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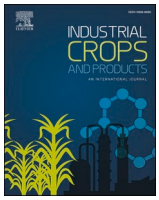

Bioactive molecules in wood extractives: Methods of extraction and separation, a review

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ABSTRACT

Bioactive molecules are those capable of interacting with living organisms, causing changes in them. Wood extractives contain important amounts of these molecules, and some of them have good antioxidant and antimicrobial activity, which favors their use as preservatives. Several different extraction methods are employed to obtain the extractives, some of which have been used for a long time. However, these conventional methods have significant disadvantages, being the most important ones high solvent, energy, and time consumption. To overcome these drawbacks, new extraction techniques are being developed whose aim is also the optimization of the process. Separation techniques such as chromatography and molecular distillation allow extractives purification and the acquisition of the desired molecules. This review aims to provide an overview of the extraction and purification methods used for wood bioactive molecules. To this end, issues such as raw material, solvent type, solid/liquid ratio (SLR), temperature, pressure, and extraction time are discussed. The application of extractives as preservatives for low durability woods is also analyzed. The study concludes that the quality and quantity of bioactive molecules, besides depending on the raw material, are determined by the employed methods and solvents to obtain these molecules. Therefore, the choice of method and solvent is of fundamental importance to achieve the desired results.

1. Introduction

Bioactive molecules are those capable of interacting with living organisms causing changes in them. These molecules are obtained from natural sources as plants and food, and they can also be produced synthetically. Due to society's growing concern for the environment, and the depletion of fossil fuel, numerous studies have been conducted to replace synthetic substances with others from renewable sources. This substitution can bring economic advantages, as the existence of large quantities of agricultural by-products constitutes a potential source of raw material for the extraction of plant bioactive molecules at a reduced cost (Meullemiestre et al., 2016). It is recognized that the bioactive molecules coming from plants have a great antibiotic, antioxidant and anti-cancer potential (Essien et al., 2020). Therefore, plant extracts are commonly used by the pharmaceutical, cosmetic and food industries (Wang and Weller, 2006).

Many studies have evaluated the use of wood and forest residues as source of bioactive molecules (Bostyn et al., 2018; Das et al., 2020; Fernández-Agulló et al., 2015; Meullemiestre et al., 2016; Santos et al., 2019; Zhou et al., 2020; Zwingelstein et al., 2020). Thus, wastes such as wood chips, knots, branches and sawdust can provide a source for the production of these molecules on a large scale (Zule et al., 2016). Wood extractives are non-structural components of the lignocellulosic material, but they are very important for the survival of the plant. The extractives are found in larger quantities in the heartwood, and they are produced by the tree as a defense against environmental stress (Kirker et al., 2013). Molecules from wood extracts can be used for a variety of purposes, from natural dyes to preservatives, with the advantage that once these high value-added compounds have been extracted, the remaining fractions of the lignocellulosic biomass can be revalorized (Zule et al., 2016). Thus, the residues could be used to reduce the environmental impact of forest activity and also to generate economic benefits (Wang et al., 2011; Wen et al., 2019).

Wood extractives are a complex mixture of compounds, among which phenolic compounds, terpenoids, alkaloids, terpenes and saponins stand out (Kadir and Babar, 2020). Some of this polyphenolic

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compounds have antioxidant and antimicrobial activity, thereby increasing the interest for the extraction of these compounds (Fernández-Agulló et al., 2015). In many wood species, different subclasses of flavonoids are linked to the natural durability of the heartwood. Accumulated in large quantities in wood, these flavonoids often have antioxidant and antifungal properties, which favors their use as preservative (Bostyn et al., 2018). Thus, the extractives isolated from naturally durable wood contain bioactive molecules that can replace synthetic preservatives for wood (Kadir and Babar, 2020), such as creosote, copper chromium arsenate (CCA) and pentachlorophenol (PCP). This compounds are related to serious soil contamination, besides bringing risk to the people who apply them, due to their persistent effect (Zulfiqar et al., 2020). Therefore, their replacement is required.

The extraction is the first step for isolation and utilization of bioactive molecules contained in plants. Consequently, the choice of the adequate extraction method is a critical stage to increase the extraction yield of these molecules (Wen et al., 2019) and their quality. In addition to the selection of the extraction process and their operation conditions, pre-treatment methods, chemical composition and physical characteristics of the plant and compound of interest are also important for the process yield (Essien et al., 2020).

Solvent extraction is the most widespread procedure for obtaining bioactive molecules, where usually the most important factors in the process are the type of solvent, time and temperature. Commonly, bioactive molecules can be extracted by different organic solvents or a mixture of organic solvents and water (Gullón et al., 2017). There are different techniques for performing extractions, and their selection also affects the efficiency of the process. Classic techniques, such as maceration and Soxhlet extraction, generally require long extraction times and large amounts of solvent. However, alternative techniques, such as microwave assisted extraction and ultrasound assisted extraction, improved the efficiency of the extraction and reduced the environmental impact of the extraction process. The alternative techniques result in reduced extraction time, energy consumption, and the amount of solvent used, providing a high yield of extract recovery (Fernández-Agulló et al., 2015).

Whether in classical or alternative extraction techniques, the process of extracting bioactive molecules from plant species involves a series of steps, such as technique selection, screening and identification, extraction, isolation, characterization and mass production (Patra et al., 2018). During the technique selection stage, the solubility of the desired substances in the selected solvents, the process conditions and the co-extraction of undesirable compounds must be considered (Essien et al., 2020). Another factor to take into account is that high extraction yield does not guarantee a high concentration of bioactive molecules in the extracts, because some bioactive components are very sensitive to oxygen and heat. Thus, one of the most important aspects to consider is the preservation of the bioactivity of the extracts, avoiding their degradation during the extraction (Todaro et al., 2017; Wang and Weller, 2006).

In order to make better use of lignocellulosic raw materials it is necessary to take into account that the obtained extracts contain complex mixtures of various metabolites (Selvamuthukumaran and Shi, 2017). Therefore, there is a need to purify the extracts through techniques that allow separation into different fractions, and the acquisition of the desired molecules in concentrated form (Chan et al., 2020). This separation can be performed through chromatography techniques (Wen et al., 2019) and molecular distillation (Ali et al., 2019).

Chromatography consists of a physical method for the separation of the parts that constitute a solution. In this technique, the components to be separated are distributed between a phase that is stationary and another that moves in a defined direction. The moving phase can be in the gaseous or liquid state, while the stationary phase may be in the liquid or solid state (Miller, 2009). Chromatographic separation is effective in obtaining the desired substances, however, its high consumption of solvents results in environmental impact and high coast of the process (Wang et al., 2009). Another separation technique is the molecular distillation, which according to Ali et al. (2019) allows the fractionation at temperatures below the boiling point by using vacuum evaporation. Therefore, molecular distillation is widely used for thermally sensitive materials, being the most economically viable purification technique (Wang et al., 2009).

This review aims to provide a comprehensive overview of the extraction and purification methods used for bioactive molecules from wood extractives. Issues such as raw material characteristics, type of solvent, solid/liquid relationship, temperature, pressure and extraction time are discussed. The application of the extractives up to now is also analyzed, focusing on the application of these extractives as preservatives for low durability woods.

2. Methods of extraction

2.1. Conventional extraction techniques

Classic extraction methods consist of solid/liquid extraction procedures and have been widely applied to isolate solid matrix compounds in both laboratory and industry. These methods are based on the extraction of the compounds according to their differences in polarity, using the appropriate solvents. Thus, the solvent is an important factor. Some of the most commonly used solvents are ethanol, methanol, acetone and dichloromethane, which are often used in different proportions with water (Wen et al., 2019). Other factors that most influence the classic methods are the characteristics of the matrix, the solid/liquid ratio, the temperature, the pressure and the extraction time (Wang and Weller, 2006). Some of the most used classic extraction methods are steam distillation, hydrodistillation, Soxhlet, maceration and infusion (Swamy and Akhtar, 2019).

