separate lipophilic compounds by liquid-liquid extraction, crystallisation, and desalting, which could be further separated by preparative chromatographic methods (see Table 4, S3).

34 individual compounds were found in pine branches, 6 of which could not be identified. Similar to spruce branch extracts, pine branch extracts do not contain distinct individual compounds, but they contain similar major compounds: isopimaric acid (1.71–5.68 %), dehydroabietic acid (1.38–8.14), dehydroabietinol (0.29–3.64 %), stigmast-5-ene, 3.β-(24S) (0.85–2.97 %) and C-22:0 (0.78–2.26 %). Unlike needles, pine branches contain carbohydrates (pentoses and hexoses), which were found in acetone (1.18–1.68 %), isopropanol (0.57–1.69 %) and butan-1-ol (0.52–0.88 %) extracts. Carbohydrates mainly fulfil various functional roles, including transport, energy metabolism and providing substrates for synthesising or exchanging protective compounds for nutrients [32]. This is the main reason why the concentration of carbohydrates is higher in branches than in needles. Pine branches contain the essential tannin structure elements - procyanidins (catechin and epigallocatechin). Tannins are mainly found in the bark and provide antibacterial and antifungal protection. In recent years, research into the use of tannins has been expanding, where they could be used as food additives to improve the shelf life of meat products without altering their taste [33]. In the extracts obtained, tannins were identified only in acetone extracts (1.48–2.26 %). In previous studies on procyanidins, aqueous acetone was the most

suitable solvent for extracting compounds from cranberry press residues [34], supporting the presence of the identified compounds in acetone extracts of pine branches (see Table 4, S3).

TABLE 4. MAJOR COMPOUNDS FOUND IN CONIFER EXTRACTS

RT, min	RI exp.	RI lib.	Name	Compound group	Main fragmentation ions, m/z
27.886	1843	1843	Shikimic acid, 4TMS derivative	Cyclohexanecarboxylic acid	204.00-73.00-147.00-205.00-357.00
28.704	1869	1815	D-Pinitol, 5TMS derivative	Cyclohexanol	73.00-147.00-217.00-260.00-318.00
33.595	2051	2036	C16:0 acid, TMS derivative	Fatty acid	117.00-73.00-75.00-313.00-132.00
35.768	2137	N/A	Labda-8(20),14-dien-13-ol, (13S)-, TMS derivative	Diterpene	143.00-73.00-75.00-81.00-257.00
37.691	2218	2209	C18:1 acid, TMS derivative	Fatty acid	117.00-339.00-354.00
39.615	2304	N/A	Dehydroabietinol, TMS derivative	Diterpene	173.00-239.00-73.00-131.00-358.00
40.444	2342	2329	Isopimaric acid, TMS derivative	Diterpene	44.00-241.00-256.00-359.00-374.00
41.443	2389	2385	Dehydroabietic acid, TMS derivative	Diterpene	239.00-73.00-240.00-357.00-372.00
42.059	2418	2422	Abietic acid, TMS derivative	Diterpene	241.00-73.00-185.00-359.00-374.00
46.513	2640	2638	C22:0 acid, TMS derivative	Fatty acid	73.00-43.00-397.00-117.00-245.00
51.671	2922	2838	Catechine, (2R-cis)-, 5TMS derivative	Flavan-3-ol	368.00-355.00-650.00-147.00-450.00
53.890	3054	2903	Epigallocatechin, 6TMS derivative	Flavan-3-ol	73.00-456.00-369.00-267.00-147.00
58.682	3358	3349	Stigmast-5-ene, 3.beta.-(trimethylsiloxy)-, (24S)-	Sterol	129.00-43.00-73.00-95.00-57.00

Note: RT (min) - retention time in minutes; RI exp. - experimentally measured retention index; RI lib. - reference retention index from a database; N/A - library retention index (RI lib.) was not found in the database.

4. CONCLUSIONS

This study highlights the potential of green solvents as sustainable alternatives to traditional hydrocarbon-based solvents for biomass extraction. Among the tested solvents, isopropanol, acetone, ethyl acetate, and dimethyl carbonate demonstrated the highest extraction efficiency while maintaining a favourable environmental and health profile. These solvents align with industrial safety standards, offering reduced toxicity, lower environmental persistence, and improved solvent recovery potential.

