FIMEK

Journal of Agronomy, Technology and Engineering Management

Review

Spruce (*Picea abies* (L.). H. Karst): Different Approaches for Extraction of Valuable Chemical Compounds

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Received: 13 April 2020; Accepted: 7 June

Abstract: Recent years have seen enormously increased utilization of wood-based compounds, extracts, and biomass, as well as worldwide interest in manufacturing health-promoting pharmaceuticals, agrochemicals, food additives, and bioenergy. Available literature indicates that spruce-based materials represent a source of wide range of valuable compounds, such as phenolic acids, flavonoids, lignans, stilbene glucosides, resin acids, terpenes, fatty acids, sterols and polysaccharides. A great deal of attention has been given to extraction routes towards the valorization of spruce and its residues. Accordingly, the objective of this review was to collect aspects related to the technologies employed to obtain and isolate different compounds from spruce-based materials, integrating conventional and non-conventional methods investigated in the literature.

Keywords: *Picea abies*; spruce residues; conventional extraction techniques; non-conventional extraction techniques; phytochemicals.

1. Introduction

Wood is a valuable source of energy and phytochemicals and a sustainable material for various industrial applications. Chemically complex structure of the cell wall and its morphological, and physical characteristics determine biological and technical properties of wood. Structural components of wood cell walls are, primarily, cellulose, hemicelluloses and lignin, the basic materials of biorefineries. The properties of wood are also influenced by extractives, such asstilbenes, flavonoids, proanthocyanidins, phenolic acids, lignans, terpenes, carbohydrates, fatty acids and others. Increasing knowledge and awareness about the health promoting impact of these compounds in plant material, combined with the cognition that a number of common synthetic substances may have detrimental effects [1; 2; 3], have led to multiple investigations in the field of natural phytochemicals in wood based materials. Current evidence firmly supports a contribution of natural compounds in wood based materials, to prevent or reduce the effect of the oxidative stress caused by ROS and associated with the pathogenesis of various diseases including atherosclerosis, cancer, diabetes mellitus, and inflammatory and neurodegenerative disease, as well as obesity and ageing [4; 5; 6]. Different biological activities including antitumor, antibacterial, antifungal and anti-inflammatory [7; 8; 9; 10; 11] were determined in spruce-based materials.

Norway spruce (*Picea abies* (L.). H. Karst) is a widely used softwood species in the wood industry and one of the most abundant conifer species in boreal Eurasian forests. Annually, a considerable amount (6-8 million tonnes) of spruce biomass is generated as by-product in the stages of forest

maintenance and initial wood processing [12]. The biomass residues are usually discarded or used for biofuel production, or animal feed. A total of about 12 million m^3 of biofuel in Europe originates from residues burnt in the mills where logs are debarked [13]. Efficient exploitation of this abundant wood material could create novel applications and wide range of high value-added products. In the last decade, great efforts have been bestowed by different investigation groups to find the best approaches to a possible valorization of spruce-based materials. A number of studies on various chemical compounds (polyphenols, terpenes, polysaccharides, fatty acids, among others) extracted from spruce using different extraction techniques have been published.

2. Extraction of spruce-based materials

In general, the quality of plant extracts depends on sample pre-treatment, type of solvents with varying polarities, extraction time and temperature, sample-to-solvent ratio as well as on the chemical composition and physical characteristics of the plant sample. Large variation in extractives recovery and composition is affected by species, age, edaphoclimatic conditions, harvesting time, and tree health [14]. The recoveries are also affected by the wood parts (cones, branches, needles, roots, barks, heartwood, and phloems). However, finding an optimal extraction method and understanding the effect of extraction parameters on the component yields leads to the acquisition of extracts with the highest content of active compounds and the lowest content of interfering substances.