2.1.1. Steam distillation and hydrodistillation

In these methods, the heat applied is the main cause of rupture of the cellular structure of the vegetable material, which allows the essential oils to be released. Thus, the heating temperature must be sufficient to break down the plant material and release the desired compounds (Tongnuanchan and Benjakul, 2014). In steam distillation, the water is boiled in one compartment and the resulting steam passes through the plant material, which is in another compartment. Alternatively, direct steam can be introduced into the sample for extraction. In hydrodistillation, however, the water and plant material are placed in the same recipient and boiled together. In both methods the extracts are dissolved in the steam for extraction. After extraction, the steam is then condensed by indirect contact with water and the mixture is separated, resulting in the extracted compounds on one side and water on the other. This separation is usually carried out by decantation based on the difference in specific weights (Selvamuthukumaran and Shi, 2017).

Despite the similarities between the methods, steam distillation allows a higher yield of essential oils and a better collection of volatile compounds. The small number of volatile compounds that is recovered in hydrodistillation is due to the hydrolysis reactions caused by the combined action of water and high temperature, which can result in the degradation of some compounds. Nevertheless, steam distillation can avoid this degradation allowing the production of better-quality essential oils, since the plant material is not in direct contact with the water (Ali et al., 2019).

To maximize the extraction yield, the wood is usually ground and then distilled, so sawdust is used as the raw material (Kumar et al., 2011; Meullemiestre et al., 2017, 2014; Santos et al., 2019). For example, to perform the hydrodistillation of *Eremanthus erythropappus* (DC.) Macleish wood, Santos et al. (2019) used the particles retained in Tyler 28 and 32 mesh (30% and 70% of the sample weight, respectively), obtaining an average diameter of 520 μm for the particles. The extraction time is also an important factor for the extraction yield, varying on the scale of hours. Thus, while Santos et al. (2019) used only 6 h, Meullemiestre

et al. (2017) spent 8 h for the extraction.

Both techniques, hydrodistillation and steam distillation, permit to obtain compounds with different properties, mainly according to the used raw material. A study about the biological activities of the essential oil from *Cedrus deodara* (Roxb.) Loud. wood conducted by Kumar et al. (2011) showed that hydrodistillation allows to obtain an oil capable to assist in the treatment of gastric ulcers. On the other hand, a study with the steam distillation of *Juniperus thurifera* L. wood, using the Clevenger apparatus, allowed Barrero et al. (2000) to obtain seven new sesquiterpenes.

2.1.2. Soxhlet extraction

This technique was named after Baron Von Soxhlet, who created this procedure in the mid-19th century (Mitra, 2004). This method is based on reflux and siphoning to constantly extract the plant sample with fresh solvent, thus combining percolation and reflux processes, through the use of heat (Patra et al., 2018). In Soxhlet extraction, the solvent is vaporized through the application of a heat source and then condensed, in a reflux condenser and drips onto the sample compartment. When the solvent loaded with the extracted material reaches the top of the sample chamber, it is drained back to the bottom through a siphon. This process is repeated several times over a predetermined period (Mitra, 2004), or can be maintained for a certain number of Soxhlet cycles, independently of time (Bukhanko et al., 2020). The extraction temperature varies according to the used solvent, since the extraction is performed at the boiling temperature of the solvent (Santos et al., 2019). As the extracted materials have higher boiling temperatures than the extraction solvent, they accumulate in the vessel while the solvent is recirculated. This extraction can be used for the isolation and concentration of various water-soluble and water-insoluble substances and allows a wide variety of chromatographic procedures (Mitra, 2004).

Some of the advantages of Soxhlet extraction are the preservation of the sample in contact with fresh solvent, the maintenance of a relatively high extraction temperature due to the heat of the distillation flask, the lack of need of filtration after lixiviation, the operational simplicity and the low cost. Nevertheless, the main disadvantages of Soxhlet extraction are the high the extraction time, the use of a large amount of solvent, the impossibility to use agitation to speed up the process, the need for concentration stage by evaporation, and the possibility of thermal decomposition of the target compounds (Wang and Weller, 2006).

Soxhlet is one of the most used techniques for extraction. This method was applied by Bukhanko et al. (2020) on branches, cones, needles and bark of *Picea abies* L. with the objective of obtaining compounds of interest from a biorefinery point of view. This process allowed to extract for example, terpenes, stilbenes and sterols, employing a mixture of petroleum ether and acetone as solvent. On the other hand, Santos et al. (2019) analyzed the effect Soxhlet extraction of *Eremanthus erythropappus* wood using solvents with different polarities, reporting a yield of 1.57 wt% for n-hexane, 5.10 wt% for ethyl acetate and 7.23 wt% for ethanol. This probably occurred because the yield of Soxhlet extraction, usually, increases with the polarity of the solvent, which can be explained by the low selectivity of polar solvents. This low selectivity causes an increase in the extraction of non-volatile compounds such as flavonoids, tannins, saponins, coumarins, triterpenes and steroids (Santos et al., 2019). The results obtained by Kirker et al. (2013) and Lipeh et al. (2019) demonstrated that Soxhlet extraction using a mixture of ethanol-toluene as a solvent, followed by a Soxhlet extraction with ethanol and complemented by a boiling water extraction is sufficient to remove the extractives from several naturally durable wood, which leaves them susceptible to the action of fungi and termites.

2.1.3. Maceration

It is considered the simplest extraction process, consisting of immersing the plant sample for a long period of time in a solvent to extract the bioactive components (Patra et al., 2018). The vegetable materials must be ground to increase the contact surface with the solvent, and thus, the extraction efficiency. At the end of the process, the liquid is strained and the solid residue from the bottom is pressed to recover as much of the dispersed compounds as possible (Selvamuthukumaran and Shi, 2017). The greatest advantage of this method is its simplicity, but does not completely remove bioactive molecules from plants. Some of the most typical solvents used are toluene, hexane and benzene (Raaman, 2006). It is also known that the sample/solvent ratio is a parameter of great influence in the extraction yield (Bostyn et al., 2018). The maceration can be improved with agitation to provide a constant homogenization during the process, which increases the extraction yield (Bostyn et al., 2018). The time employed for the maceration is quite varied, for example, Martínez-Gil et al. (2018) used 15 days, while Todaro et al. (2017) only needed one hour. In general, the extraction yield increases with time until the extract concentration equilibrium is reached between the raw material and the solvent. Therefore, the extraction process can be optimized by collecting at set times to determine the time until equilibrium (Bostyn et al., 2018).

Meullemiestre et al. (2016) used maceration to valorize industrial waste. In this work, the researchers carried out the extraction of sawdust from *Pinus pinaster* Ait., a byproduct of wood industry, obtaining polyphenols, such as catechin. On the other hand, Rodríguez-Cabo et al. (2018) used alcoholic distillates obtained from grape pomace and low-quality wine, both residues generated during the winemaking process, to perform the maceration of *Vitis vinifera* L. canes. The authors found that the alcoholic distillates achieved extraction yields close to the yields of commercial solvents such as ethanol, ethanol-water, methanol, and acetone.

Wood extractives, which can be obtained by macerating sawdust, are a source of several interesting compounds for different industries. The maceration, using an ethanol-water solution as solvent, was used by Bostyn et al. (2018) to obtain robinetin and dihydrorobinetin, two of the main flavonoids of *Robinia pseudoacacia* L. wood, achieving 3000 mg/L and 670 mg/L, respectively. Todaro et al. (2017) obtained flavonoids, polyphenols and tannins from *Populus nigra* L. wood sawdust using maceration, with n-hexane, and then with ethanol-water solution (70:30 v/v), as solvents. The results obtained by Martínez-Gil et al. (2018) showed that maceration of sawdust, using a hydroalcoholic solution as solvent, was able to obtain from *Quercus humboldtii* Bonpl. toasted wood the following substances: 5-methylfurfural, guaiacol, trans-isoeugenol, 4-vinylguaiacol, cis-isoeugenol, syringol, furfural, 5-hydroxymethyl-furfural, cis-β-methyl-γ-octalactone, vanillin, eugenol and trans-β-methyl-γ-octalactone. It makes this species of wood suitable for wine industry. Maceration also provides extracts with good values of total phenolic content (TPC), which was confirmed by Diouf et al. (2009), St-Pierre et al. (2013), Todaro et al. (2017), and Cetera et al. (2019) (Table 1). In the same way, maceration provides extracts with good antioxidant capacity (Fernández-Agulló et al., 2015; Todaro et al., 2017).