Isopropanol and acetone, widely used in the pharmaceutical and food industries, showed the highest efficiency in extraction yields, potentially extracting more total polyphenols and carbohydrates, making them ideal for applications in nutraceuticals and functional food ingredients. Propyl acetate, with its moderate polarity and low toxicity, exhibited excellent selectivity for bioactive extractions and is well-suited for industrial applications requiring solvent recyclability and minimal health risks. Dimethyl carbonate, recognized for its non-

toxic and biodegradable nature, proved an effective alternative to hexane for non-polar compound extraction, balancing efficiency and sustainability.

Integrating these green solvents into large-scale biorefinery processes presents a viable pathway for sustainable forestry biomass utilization. Their industrial scalability and ability to selectively extract valuable compounds support the development of high-value bio-based products while minimizing environmental impact.

Future research should focus on optimizing process parameters, including solvent concentration, extraction time, and temperature, to enhance extraction efficiency further. Additionally, techno-economic and life cycle assessments will be crucial in evaluating the feasibility of implementing these green solvent-based extraction methods in commercial biorefineries.

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ANNEX

TABLE S1. YIELD OF EXTRACTIVES OF CONIFEROUS GREENERY BY CONVENTIONAL EXTRACTION

Solvent	Spruce needles, %	Spruce branches, %	Pine needles, %	Pine branches, %
Ethyl acetate	3.80±0.11	5.50±0.17	8.0±0.2	11.2±0.3
t-Butyl methyl ether	2.77±0.08	4.32±0.13	7.3±0.2	10.6±0.3
Propylacetate	3.23±0.10	4.13±0.12	7.3±0.2	10.6±0.3
3-pentanoate	3.36±0.10	4.26±0.13	7.8±0.2	10.5±0.3
Acetone	6.6±0.2	8.6±0.3	10.0±0.3	10.5±0.3
Ethyl propanoate	3.01±0.09	4.78±0.14	7.7±0.2	10.3±0.3
Isopropanol	8.7±0.3	4.20±0.13	9.0±0.3	10.2±0.3
Isopropyl acetate	2.81±0.08	4.41±0.13	7.4±0.2	10.0±0.3
Dichloromethane	3.76±0.11	4.18±0.13	7.3±0.2	9.9±0.3
Dimethyl carbonate	3.50±0.10	3.22±0.10	6.40±0.19	9.7±0.3
Butan-1-ol	5.13±0.15	3.90±0.12	8.6±0.3	9.5±0.3
Hexane	1.59±0.05	2.93±0.09	5.66±0.17	6.8±0.2

TABLE S2. MAJOR COMPOUND GROUPS DETECTED IN DIFFERENT BIOMASS ACETONE EXTRACTS

Compound groups	Pine branches	Pine needles	Spruce needles	Spruce branches
Terpenes, %	13.5	8.3	3.6	5.8
Unidentified, %	0.5	1.4	5.0	0.2
Carbohydrates, %	6.0	1.5	12.8	8.9
Cyclohexanols, %	3.5	3.4	5.4	2.8
Cyclohexanecarboxylic acids, %	0.7	5.3	18.4	
Fatty acids, %	4.0	3.5		7.6
Sterols, %	1.7	2.2	2.8	2.8

TABLE S3. LIST OF COMPOUNDS IDENTIFIED IN CONIFEROUS BIOMASS

Comp. Nr.	RI experimental	Name	Compound group
1	1229	exo-Borneol, TMS derivative	Terpene
2	1269	N-(Trimethylsiloxycarbonyl)piperidine	Heterocyclic amine
3	1278	Glycerol, 3TMS derivative	Alcohol
4	1289	Bornyl acetate	Mono terpene
5	1464	4'-Hydroxyacetophenone, TMS derivative	Alkyl-phenyl ketone