The application of conventional extraction techniques: solid-liquid extraction (SLE), hydro-distillation (HD), steam distillation (SD), simultaneous distillation extraction (SDE), Soxhlet) is the most common procedure for isolation of extractives from wood-based material. In order to overcome certain drawbacks of standard extraction methods such as huge energy consumption, time-consumption and cost-consumption, non-conventional extraction techniques, such as hot water extraction (HWE),supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), subcritical water extraction (SWE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), extraction with deep eutectic solvents (DESs) and switchable ionic liquids (SILs),have been emerged. Even if these techniques are the main novel methods used to extract spruce-based materials, other emerging techniques are also studied, electrical treatments like pulsed electric fields (PEF) and high-voltage electrical discharges (HVED). Accordingly, Table 1 summarizes the studies in the field of the phytochemicals' extraction from spruce and its residues.

Table 1. Studies of chemical compounds extracted from *P. abies* and its residues by different extraction techniques.

Raw material	Compound	Extraction technique	Reference
wood residues	phenolic acids, flavonoids, lignans, stilbene glycosides	SFE	[11]
cones, branches, needles and bark	fatty acids, terpenes, stilbenes, sterols, long chain alcohols	SFE, Soxhlet	[15]
bark, phloem	stilbene glycosides	UAE	[16]
phloem	stilbenes, terpenes	UAE	[17]
root bark	stilbenes	PLE	[18]
bark	resin acids, fatty acids, terpenes, stilbenes	Soxhlet	[9]
bark	fatty acids, alcohols, waxes, terpenes, resin acids	SFE, PLE	[19]
bark	stilbenes, tannnins, lignin	SLE	[20]
bark	stilbene glycosides	SLE	[21]
bark	stilbene glycosides	PLE, SLE, SFE	[22]
bark	stilbene glycoside dimers	SLE	[23]
bark	trans-resveratrol	UAE, PLE, SFE	[24]
bark	polyphenols	DESs	[25]
bark	polyphenols	MAE, PLE, DESs	[26]
bark	polyphenols	HWE, UAE	[27]
bark	polyphenols	SFE, UAE	[28]

bark	polyphenols	MAE	[29]
bark	polyphenols	UAE	[30]
bark	polyphenols	PEF	[31]
bark	polyphenols, fatty acids, aliphatic hydrocarbons, terpenes, sterols, steroids	Soxhlet, PLE	[32]
bark	tannins, non-cellulosic polysaccharides	HWE	[33]
bark	non-cellulosic polysaccharides	PLE	[34]
bark	stilbene glycosides, tannins, lignin, non-cellulosic polysaccharides	PLE	[35]
bark	suberin, Klason and acid soluble lignin, holocellulose, monosaccharides	SLE	[13]
sapwood, heartwood	cellulose, hemicelluloses, lignin, pectins ,lipophilic extractives	Soxhlet, UAE	[36]
sapwood	hemicelluloses	HWE	[37]
sapwood	hemicelluloses	SWE	[38]
sapwood	hemicelluloses	PLE	[39]
wood	hemicelluloses	MAE	[40]
wood	hemicelluloses	SWE	[41]
wood	hemicelluloses	HWE	[42]
wood	hemicelluloses	SWE	[43]
wood	hemicelluloses	PLE	[44]
wood	hemicelluloses	SILs	[45]
wood	hemicellulosic oligosaccharides	MAE	[46]
wood	terpenes	SFE, SLE, SD	[47]
sawdust	hemicelluloses	HVED	[48]
sawdust	hemicelluloses	MAE	[49]
saw meal	hemicelluloses	SWE	[50]
needle	wax	SFE	[51]
needle	terpenes	SDE, SFE	[52]
needles	essential oil	MAE, HD	[53]
stemwood, knot	lignans, resin acids, fatty acids, sterols, diterpenyl alcohols	PLE	[54]
shoots	terpenes	HD	[55]