2.1.4. Infusion

It is a conventional extraction technique, which is widely used in traditional medicine (Patra et al., 2018). The infusion is prepared by adding a small amount of plant material to the solvent at high temperature, which allows the solution to be ready in a short period of time (Gamboa-Gómez et al., 2017). Water is often used as a solvent, and it is common to use it at or just below the boiling point, with extraction times between 5 and 60 min (Bastos et al., 2015; Coelho et al., 2016; Jiménez-Moreno et al., 2018; Malca-Garcia et al., 2019; Rakotoniaina et al., 2018). To facilitate the extraction of the compounds, the plant material should be cut into small pieces (Rakotoniaina et al., 2018). This method produces extracts with abundance in glycosides and essential oils (Malca-Garcia et al., 2019). However, it has the disadvantage of using large amounts of solvent (Tiwari, 2015). In addition, the use of high temperature can cause the degradation of thermolabile compounds (Malca-Garcia et al., 2019).

Bastos et al. (2015) found that several flavonoids can be obtained by

Table 1
Overview of the different extraction methods used for wood.

	Conventional extraction methods			Alternative extraction methods			
Raw material	**Technique**	**Experimental data**	**Results**	**Technique**	**Experimental data**	**Results**	**Reference**
Eremanthus erythropappus	H	6 h	Essential oil yield: 1.33% α-bisabolol content: 79.89%	UAE	Solvent: HX SLR: 1:10 (w:v) 60 °C, 7 min	Essential oil yield: 1.17% α-Bisabolol content: 63.52%	Santos et al. (2019)
	SE	Solvent: HX, SLR: 1:25 (w:v) 68 °C, 6 h	Essential oil yield: 1.57% α-bisabolol content: 59.89%	PLE	Solvent: HX 70 °C, 20 min, 10 MPa	Essential oil yield: 0.85% α-Bisabolol content: 63.40%	
Pinus pinaster	H	SLR: 1:90 (w:v) 480 min	Essential oil yield: 0.28% TPC: 54.14 mg of GAE/g extract	MAE	Solvent free IP: 668 W 43 min	Yield: 0.43% TPC: 74.62 mg GAE/g extract	Meullemiestre et al. (2017)
Pinus pinaster	H	SLR: 1:18 (w:v) 480 min	Yield: 0.26% (β-caryophyllene, longifolene, and α-pinene)	MAE	Solvent free IP: 600 W 60 min	Yield: 0.27% (β-caryophyllene, longifolene and α-terpineol)	Meullemiestre et al. (2014)
				UAE	Solvent: water SLR: 1:18 (w:v) 150 min	Yield: 0.28% (β-caryophyllene, α-terpineol and longifolene)	
Juniperus thurifera	SD	Circulatory Clevenger apparatus	Essential oil yield: 2.7% (identified seven new sesquiterpenes)				Barrero et al. (2000)
Picea abies branches	SE	Solvent: PET-ACTN (90:10) 1–2 h	Yield: 4–5%	SFE	Solvent: CO_2 50 °C, 2 h, 30 MPa Flow rate: 40 g/min	Yield: 5.3%	Bukhanko et al. (2020)
Vitis vinifera canes	SE	Solvent: EtOH-water (1:1) and AD SLR: 1:20 (w:v) 3 h	Catechin yield: 0.65 mg/g (EtOH-water), 0.82 mg/g (AD)	PLE	Solvents: EtOH-water (1:1) and AD 100 °C, 3 × 5 min, 10.34 MPa	Catechin yield: 0.54 mg/g (EtOH-water) and 0.51 mg/g (AD)	Rodríguez-Cabo et al. (2018)
	M	Solvents: EtOH, EtOH-water, MeOH, ACTN and AD SLR: 1:25 (w:v) Overnight	Good extraction yield, especially using AD				
Eremanthus erythropappus	SE	Solvent: DCM and HX 20 h	Yield: 3.6% (DCM) and 1.18% (HX) α-bisabolol: 29.07% (DCM) and 37.11% (HX)	PLE	Solvent: propane 80 °C, 1 h, 12 MPa Flow rate: 2 ml/min	Yield: 0.59% α-Bisabolol: 37.36%	Ribas et al. (2014)
				SFE	Solvent: CO_2 60 °C 1 h, 12 MPa Flow rate: 3 ml/min	Yield: 0.36% α-Bisabolol: 58.02%	
Betula pendula Roth	SE	Solvent: MeOH	Yield: 2.0%	ILs	IL: 1-butyl-3-methylimidazolium chloride 120 °C	Yield: 6.0%	Strehmel et al. (2017)
Caesalpinia sappan L.	SE	Solvent: EtOH-water (96:4) SLR: 1:20 (w:v) 3 h	Yield of brazilin: 5.43 mg/g of extract	DES	DES: betain:lactic acid with 60% water SLR: 1:20 (w:v)	Yield of brazilin: 4.49 mg/g of extract	Setiawan et al. (2020)
	M	Solvent: EtOH-water (80:20) SLR: 1:20 (w:v) 72 h	Yield of brazilin: 3.12 mg/g of extract				
		Solvent: EtOH-water (96:4) SLR: 1:20 (w:v) 72 h	Yield of brazilin: 4.58 mg/g of extract				
Populus nigra	M	Solvent: EtOH-water (70:30) SLR: 1:12 (w:v) RT, 1 h	TPC: 334.87 mg of GAE/g extract FRAP antioxidant activity: 599.33 mg TE/g extract	UAE	Solvent: EtOH-water (70:30) SLR: 1:12 (w:v) 1 h	TPC: 303.25 mg GAE/g extract FRAP antioxidant activity: 752.29 mg TE/g extract	Todaro et al. (2017)
				PLE	Solvent: EtOH-water (70:30) 100 °C, (3 ×5 min), 10.34 MPa	FRAP antioxidant activity: 628.03 mg TE/g extract	
Pinus pinaster	M	Solvent: acidified water SLR: 1:17 (w:v) 40 °C	Yield of catechin: 2.34 mg/g of wood	UAE	Solvent: acidified water SLR: 1:17 (w:v) 40 °C, 43 min	Yield of catechin: 3.42 mg/g of wood	Meullemiestre et al. (2016)
Betula alleghaniensis Britton	M	Solvent: EtOH-water (95:5)	Yield: 1.3% of wood TPC: 240.1 mg of GAE/g extract	UAE	Solvent: EtOH-water (95:5)	Yield: 1.3% of wood TPC: 205.2 mg GAE/g extract	Diouf et al. (2009)

(*continued on next page*)

Table 1 (*continued*)