6	1745	(((1S,4R)-4-isopropyl-1,6-dimethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalen-1-yl)oxy) TMS derivative	Terpene
7	1836	Oplopanone, TMS derivative	Sesquiterpenoid
8	1843	Shikimic acid, 4TMS derivative	Cyclohexane-carboxylic acid
9	1844	Pentose, 5TMS derivative (27.800)	Carbohydrate
10	1854	Pentose, 5TMS derivative (28.200)	Carbohydrate
Comp. Nr.	**RI experimental**	**Name**	**Compound group**
11	1869	D-Pinitol, 5TMS derivative	Cyclohexanol
12	1898	Quininic acid, 5TMS derivative	Alcoloid
13	1918	Hexose, 5TMS derivative (30.000)	Carbohydrate
14	1930	Hexose, 5TMS derivative (30.400)	Carbohydrate
15	2027	Hexose, 5TMS derivative (33.000)	Carbohydrate
16	2051	C16:0 acid, TMS derivative	Fatty acid
17	2127	? (35.400)	Unidentified
18	2137	Labda-8(20),14-dien-13-ol, (13S)-, TMS derivative	Diterpene
19	2182	Phytol, TMS derivative	Terpenoid
20	2187	? (36.900)	Unidentified
21	2194	? (37.100)	Unidentified
22	2212	18:2 acid, TMS derivative	Fatty acid
23	2218	C18:1 acid, TMS derivative	Fatty acid
24	2304	Dehydroabietinol, TMS derivative	Diterpene
25	2323	Cryptopimaric acid, TMS derivative	Diterpene
26	2336	? (40.400)	Unidentified
27	2342	Isopimaric acid, TMS derivative	Diterpene
28	2378	Levopimaric acid, TMS derivative	Diterpene
29	2389	Dehydroabietic acid, TMS derivative	Diterpene
30	2396	? (41.500)	Unidentified
31	2401	? (41.700)	Unidentified
32	2418	Abietic acid, TMS derivative	Diterpene
33	2441	? (42.500)	Unidentified
34	2443	C20:0 acid, TMS derivative	Fatty acid
35	2444	? (42.600)	Unidentified

36	2489	7-Hydroxy-1,4a-dimethyl-9-oxo-7-propan-2-yl-2,3,4,4b,5,6,10,10a-octahydrophenanthrene-1-carboxylic acid, TMS derivative	Diterpene
37	2513	? (44.000)	Unidentified
38	2532	(1R,4aR)-6-Hydroxy-1,4a-dimethyl-7-propan-2-yl-2,3,4,4b,5,6,10,10a-octahydrophenanthrene-1-carboxylic acid, 2TMS derivative	Diterpene
39	2547	7-Hydroxy-8,11,13-abietatrien-19-oic acid, 2TMS	Diterpene
40	2551	? (44.700)	Unidentified
41	2562	1-Naphthalenecarboxylic acid, decahydro-5-(5-hydroxy-3-methylpentyl)-1,4a-dimethyl-6-methylene-, (1R,4aS,5R,8aS)-, 2TMS	Diterpene
42	2586	? (45.400)	Unidentified
43	2595	15-TMS dehydroabietic acid, TMS	Diterpene
44	2597	5-(5-Methoxycarbonyl-5,8a-dimethyl-2-methylidene-3,4,4a,6,7,8-hexahydro-1H-naphthalen-1-yl)-3-methylpentanoic acid, TMS	Diterpene
45	2625	Maesopsin, 4TMS derivative	Benzofuran derivative
46	2640	C22:0 acid, TMS derivative	Fatty acid
Comp. Nr.	**RI experimental**	**Name**	**Compound group**
47	2641	? (46.500)	Unidentified
48	2648	? (46.600)	Unidentified
49	2649	? (46.700)	Unidentified
50	2734	(3S,4bS,8R,10aS)-4b,8-Dimethyl-2-(propan-2-yl)-4,4a,4b,5,6,7,8,8a,9,10-decahydro-3H-3,10a-epidioxyphenanthrene-8-carboxylic acid	Phenanthrene derivative
51	2746	Tetracosanol, TMS	Alcohol
52	2763	? (48.900)	Unidentified
53	2823	? (49.800)	Unidentified
54	2836	C24:0 acid, TMS derivative	Fatty acid
55	2915	? (51.500)	Unidentified
56	2922	Catechine, (2R-cis)-, 5TMS derivative	Flavan-3-ol
57	2965	Dihydroconiferin, 5TMS derivative	Alcohol
58	3054	Epigallocatechin, 6TMS derivative	Flavan-3-ol
59	3070	Nonacosan-10-ol, O-TMS	Alcohol
60	3276	Nonacosan-4,10-diol, 2TMS derivative	Alcohol
61	3315	? (58.200)	Unidentified
62	3349	Pinoresinol, 2TMS derivative	Polyphenol
63	3358	Stigmast-5-ene, 3.beta.-(trimethylsiloxy)-, (24S)-	Sterol

64	3483	? (60.500)	Unidentified
65	3570	? (62.200)	Unidentified
66	3745	? (65.500)	Unidentified
67	3858	? (68.300)	Unidentified