3. Essential oils

Spruce essential oils are complex mixtures of the volatile aromatic compounds. The most widespread compounds in wood, and essential oils and resins constituents are terpenes [56]. It is reported that terpenes produced by conifers contributed increasingly to the protection of wood against fungal pathogens and bark beetle, and possessed antibacterial, antioxidant and cytotoxic effects [57; 58]. In order to evaluate antimicrobial activity of *P. abies,* Radulescu et al. [55] investigated the chemical composition of volatile oil isolated by hydro-distillation (without organic solvent for 4 h) from spruce shoots. Fifty-four compounds were identified adding up between 96.30-98.42% of the oil. The main compounds belonged to monoterpenic hydrocarbons (α-pinene, camphene, limonene, myrcene), oxigenated monoterpenes (bornyl acetate), sesquiterpene hydrocarbons (δ-cadinene, muurolene), sesquiterpenic alcohols (cadinol, muurolol) and diterpenic alcohol (manool). In the study by Miletić et al. [53], microwaves pre-treatment for 10 min of spruce needles was applied in order to improve the kinetics of the essential oil hydro-distillation. Application of microwaves for the pre-treatment of wood material before hydro-distillation caused higher essential oil yield (0.63 and 0.78%). In addition, duration of the hydro-distillation process proceeded by the microwave pre-treatment was shorten for up to 50%.

In the study of Orav et al. [52], application of supercritical carbon dioxide for isolation of volatile compounds from spruce needles was investigated. Comparison to the simultaneous distillation extraction techniques for volatile compounds isolation was also provided. According to the authors, supercritical carbon dioxide extraction gave higher yields of oxygenated mono- and sesquiterpenoids. The monoterpene yields were similar for both systems. However, the addition of

organic modifiers (methylene chloride, ethanol) increased the terpenes yield using SFE. Additionally, the SFE extracts of spruce needles contained semi-volatile (sesquiterpene alcohols, diterpenes, diterpene alcohols, alkanes and squalene) compounds not obtained in SDE extracts.

Bertaud et al. [47] examined the different extraction techniques, Soxhlet, steam distillation, PLE and SFE using CO_2, for isolation of volatile terpene from spruce with two different particle size, grounded and coarse-crushed. According to the results, PLE applied on spruce biomass with successive extraction with n-hexane and acetone/water (95/5) was not suitable for volatile compounds. The highest yield of terpenes from spruce wood was achieved using Soxhlet extraction with successive application of acetone and acetone/cyclohexane (1150±140 mg/kg). SFE using CO_2 (20 and 30 MPa, 60°C modified by 5% ethanol) was able to extract most of the terpenes and terpenoids (66.77 and 85 mg/kg, respectively), but the extraction rates of volatiles were very limited because of a pre-drying step (freeze-drying and crushing). Terpene content in extract obtained with steam distillation was 44±40 mg/kg. α-pinene and β-pinene were found as major compounds of all extracts independently from the extraction technique.

Another research by Bukhanko et al. [15] reported similar results for Soxhlet of four low-value spruce tree fractions (cones, branches, needles and bark). Conventional Soxhlet and supercritical carbon dioxide extraction techniques were used for extraction of volatile compounds (e.g. terpenes and aromatic compounds), among others, from these waste fractions. Terpenes were the major extractives detected in spruce needles extracts obtained by SFE, with a total content of 19.7 g/kg, which is essentially higher than in previous work (2 g/kg) derived by SDE [52]. On the contrary, the Soxhlet technique was more effective for delivering terpenes and diterpenoids (20.8 g/kg) from spruce bark.

Efficient isolation of volatile compounds from spruce biomass by different extraction techniques has also been reported in another studies (Table 1).

4. Polyphenolic compounds

The spruce residues provided other important biologically active substances. The most abundant polyphenolic compounds such as phenolic acids, flavonoids, lignans, stilbene glycosides and tannins were reported (Table 1). According to several authors, phenolic metabolites were considered as tree constitutive compounds with an important protective role [59; 60]. The glycosides of stilbenes (piceid, astringin, isorhapontin), which are concentrated in the spruce bark (5-10%), had an important function of chemical protection against beetle and pathogens, and were also responsible for the antileukemic activity [16; 23; 61].

Several studies report use of solvents, pressure, or other types of extraction systems for isolation of polyphenols from spruce biomass. The conventional methods of polyphenols extraction from spruce wood such as SLE [21] and Soxhlet extraction [9] had been applied. The authors reported astringin and isorhapontin as the main glycosylated stilbenes identified in the spruce bark extracts obtained by ethanol-water mixture (85/15, v/v) at 60 °C for 2 h.On contrary, low amounts of piceid and astringin in the ethanol extract of spruce bark prepared by Soxhlet extraction (78 °C, 8 hours) were obtained.