	Conventional extraction methods			Alternative extraction methods			
Raw material	**Technique**	**Experimental data**	**Results**	**Technique**	**Experimental data**	**Results**	**Reference**
		SLR: 1:10 (w:v) RT, 24 h			SLR: 1:10 (w:v) 30 min		
Eucalyptus globulus Labill.	M	Solvent: EtOH SLR: 1:10 (w:v) 50 °C, 90 min	Yield: 2.87% TPC: 85.71 g of GAE/100 g extract FRAP antioxidant activity: 7787 nmol AAE/mg extract	MAE	Solvent: EtOH SLR: 1:10 (w:v) 50 °C, 10 min	Yield: 1.34% TPC: 67.49 g GAE/100 g extract FRAP antioxidant activity: 5101 nmol AAE/mg extract	Fernández-Agulló et al. (2015)
Quercus cerris L.	M	Solvent: EtOH-water (70:30) SLR: 1:5 (w:v) RT, 1 h	Yield: 1.09% TPC: 270.41 mg of GAE/g extract	UAE	Solvent: EtOH-water (70:30) SLR: 1:5 (w:v) 1 h	Yield: 1.20% TPC: 350.28 mg GAE/g extract	Cetera et al. (2019)
				PLE	Solvent: EtOH-water (70:30) 100 °C, 3 × 5 min, 10.34 MPa	Yield: 2.90%	
Acer saccharum Marsh	M	Solvent: EtOH-water (95:5) SLR: 1:10 (w:v) RT, 24 h	Yield: 2.2% TPC: 296 mg of GAE/g extract	UAE	Solvent: EtOH-water (95:5) SLR: 1:10 (w:v) 30 min	Yield: 2.3% TPC: 286 mg GAE/g extract	St-Pierre et al. (2013)
Pseudotsuga menziesii (Mirb.) Franco	M	Solvent: ACTN-water (70:30 v:v) SLR: 1:5 (w:v)	Yield: 2.0% for sapwood and 5.4% for heartwood	PLE	Solvent: ACTN-water (70:30) SLR: 1:4.4 (w:v) 100 °C, 3 × 5 min, 1 MPa	Extracted molecules: flavonoids, phenolic acids, procyanidins, flavonolignans, lignans, and terpenes	Mbakidi-Ngouaby et al. (2018)
Caesalpinia sappan L. (CS)	M	Solvent: EtOH-water (96:4) SLR: 1:10 (w:v) RT, 12 h	Yield of brazilin: 57.38 μg/ml	DES	Solvent: ChCl:glycerol + 50% water SLR: 1:2 (w:v) 50 min	Yield of brazilin: 368.67 μg/ml	Sakti et al. (2019)
Thujopsis dolabrata (L. f.) Siebold & Zucc	M	Solvent: HX SLR: 1:26.7 (w:v) 80 °C, 1 h Stirring: 1200 rpm	Yield of (−)-thujopsene: 0.65% of wood	ILs	Solvent: [EMIM] methylphosphonate SLR: 1:13.3 (w:v) 80 °C, 40 min	Yield of (−)-thujopsene: 0.75% of wood	Syahmina and Usuki (2020)
Pinus taeda L.	M	Solvent: HX SLR: 1:10 (w:v) RT, 5 min	α-pinene yield: 11 mg/g of wood	ILs	Solvent: [EMIM] acetate SLR: 1:3 (w:w) 120 °C, 3 h	α-pinene yield: 28 mg/g of wood	Papa et al. (2017)
French oak	INF	Wood chips toasted and untoasted Solvent: water 100 °C	Main components: furfural, γ-butyrolactone, trans-oak lactone, acetovanilone, vanillin, syringaldehyde, coniferaldehyde, 2-phenylethanol				Jiménez-Moreno et al. (2018)
Prunus avium L. stems	INF	Solvent: water SLR: 1:200 (w:v) 100 °C, 5 min	Flavonoids: quercetin derivatives, kaempferol derivatives and catechin				Bastos et al. (2015)
Larix gmelini (Rupr.) Rupr.				EAE	Solvent: Water 0.5 mg/ml cellulase 0.5 mg/ml pectinase 32 °C, 18 h	Taxifolin and total flavonoids yields: 1.35 mg/g and 4.96 mg/g, respectively.	Wang et al. (2011)
Castanea sativa Mill.	SE	Solvent: EtOH-TO (1:2) SLR: 1:300 (w:v) 7 h	Yield: 7.4%	PLE	Solvent: EtOH-water (70:30) 110 °C, 3 × 5 min, 1 MPa	Yield: 12.5%	D'Auria et al. (2021)
Olea europaea L.	M	Solvent: EtOH-water (70:30) SLR: 1:5 (w:v) RT, 1 h, stirring	Yield: 7.0% TPC: 130 mg GAE/g extract	UAE	Solvent: EtOH-water (70:30) SLR: 1:5 (w:v) RT, 1 h	Yield: 9.0% TPC: 156.04 mg GAE/g extract	Faraone et al. (2021)
				PLE	Solvent: EtOH-water (70:30) 100 °C, 3 × 5 min, 10.34 MPa	Yield: 9.0% TPC: 101.60 mg GAE/g extract	

AAE: Ascorbic acid equivalent; ACTN: Acetone; AD: Alcoholic distillates; ChCl: Choline chloride; DCM: Dichloromethane; DES: Deep eutectic solvents; EAE: Enzymatic assisted extraction; [EMIM]: 1-ethyl-3-methylimidazolium; EtOH: Ethanol; FRAP: Ferric reducing antioxidant power; GAE: Gallic acid equivalent; H: Hydrodistiliation; HX: Hexane; ILs: Ionic liquids; IP: Irradiation power; INF: Infusion; M: Maceration; MAE: Microwave assisted extraction; PET: Petroleum ether; PLE: Pressurized liquid extraction; RT: Room temperature; SD: Steam distillation; SE: Soxhlet extraction; SFE: Supercritical fluid extraction; SLR: Solid/liquid ratio; Temp.: Temperature; TE: Trolox equivalent; TO: Toluene; TPC: Total phenolic content; UAE: Ultrasound assisted extraction.

infusion of *Prunus avium* stems, adding 1 g of sample to 200 ml of boiling water and leaving it at room temperature for 5 min. The found flavonoids were: catechin, quercetin-3-O-rutinoside, quercetin-3-O-glucoside, kaempferol-3-O-rutinoside and kaempferol-3-O-glucoside. The results obtained by Jiménez-Moreno et al. (2018) indicated that infusion with water at 100 °C permits to obtain oak wood extractives, which being applied in vineyards can be absorbed by the plants.

2.2. Alternative methods

Classic extraction methods present the advantages of reduced cost and ease of operation. However, they generally use large amounts of solvents, which require an evaporation step for recovery, but are difficult to remove completely. In addition, there is the possibility of thermal degradation of bioactive components due to high solvent temperatures during a long extraction time. To try to overcome the inherent disadvantages of the conventional techniques, alternative extraction methods have been developed (Tiwari, 2015; Wen et al., 2019). Some of the main alternative methods are microwave assisted extraction, ultrasound assisted extraction, pressurized liquid extraction, supercritical fluid extraction, ionic liquids, deep eutectic solvents and enzyme assisted extraction (Ali et al., 2019). Alternative extraction techniques can minimize or eliminate the use of organic solvents and usually work at low temperature during the extraction process, which prevents the extracted compounds from being affected in terms of stability (Tiwari, 2015). Moreover, alternative methods are generally more efficient, with a reduction in extraction time and energy consumption, and an increase in the yield and the quality of obtained products (Santos et al., 2019).

2.2.1. Microwave assisted extraction (MAE)

Microwaves are electromagnetic waves with frequencies between 0.3 and 300 GHz, and, in commercial systems, are generally used at 2.45 GHz (Selvamuthukumaran and Shi, 2017; Zwingelstein et al., 2020). Microwave assisted extraction is based on the physical mechanisms of ionic polarization and reorientation of molecules. In the presence of an electric field generated by microwave radiation, the polar molecules begin to vibrate so that the dipoles of the molecules are continuously aligned with the electric field (Ali et al., 2019). This vibration of the molecules causes a uniform heating of the material (Wang and Weller, 2006). Due to heating, a great pressure appears inside the vegetable cell wall causing it to break. As a result, the constituents inside the cells are released into the extraction medium, and the penetration of solvents in the vegetable matrix is facilitated, leading to faster extraction (Ali et al., 2019). Solvents with low viscosity offer an advantage for extraction, because a lower viscosity of the medium facilitates molecular rotation which favors the microwave heating mechanism (Selvamuthukumaran and Shi, 2017).

Generally, MAE is performed through one of the following systems: closed extraction containers at controlled pressure and temperature, or open vessel at atmospheric pressure. The MAE closed system is normally used for extraction under intense conditions, such as high extraction temperature and under pressure. In this system, the pressure in the vessel basically depends on the volume and boiling point of the solvents. On the other hand, the open vessel system has as maximum temperature the boiling point of the solvents at atmospheric pressure (Wang and Weller, 2006).

The yield of MAE depends on several factors, such as dielectric susceptibility of the solvent and the matrix, solvent concentration, particle size, SLR, temperature, extraction time and extraction power. The use of microwaves in extraction leads to a reduction in extraction times, an increase in extraction yield and a reduction in the amount, or even dispensation, of solvents (Zwingelstein et al., 2020), as well as allowing the obtaining of extracts with high antioxidant activity (Fernández-Agulló et al., 2015). The MAE was used by Moreira et al. (2017) to extract phenols from *Malus domestica* Borkh heartwood using ethanol-water as solvent. The authors reported a yield of 23 mg of GAE/g dry wood extracted.

Fernández-Agulló et al. (2015) showed that the MAE using ethanol as solvent, 1:10 (w:v) SLR, 50 °C and microwave power of 150 W allows obtaining an extraction yield of 1.34%, with total phenol content of 67.49 g per 100 g of extracts, from Eucalyptus globulus wood. The authors used the same parameters, but without microwave, to extract the wood by maceration, achieving a yield of 2.87%, with total phenol content of 85.71 g per 100 g of extracts. The better performance of the maceration was possibly due to the extraction time, because while the MAE had a time of 10 min, the maceration had a time of 90 min. In addition, they concluded that microwaves could have caused degradation of part of the extracts.

Meullemiestre et al. (2014) obtained similar yields for MAE and hydrodestilation of *Pinus pinaster* wood., 0.27% (w/w) and 0.26% (w/w) extraction yield, respectively. The MAE was carried out with water during one hour, and the hydrodistillation takes 8 h, being the SLR in both extractions 1:18 (w:v). In another study with *Pinus pinaster* wood, Meullemiestre et al. (2017) obtained a yield of 0.43% (w/w) and TPC of 74.62 mg of GAE/g extract, for a 43 min extraction using MAE. However, conducting an 8 h hydrodistillation, the achieved yield was 0.28% (w/w) and the TPC was 54.14 mg of GAE/g extract. In both studies the use of MAE was time savings and promoted a similar or higher extraction yield compared to hydrodistillation.

2.2.2. Ultrasound assisted extraction (UAE)

Ultrasonic waves, when passing through a medium, cause cycles of expansion and compression in the molecules that constitute this medium. These alternate changes in pressure cause the formation, expansion and collapse of bubbles in a liquid medium, a so-called cavitation phenomenon (Ali et al., 2019; Selvamuthukumaran and Shi, 2017). Ultrasound assisted extraction consists of using ultrasonic waves, in a pre-treatment step or during the solid/liquid extraction itself (Tiwari, 2015), so that cavitation favors the liberation of the desired compounds by cellular disruption of the plant cells (Meullemiestre et al., 2014; Wen et al., 2019). The ultrasound also allows a greater penetration of solvent in the sample matrix, increasing the contact surface area between the solid and liquid phases. As a result, heat and mass transfer are increased, and the solute diffuses more rapidly from the solid phase to the solvent (Meullemiestre et al., 2016). The ultrasound assisted extraction has been proven to be efficient in obtaining various products, such as essential oils, polysaccharides, proteins, dyes, pigments and bioactive molecules (Santos et al., 2019).

Several factors interfere in the efficiency of the UAE, in particular the frequency and intensity of the ultrasound, viscosity, temperature and pressure of the medium, moisture content and particle size of the plant sample, used solvent, sonication time and nature of the plant matrix (Selvamuthukumaran and Shi, 2017; Tiwari, 2015; Wang and Weller, 2006; Zwingelstein et al., 2020). The main advantages of using ultrasound for solid/liquid extraction are increased extraction yield, reduced time, energy and solvent consumption, reduced operating temperature, which allows the extraction of thermosensitive compounds, relatively low cost, ease of operation and possibility of using a wide range of solvents (Santos et al., 2019; Selvamuthukumaran and Shi, 2017; Wang and Weller, 2006; Zwingelstein et al., 2020). The use of ultrasound energy for extraction also leads to more effective mixing and faster transfer of energy, which contributes to the extraction efficiency (Wen et al., 2019). Moreover, UAE allows to obtain extracts with good antioxidant activity, as Todaro et al. (2017) illustrated in the characterization of the *Populus nigra* extracts obtained by UAE, obtaining an antioxidant activity value of 752.29 mg TE/g extract (FRAP).

Meullemiestre et al. (2016) found that catechin can be extracted from *Pinus pinaster* wood by UAE using acidified water as solvent. As a comparison, the authors also performed the extraction by maceration, using the same conditions, only without ultrasound. The yield of catechin was 47% higher using UAE. Diouf et al. (2009), on the other hand, used ethanol-water for the extraction of *Betula alleghaniensis* wood by

UAE. 30 min extraction was performed with a SLR of 1:10 (w:v), achieving a 1.3% yield. The authors also performed the extraction of the wood by maceration, under similar conditions, but the same yield was achieved with a much longer extraction time, 24 h.

UAE was applied by St-Pierre et al. (2013) to valorize forest industry residues by obtaining extractives, especially phenols, from *Acer saccharum* wood. A yield of 2.3% extractives with a TPC of 286 mg of GAE/g extract was obtained with only 30 min. Extraction by maceration was also performed as a way to compare the results, and it was found that maceration needs about 24 h to obtain similar yield values.

In a recent study, UAE was used by Cetera et al. (2019) to obtain extractives from *Quercus cerris* wood with ethanol-water as solvent, and extraction time of 1 h. The extraction yield was 1.20% with a TPC of 350.28 mg of GAE/g extract. These results were better than those obtained by maceration.

2.2.3. Pressurized liquid extraction (PLE)

In this extraction method, high pressure is used to keep the solvents in the liquid state even under elevated temperature (Zwingelstein et al., 2020). The increased temperature improves solvent diffusivity and the high pressure allows the extraction cell to be filled more rapidly, and facilitates the penetration of the solvent into the solid matrix, which speeds up the extraction process. In general temperatures between 50 and 200 °C, and pressures between 10 and 15 MPa are used (L. Wang and Weller, 2006). PLE has been successfully applied to the extraction of bioactive compounds from different plant materials, being considered an alternative to conventional methods, because it is a faster process with less amount of solvents (Ali et al., 2019; Selvamuthukumaran and Shi, 2017; Wen et al., 2019). PLE is also named accelerated solvent extraction, enhanced solvent extraction, and high-pressure solvent extraction. However, when water is employed as solvent, this method is called pressurized hot water extraction, subcritical water extraction or superheated water extraction (Zwingelstein et al., 2020).

Several factors determine the efficiency of PLE, such as the nature of the solvent or solvent mixture, solid/liquid ratio, particle size, pressure, temperature, number of extraction cycles, duration of each cycle and the flow rate (Essien et al., 2020; Wen et al., 2019). The use of PLE provides reduced extraction time, low solvent consumption, high selectivity and high biologically active extracts (Essien et al., 2020). Another significant advantage is the possibility of using non-toxic solvents, such as carbon dioxide (CO_2) and water (Wang and Weller, 2006). However, special attention should be given to high temperature extraction, as it can lead to the degradation of thermolabile compounds (Essien et al., 2020; Nastić et al., 2018; Wang and Weller, 2006).

The n-hexane PLE with an extraction time of 20 min allowed Santos et al. (2019) to obtain essential oil from *Eremanthus erythropappus* wood with a yield of 0.85%, and with a concentration of 63.40% of α-bisabolol. To compare the yield of PLE, a 6 h hydrodistillation and n-hexane Soxhlet extraction were performed, achieving a yield of 1.33% and 1.57%, with a concentration of 79.89% and 59.89% of α-bisabolol, respectively. The results were also compared with the ones measured with UAE using n-hexane as solvent. This method showed a yield of 1.17% with a concentration of 63.52% of α-bisabolol, with only 7 min for extraction. The comparison of the techniques makes evident the time savings provided by the alternative PLE and UAE methods in relation to conventional methods.