New extraction techniques have emerged in recent years in order to increase extraction efficiency. Extraction of phenolic compounds from spruce wood bark with UAE was investigated by Ghitescu et al. [30]. Key processing parameters included extraction time (30-60 min), temperature (40-60 ºC) and ethanol concentration (50 and 70%, v/v). The maximum extraction yield of total polyphenols (13.232 mg gallic acid equivalents (GAE)/g of spruce bark) was obtained using a process time of 60 min, an extraction temperature of 54 °C and a concentration of ethanol of 70%. According to Zhang et al. [62], the enhanced yield of phenolic compounds with the increase of sonication temperature might be due to the fact that higher temperature decreased the surface tension at the solvent and increased the formation of cavitation bubbles, allowing the enhanced desorption and solubility of phenolic compounds from the cells. However, further increase in temperature was not regarded efficient due to the loss of solvent and degradation of phenolic compounds. Recently, PEF was also used for extraction of molecules of interest from wood materials. In the study of Bouras et

al. [31], the PEF treatment using a solvent composed of 0.04% of sodium hydroxide was employed in order to enhance the aqueous solid/liquid extraction of polyphenols from spruce bark. The maximum phenolic content for untreated spruce bark was 9.60 mg GAE/g, whereas the PEF treatment increased this value of more than eight times (88.90 mg GAE/g). The improvement of extraction with PEF treatment was attributed to the electroporation i.e. permeabilisation of the cell membrane caused by external electric field, resulting in disruption of the cell membrane [63]. Haman et al. [11] investigated the composition of spruce essential oils obtained by SFE using 10% ethanol as co-solvent. The extraction led to the superior recovery generally achievable by solvent extraction and steam distillation [64]. The mild SFE conditions (45 °C, 20 MPa) enabled the extraction of phenolic acids (cinnamic, protocatechuic, *p*-coumaric, gallic and ferulic acid), flavonoids (catechin, dihydroquercetin), lignan (hydroxypinoresinol) and stilbene glucosides (astringin, isorhapontin). In another study, PLE has proven to be a superior extraction technique to the SLE and SFE when it comes to the separation of phenolic compounds from spruce bark [22]. PLE using water and ethanol as solvent at 160 and 180 °C gave extracts rich inisorhapontin, piceid and astringin. Stilbene glucosides isorhapontin, astringin, and piceid accounted for up to 7.2% of *Picea abies* bark successively extracted using PLE with different solvents (hexane, acetone, ethanol and water) at 10-160 ºC and 103 bar [35].

Tannins are main water-soluble polyphenolic compoundsin spruce bark. Tannins of proanthocyanidin typeare condensed tannins with flavan-3-ol units, while hydrolysable tannins are based on gallic or hexahydroxydiphenic acid esters linked to a sugar. Extracts of spruce bark with up to 50% condensed tannin content were produced in bench- and pilot-scale in unpressurized conditions using hot water as solvent [33]. Extraction temperature (60-90 °C) affected the tannin yield increasing it 3.5-4.2 percentage points. Lower tannin yields were observed in spruce bark extracts (4-6%) obtained by successive PLE [35], whereas Zhang and Gellerstedt [20] reported 1.7-8.0% in bark extracts obtained by acetone water (2:1, v/v) extraction, after extractions at room temperature with petroleum ether, dichloromethane, acetone and water.