Mbakidi-Ngouaby et al. (2018) obtained a variety of bioactive molecules, including flavonoids, phenolic acids, procyanidins, flavonolignans, lignans, and terpenes, with PLE from *Pseudotsuga menziesii* wood using acetone-water as solvent. Catechin, another bioactive molecule, was extracted from *Eucalyptus camaldulensis* Dehnh wood by Benouadah et al. (2018) using PLE. In this case, the authors carried out an extraction employing acetone-water as solvent, 138 MPa, 90 °C and 15 min, reaching 5.39 mg/g catechin from sapwood, and 16.61 mg/g from heartwood. Rodríguez-Cabo et al. (2018) also extracted catechin, but from *Vitis vinifera* canes. The solvents use in this work were ethanol-water and alcoholic distillates. After 15 min, the yield of catechin was 0.54 mg/g for ethanol-water, and 0.51 mg/g for alcoholic distillates. In order to compare the results, *Vitis vinifera* was also extracted with Soxhlet method, achieving a yield of 0.65 mg/g and 0.82 mg/g with ethanol-water and alcoholic distillates, respectively, but this time it took 3 h.

Ribas et al. (2014) found that PLE, with propane as solvent, allows obtaining α-bisabolol from *Eremanthus erythropappus* wood. Using 12 MPa and 60 min, an extraction yield of 0.59% with a concentration of 37.36% of α-bisabolol was obtained. To compare the results, a 20 h Soxhlet extraction was carried out using dichloromethane and hexane. The achieved yields were 3.6% and 1.18%, with a concentration of α-bisabolol of 29.07% and 37.11%, respectively. Thus, the PLE had a slightly lower extraction yield, but with considerably shorter extraction time.

2.2.4. Supercritical fluid extraction (SFE)

The supercritical state is achieved when a substance is subjected to temperature and pressure above its critical point. In this condition, there are no two distinct phases, instead the whole substance acquires the properties of viscosity, surface tension and diffusion of the gas, and the density and solvency of the liquid. (Ali et al., 2019; Wen et al., 2019). Supercritical fluids have high diffusivity and low viscosity, properties that can be modified by adjusting pressure and temperature to provide a better extraction yield (Essien et al., 2020). The solubility of a solid in a supercritical fluid is enhanced by the increase of the density of the fluid, which can be achieved at high pressures. This mechanism can be also used in reverse to recover dissolved compounds (Wang and Weller, 2006). CO_2 is the most commonly used substance as supercritical fluid due to its moderate temperature and pressure conditions to reach the critical point, 31 °C and 7.4 MPa. Under these conditions, CO_2 turns into a liquid and presents properties similar to those of the organic solvent promoting its use as an inert and safe medium for the extraction of molecules from different raw material. Another important advantage of using CO_2 is that it does not remain in the final product, as the liquid CO_2 turns back into a gas and evaporates at atmospheric pressure and room temperature (Chan et al., 2020; Tongnuanchan and Benjakul, 2014). CO_2 is a non-toxic, reusable and recyclable solvent (Ali et al., 2019). The main factors that determine the SFE extraction efficiency are temperature, pressure, raw material particle size and moisture, extraction time, fluid flow rate and solvent to feed ratio (Selvamuthukumaran and Shi, 2017).

The use of SFE presents several advantages, such as selective and rapid extraction, moderate operating temperatures, avoidance of product degradation, and the possibility of waste-free extractions and fractionations (Ribas et al., 2014; Selvamuthukumaran and Shi, 2017; Wen et al., 2019). The disadvantage of SFE is its high cost, due to the high energy consumption for compression and decompression, and the cost of the equipment itself (Choi and Verpoorte, 2019; Essien et al., 2020). Another disadvantage is the inefficiency of CO_2 to solubilize polar compounds (Ali et al., 2019), but this limitation can be overcome by adding a small amount of a modifier, such as dichloromethane (Selvamuthukumaran and Shi, 2017) or ethanol, to increase the polarity of the CO_2, improving the extraction (Guedes et al., 2020).

SFE with CO_2 as fluid allowed Eller et al. (2018) to obtain an essential oil with ability against fungi and termites from *Juniperus virginiana* L. wood. Zhou et al. (2020), on the other hand, found that SFE with CO_2 is appropriate for the extraction of elemicin from wood sawdust *Dalbergia pinnata* (Lour.) Prain wood. The authors achieved a yield of 4.75% essential oil with a 91.06% of elemicin. Experimental conditions were 35 MPa, 75 °C and 4 h.

Bukhanko et al. (2020) performed a SFE with CO_2 for *Picea abies* wood extractive obtention. After 2 h of extraction, 5.3% of yield was achieved. In order to compare the yield of the method, the authors also performed Soxhlet extraction with petroleum ether-acetone, between 1 and 2 h, until 12 cycles were completed, reaching an extraction yields

between 4% and 5%, thus the SFE had a better yield, for a similar extraction time.

In a recent study, SFE was utilized by Surup et al. (2020) to obtain chemicals from Scots pine branches. Using CO_2 at 40 MPa and 60 °C during 2 h, an extraction yield of 8.0% was obtained. The obtained extract which was formed mainly by terpenes, resin acid, steroids, fatty acid, and aromatics.

2.2.5. Ionic liquids (ILs)

The ILs are mixtures of salts in which individual ionic components bind to each other and constitute liquids at room temperature (Choi and Verpoorte, 2019). This confers them unique properties, such as extremely low vapor pressure, high thermal stability, and high conductivity (Choi and Verpoorte, 2019). One of the great advantages of ILs is that some of their properties, such as polarity, viscosity and density, can be adjusted according to requirements varying the anion-cation pairs (Chan et al., 2020). The ionic nature of ILs enables them to accept the hydrogen bonds that constitute biopolymers such as cellulose, lignin, and chitin. In this way, ILs can dissolve these biopolymers, allowing solvent access to the extractives (Syahmina and Usuki, 2020).

ILs are often considered environmentally friendly solvents due to their low volatility, low flammability and good reusability (Syahmina and Usuki, 2020). Furthermore, other qualities, such as their good dissolution properties (Strehmel et al., 2017) and high thermal stability (Chan et al., 2020), also make ILs attractive as an alternatives solvents. However, the removal of ILs from extracts is not an easy task (Strehmel et al., 2017). This coupled with their toxicity, expensive synthesis, and poor degradability make their application in industry difficult (Choi and Verpoorte, 2019).

The ILs were utilized by Syahmina and Usuki (2020) as a pretreatment step to extend the extraction of (-)-thujopsene from *Thujopsis dolabrata* wood. IL 1-ethyl-3-methylimidazolium methylphosphonate, with a SLR of 1:13.3 (w:v), was used for the extraction followed by hexane (SLR of 1:20 (w:v)), achieving a 0.75% yield of (-)-thujopsene. This result was better than the one obtained by conventional extraction with hexane, 0.65%.

Papa et al. (2017) achieved a significant increase in the yield of α-pinene from *Pinus taeda* wood by ILs extraction. A pretreatment was performed using 1-ethyl-3-methylimidazolium acetate for 3 h resulted in a 2.55 times higher extraction yield of α-pinene than with n-hexane. Strehmel et al. (2017), on the other hand, first extracted *Betula pendula* wood with methanol using the Soxhlet method, obtaining a yield of 2.0% extractives, and subsequentially, the non-soluble fraction was subjected to an extraction with 1-butyl-3-methylimidazolium chloride, obtaining an additional yield of 4.0% extractives.

2.2.6. Deep eutectic solvents (DES)

The deep eutectic solvents consist of two or more solid components that form an eutectic mixture, which is a mixture that has a melting point below the melting point of each of the components. In this way, DES remains in the liquid state at a temperature where each of its components would be in solid state (Choi and Verpoorte, 2019). DES are formed by non-ionic species (salts or molecular components) linked by hydrogen bonds, and can have their composition varied according to the characteristics of the plant matrix that will be extracted, in order to increase the efficiency of extraction. This efficiency is linked to physical and chemical properties of DES, such as density, viscosity, polarity, miscibility (Ali et al., 2019), acidity and hydrogen bonds (Sakti et al., 2019). The natural deep eutectic solvents (NADES) are produced using natural-based components which improved the environmental safety of the solvent. Thus NADES are considered one of the main green solvents (Sakti et al., 2019). For the preparation of NADES, primary metabolites (amino acids, sugars, organic acids, and sugar alcohols) are usually combined with an ammonium salt and other hydrogen bond donors (Setiawan et al., 2020).

Some DES can be used as alternatives to replace organic solvents that are toxic, flammable and harmful to the environment (Setiawan et al., 2020). The most outstanding advantages of DES are biodegradability, sustainability, low toxicity, simple preparation, low cost, recyclability, high stability, low volatility, extremely low vapor pressure, easy to use and efficient in extracting active compounds (Sakti et al., 2019; Setiawan et al., 2020). However, DES also have some disadvantages as the difficulty of removing the solvent from the extracts obtained. Considering that most DES are non-toxic, this should not be a problem since they could be used as together with the extracts, without purification stage (Choi and Verpoorte, 2019).

DES can also increase the yield of extractives, as it was confirmed by Sakti et al. (2019). These authors obtained brazilin from *Caesalpinia sappan* heartwood through extraction with a choline chloride: glycerol-based DES, and with the aid of an ultrasound. The yield of brazilin was 368.67 μg/ml, 6.4 times higher than the value achieved by maceration with ethanol-water (57.38 μg/ml), even though solvent consumption and time were greater.

Setiawan et al. (2020) also performed the extraction of brazilin from *Caesalpinia sappan* wood employing DES. They used betain:lactic acid during 30 min achieving a brazilin yield of 4.49 mg/g. The authors also performed an extraction by maceration with ethanol-water for 72 h obtaining a yield of 4.58 mg/g, and a Soxhlet extraction with ethanol-water for 3 h reaching a yield of 5.43 mg/g. Thus, the extraction with DES proved to be very attractive due to the reduced time required and the achieved competitive extraction yield.

2.2.7. Enzyme assisted extraction (EAE)

This technique is an alternative to extend the release of extractives from plant materials, serving as a pre-treatment to improve the extraction of bioactive molecules by other techniques. The enzymes act by breaking down the structural integrity of the cell walls, allowing the solvents to penetrate to places where they would not arrive, which increases the amount of liberated extracts (Wang et al., 2011). The EAE often allows the elimination of organic solvents from the process. Another important issue is that enzymes act as catalysts and are specific, so, using EAE the original characteristics of the compounds to be isolated are preserved. Some of the most used enzymes are: cellulase, β-glucosidase, xylanase, β-gluconase and pectinase (Wen et al., 2019). The key parameters for the efficiency of EAE depends on factors such as enzyme composition and concentration, reaction temperature, particle size, solid/liquid ratio, system pH, extraction time and plant moisture (Selvamuthukumaran and Shi, 2017). Nevertheless, the cost of some enzymes brings limitations for industrial utilization (Xia et al., 2017). The EAE was applied by Wang et al. (2011) as a pretreatment to improve the extraction of the natural antioxidant taxifolin and flavonoids from *Larix gmelinii* sawdust by UAE. The authors determined 1.20 and 1.27 times higher yield of total flavonoid and taxifolin when EAE was employed. Regarding the antioxidant capacity, the reported values were also higher for EAE extracts, being between 1.68 and 1.8 times higher (Table 1).

3. Separation techniques for bioactive molecules

Plants are sources of a wide range of bioactive molecules. Extracts obtained from plants contain complex mixtures of various metabolites, such as alkaloids, glycosides, phenolics, terpenoids and flavonoids (Selvamuthukumaran and Shi, 2017). Therefore, there is a need to use techniques that allow the fractionation of the extracts so that the desired substances can be separated (Chan et al., 2020). Among the different possible separation methods, this review focuses on molecular distillation, macroporous resins adsorption chromatography, silica gel chromatography, gel filtration chromatography, preparative liquid chromatography and countercurrent chromatography (Table 2).

Table 2
Different techniques used to separate bioactive molecules obtained from wood.

Raw material	EP / ST	Measurement parameters	Separated compounds	Ref.	Raw material	EP / ST	Measurement parameters	Separated compounds	Ref.
Dalbergia pinnata	SFE / MD	Evaporation surface: 70 °C Condensing surface: 10 °C Vacuum: 120 Pa FR: 2 ml/min	Elemicin, methyl eugenol, whiskey lactone and 4-allyl-2.6-dimethoxyphenol	Zhou et al. (2020)	*Pseudotsuga menziesii*	M / SGC	Silica gel: 35–70 μm Eluents: MeOH and DCM-MeOH	Dihydrotodomatuic acid, taxifolin and quercetin	Mbakidi-Ngouaby et al. (2018)
Wood and bark of conifers	P / MD	Temp. 60–100 °C Vacuum: 10 kPa Periods: 0.5 and 1 h	Low molecular weight compounds (until 10.6%)	Rahman et al. (2018)	*Albizia myriophylla* Benth.	M / GFC	Eluents: DCM, ACTN, MeOH and HX	Indenoic acid and 8-methoxy-7,3',4'-trihydroxyflavone	Bakasatae et al. (2018)
Mongolian Scots Pine	P / MD	Evaporation temp. 70–130 °C Vacuum: 60 Pa FR: 1 ml/min	Light, medium and heavy fractions of bio-oil	Wang et al. (2009)	*Pinus halepensis* Mill. and *Eucalyptus camaldulensis*	PLE / GFC	Eluent: THF with 1% acetic acid Extractive/eluent ratio: 1:1000 FR: 0.8 ml/min	Resin acids, steryl esters, glycerides, triterpenes, fatty alcohols, sterols, stilbenes, cyclic polyols and flavanols	Benouadah et al. (2018)
Water used in MDF panel processing	- / MRA	Resin: polyvinylpolypyrrolidone Elution: MeOH and FA and PA mixture	Yield of hydroxymatairesinol: 37.4 mg/g of polyvinylpolypyrrolidone	Lindemann et al. (2020)	*Quercus petraea* (Matt.) Liebl.	M / PLC	Mobile phase: 0.05% trifluoroacetic acid and ACN FR: 20 ml/min	Nine lignans, including lyoniresinol	Marchal et al. (2015)
Larix gmelinii	HAC / MRA	Resin: AB-8 Loading: EtOH-water (20:80) Desorption: EtOH-water (60:40)	Dihydroquercetin adsorption: 17.4 mg/g of resin Desorption: 92.5%	Xia et al. (2017)	*Quercus petraea*	M / PLC	Mobile phases: water and ACN FR: 20 ml/min	(+) and (-) lyoniresinol (44.6% and 43.5%, respectively)	Cretin et al. (2015)
Eremanthus erythropappus	SFE / SGC	Silica gel: 35–70 mesh Solvent: CO_2	α-Bisabolol (43.69%)	Ribas et al. (2014)	Oak	- / CCC	Solvent: HX-AtOAc-MeOH-water (1:1:1:1) + 0.1% trifluoroacetic acid	Isolation of 48 phenolic compounds	Regalado et al. (2011)
Mixture of wood and bark of *Populus spp.* and *Betula spp.*	P / SGC	Solvents: pentane, benzene, DCM, AtOAc and MeOH	Bio-oils fractions (depends on the employed eluent)	Garcìa-Pérez et al. (2007)	*Tabebuia sp.*	- / CCC	Solvent: HX-ACN-MeOH (2:2:1) SLR 1:50 (w:v) Period: 5 h, FR: 2 ml/min	12 naphthoxazole derivatives	Del Rio et al. (2015)

ACN: Acetonitrile; ACTN: Acetone; AtOAc: Ethyl acetate; CCC: Countercurrent chromatography; DCM: Dichloromethane; EP: Extraction process; EtOH: Ethanol; FA: Formic acid; FR: Flow rate; GFC: Gel filtration chromatography; HAC: Homogenization-assisted cavitation hybrid rotation; HX: Hexane; M: Maceration; MD: Molecular distillation; MDF: Medium-density fiberboard; MeOH: Methanol; MRA: Macroporous resins adsorption; P: Pyrolysis; PA: Phosphoric acid; PLC: Preparative liquid chromatography; PLE: Pressurized liquid extraction; SFE: Supercritical fluid extraction; SGC: Silica gel chromatography; SLR: Solid-liquid ratio; ST: Separation technique; THF: Tetrahydrofuran.