5. Polysaccharides

Essential chemical units of woody cells are carbohydrates such as cellulose, hemicelluloses, starch and pectins. In addition to free mono-, oligo- and polysaccharides, flavonoid and stilbene glycosides degradation products (glucoside, galactoside, arabinoside, rutinoside) occur in plant. Monomeric form of wood polysaccharides has been investigated commonly for ethanol bioconversion. On the other side, due to important structural diversity and functionality of polymeric hemicelluloses, several authors investigated potential of its extraction (Table 1). Fernández et al. [41] investigated the effect of SWE extraction parameters on properties of hemicelluloses from Norway spruce. Extraction temperature (130-170 °C), extraction time (70-224 min) and solid-to-liquid ratio (1/10 and 1/20) were studied. The yield of hemicellulose increased as a function of temperature, being the highest after 70 min at 170 °C (80%). The main sugars in Norway spruce hemicelluloses were determined to be mannose, xylose and galactose. In the study of Chadni et al. [48], HVED pre-treatment was carried out in order to increase the accessibility and the penetration of the solvent into the wood matrix, allowing easier release of high molecular mass hemicelluloses. Chemical extraction and autohydrolysis induced by HVED pre-treatment at mild conditions (near neutral pH, temperature of 50 °C) allowed the extraction of hemicellulose chains from spruce sawdust. The recovery of hemicellulose increased at long pre-treatments times. In fact, after 4 ms of HVED treatment in 1 M NaOH solution, the recovery was higher in comparison to the untreated sample (19 mg/g of dry matter and 15.8 mg/g of dry matter respectively). The medium of extraction (pH) strongly affected the selectivity of the extraction: basic conditions solubilized primarily arabinoglucuronoxylans, while the extraction in pure water medium promoted the extraction of galactoglucomannans. Another non-conventional extraction technique was found efficient in hemicellulose extraction. A laboratory scale MAE at temperature <100 ºC and atmospheric pressure was found more effective than conventional extraction in extracting hemicellulose from spruce sawdust [49]. The possible reason was that the microwave radiation can penetrate and rapidly heat the wood, destabilize and trigger structural

damage of wood matrix, and consequently enhance the extraction yields of the target components. Microwaves were applied at different treatment powers (125-573 W) in water and 1 M sodium hydroxide solution for a period of 60 min. The highest yield of hemicelluloses (27.5 mg/g) has been obtained using the highest microwave power transmitted to the wood matrix.

According to Krogell et al. [35], the character and structure of lignin in spruce residues were not well established because of the Klason lignin, acid insoluble material that occurred when water soluble high molecular weight tannins and stilbene glucosides precipitate in acidic conditions. Miranda et al. [13] analyzed the Klason and acid-soluble lignin content of spruce bark after extraction in a Soxtec extractor during 1.5 h with each solvent, successively with dichloromethane, methanol, ethanol and water. Lignin content of 27.9% in this study was comparable with the values of 26.8, 12.1 and 20.8% reported in hot water (100, 140 and160 °C) extracts of spruce bark [34]. Krogell et al. [35] determined Klason lignin content in original and pre-extracted spruce barks. The barks were pre-extracted with hexane and acetone-water, and water at 160 °C, both for 15 and 60 min. Higher values were obtained for original barks (32% for inner bark, 45% for outer bark) indicating that part of the extractives (stilbene glucosides and tannins) condensed to insoluble material during the treatment. Zhang and Gellerstedt [20] determined Klason lignin of pre-extracted residues and reported 3.1% for inner bark and 34.8% for outer bark, being lower than those reported by Krogell et al. [35].

High amount of non-cellulosic glucose, varying according to the felling season (7.7-11.5% of wood-free bark) was extracted by hot water extraction [33]. Glycome profiling performed on bark and hot water extracts showed the presence of xyloglucan, pectic polysaccharides and arabinogalactan in bark. Le Normand et al. [34] reported that 8-16% of sugars in the extracts of spruce bark obtained by sequential acetone-water and pressurized hot water extractions at 100-160 °C were present as monosaccharides. Non-cellulosic polysaccharides were mainly composed of glucose, arabinose and galacturonic acid units which revealed the presence of starch, arabinose-rich hemicelluloses and pectins. The major part of non-cellulosic polysaccharides was extracted at 140 °C and started to undergo degradation at higher temperature.

Non-cellulosic polysaccharides from spruce biomass were efficiently extracted by different extraction techniques, as reported in another studies (Table 1).

6. Waxes and other lipophilic extractives

The most abundant wax found in spruce needles was nonacosan-10-ol (60% of the total wax), a natural hydrophobic molecule, with potential industrial applications in coatings as an alternative to the currently utilized non-renewable plastic coatings [Simmleit and Schulten, 1989]. McElroy et al. [51] focused their study on the combination of SFE followed by the use of a facile recrystallization technique for the recovery of nonacosan-10-ol from the complex mixture of lipophilic molecules. Waste spruce needles were extracted for 2 h with CO_2 at various pressures (200, 300 and 400 bar) and temperatures (40, 50 and 60 °C). The results showed that a significant increase in the yield was observed at elevated temperatures and pressures (1.70% of wax at 400 bar and 60 °C). According to Sin et al. [66], higher extraction yield of wax at higher temperature could be attributed to its semi-crystalline form and high melting point. However, the conditions that exibited the highest concentration of noncosan-10-ol were 200 bar and 60 °C, with approx. 8070±91.1 μg/g needles. Besides the nonacosan-10-ol, GC-MS analysis revealed the presence of other lipophilic compounds in spruce needles such as free saturated (C_{12}-C_{20}) and unsaturated fatty acids (C_{18}), unsaturated ketones (C_{28}-C_{30}), sterols, hydroxy-acids, benzoic acid and phytol. Another study also reported efficient extraction of waxes, among other extracted compounds (fatty acids and alcohols, terpenes and resin acids) in spruce bark extracts obtained by PLE using n-hexane at 100 °C for 6 min [19].

In addition to the waxes, other extractives such as resin acids, fatty acids, lignans, sterols, steryl esters and triglycerides were also quantified in spruce biomass (Table 1). Jablonský et al. [32] reported the chemical composition of the lipophilic part of extractives present in spruce bark extracts obtained by PLE with ethanol at 80, 120, 160 °C and Soxhlet extraction with ethanol for 8 h. Due to elevated temperature and pressure and shorter extraction time (36 min), PLE resulted as more efficient and faster extraction technique than Soxhlet. The main compounds identified included fatty acids, sterols

and steroids. Similar composition of spruce bark extract obtained by Soxhlet at the same conditions was reported in the study by Burčová et al. [9]. The most abundant group of compounds was resin acids, in particular the abietic and dehydroabietic acid derivatives, followed by fatty acids (9-octadecenoic and 14-methylpentadec-9-enoic acids). Bertaud et al. [47] applied conventional Soxhlet extraction and steam distillation and compared with non-conventional ones, PLE and SFE using CO_2. As for fatty acids, sterols, steryl esters and triglycerides, Soxhlet extraction gave the highest extraction yield in spruce extracts. PLE was able to extract most of the lignans, while resin acids content was highest in SFE extracts (30 MPa, 60 °C, with 5% EtOH as modifier). Steam distillation was not efficient in the extraction of these lipophilic compounds.

5. Conclusion

In the last decade numerous studies about the spruce and its residues valorization have been reported, highlighting their potential as promising source of active compounds with new properties and applications. The present review attempted to discuss the extraction techniques existing for the recovery of different chemical constituents depending on the spruce biomass and their chemical classes including polyphenols, polysaccharides, oils, etc. The Soxhlet, hot water extraction, ultrasound-assisted, microwave-assisted, supercritical fluid extraction and pressurized liquid extractions were the most frequently studied. The non-conventional methods were not necessarily more efficient than the conventional one. However, the advantages of such techniques have been lesser solvent and energy consumption and shorter extraction time. According to the literature, no single extraction technique was found effective for extraction of all compounds. Some studies reported that combining extraction techniques often had advantages to overcome the limitations of an individual technique. In addition to sample pre-treatment, extraction techniques and parameters, large variation in extractives recovery and composition is affected by age, edaphoclimatic conditions, harvesting time and tree health.

Overall, with the increasing interest in the development and production of health-promoting pharmaceuticals, agrochemicals, food additives, and biofuels, newer economical and environment friendly techniques of wood biomass extractions and their optimizations might be developed in future.

Acknowledgement

The authors would like to express their gratitude to Zorica Nijemcevic, MSc in Economics, who was the initiator of this investigation.

Conflicts of Interest: The authors declare no conflict of interest.

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