3.1. Molecular distillation (MD)

Molecular distillation (MD), also known as short path distillation, is a separation technique that uses vacuum evaporation to obtain fractionation at temperatures below the boiling point (Ali et al., 2019). MD is usually performed by spreading the liquid on the surface of a vessel forming a thin layer to promote better heat transfer performance. Then this surface turns into the evaporation surface of the system, which together with the condensation surface promotes the separation by weight of the molecules using the principle of the mean free path (Chan et al., 2020). Under low pressure, the distance for the volatile molecules to traverse between the evaporating and condensing surfaces is less than or equal to the average distance traversed by a particle between two collisions in sequence, known as the mean free path (Ali et al., 2019; Chan et al., 2020; Rahman et al., 2018; Selvamuthukumaran and Shi, 2017; Wang et al., 2009). In this condition the light volatile molecules escape from the liquid phase on the evaporating surface and then condense on the cooling surface. In the case of heavy molecules, the distance to cross between the evaporation surface and the condensation surface is greater than the mean free path, so heavy molecules are unable to reach the cooling surface and therefore return back to the liquid phase at the evaporating surface itself (Chan et al., 2020). The liquid material to be extracted is fed into an equipment composed of an evaporator, a condenser, a rotor and a vacuum pump. The volatile and heavy components are then separated by adjusting the temperature, pressure, speed and flow of the evaporator. However, as the distillation temperature increases, less volatile components evaporate, which increases the distillate yield, but changes the composition of the obtained bio-oil (Wang et al., 2009).

MD is suitable for thermally sensitive compounds, since the application of low temperature and vacuum prevents oxidative degradation. MD has the advantages of using low temperatures and a short residence time, in addition to having a good separation performance and high distillate recovery. However, the used of vacuum cause high energy consumption, and the manufacturing cost of the equipment is also high (Ali et al., 2019). The MD can be used to purify crude extracts obtained through the supercritical CO_2 extraction, as it was done by Zhou et al. (2020) in a study to evaluate the chemical composition and antioxidant and antimicrobial activities of *Dalbergia pinnata* wood essential oils. In the same way, MD reduced the amount of acids and water in the bio-oils generated by pyrolysis of wood and bark, facilitating the use of bio-oils as fuels (Rahman et al., 2018).

3.2. Column chromatography

Column chromatography is a method used to separate the components according to the polarity, the structure of the sample and the used eluents (mobile phase), and the characteristics of the employed adsorbents (stationary phase) (Chan et al., 2020). Some of the commonly applied column chromatography techniques to isolate bioactive compounds are the macroporous resins adsorption, silica gel chromatography and gel filtration chromatography (Wen et al., 2019).

3.2.1. Macroporous resins adsorption (MRA)

The macroporous polymer resin has a structure with a large specific surface area that can physically and selectively adsorb organic compounds from a liquid solution. Normally, the bonds of organic solutions with the macroporous resin involve electrostatic interactions, hydrogen bonding, van der Waals forces, hydrophobic interactions and size sieving action. The separation of the substances is based on the interactions between the substrate and the macroporous resin. The MRA constitutes an important alternative for the selection and enrichment of bioactive components. The main advantages of MRA are low cost, high chemical stability, easy regeneration, suitability for use with aqueous and non-aqueous solutions, high capacity to remove impurities and an adjustable selectivity (Wen et al., 2019).

In a recent study, Lindemann et al. (2020) performed the removal and selective recovery of lignans and stilbenes (polyphenols) from water used in the processing of medium density fiberboard (MDF). The polyphenols were adsorbed in a medium pressure liquid chromatography column with a polyvinyl polypyrrolidone based macroporous resin. The results revealed the potential of the resin for a selective adsorption and recovery of polyphenols. Furthermore, Xia et al. (2017) used the MRA to concentrate and purify dihydroquercetin, obtained from *Larix gmelinii* wood. The results indicated a dihydroquercetin absorption capacity of 17.4 mg/g of resin and a desorption ratio of 92.5%, from an extract with a dihydroquercetin concentration of 4.50 mg/g.

3.2.2. Silica gel chromatography (SGC)

This technique allows the separation of compounds according to their facility of absorption into the silica gel, which is used as a stationary phase. In most cases, substances with high polarity are easily absorbed in silica gel, while substances with low polarity present difficulties to be adsorbed. In this way, the desired products can be obtained through selective desorption, using the solvents with the appropriate polarity (Wen et al., 2019). Silica gel is a porous material with a large surface area, so it has many places for adsorption, which allows a good separation yield of the compounds. The pores of silica can be of various sizes, however, the best solution is usually to adopt a uniform size for the pores (Miller, 2009). The SGC is a widely used chromatographic method, but has some disadvantages such as the need of large amount of solvent, relatively high operating time and degradation risk of some products (Del Rio et al., 2015).

The results obtained by Mbakidi-Ngouaby et al. (2018) showed that SGC using different proportions of methanol/dichloromethane as eluent allows the separation of dihydrotodomatuic acid, taxifolin and quercetin from *Pseudotsuga menziesii* wood extractives. Similarly, Garcìa-Pérez et al. (2007) found that the mass yield and composition of each fraction depend on the solvent used as eluent. It was confirmed by a purification experiment conducted for the extraction of bio-oil from a mixture of wood and bark of *Populus spp*. and *Betula spp*.

3.2.3. Gel filtration chromatography (GFC)

GFC, which is also known as size exclusion chromatography (Yang et al., 2020), consists of a liquid chromatography method that separates molecules according to their size. The sample is introduced into a column filled with porous particles, which can have pores of one or more sizes, and then the sample is transported through the column by the solvent flow. During this transport, components are exchanged between the moving liquid and the stationary liquid within the pores of the particles. Smaller components spend more time in the pores than larger components, resulting in a size-based separation. In other words, the larger molecules elute first while the smaller ones elute last (Eghbali et al., 2019; Miller, 2009). GFC has the disadvantage of not making a good separation between similar species, so it is considered a low resolution technique and is often used as a complementary form of chromatography after the use of other techniques (Wen et al., 2019).

Bakasatae et al. (2018) used gel filtration chromatography to isolate the compounds responsible for the antioxidant activities of *Albizia myriophylla* wood, using dichloromethane, acetone, methanol and hexane as eluents. The main isolated antioxidant components were indenoic acid and 8-methoxy-7,3',4'-trihydroxyflavone. In other study, Benouadah et al. (2018) evaluated the molecular mass distribution of *Pinus halepensis* and *Eucalyptus camaldulensis* wood extractives through high performance size exclusion chromatography (HPSEC) with tetrahydrofuran with 1% acetic acid used as an eluent, which allowed the identification of resin acids, steryl esters, glycerides, triterpenes, fatty alcohols, sterols, stilbenes, cyclic polyols and flavanols.

3.3. Preparative liquid chromatography (PLC)

Preparative liquid chromatography (PLC) is used to purify target

compounds from a mixture. In the PLC the sample is conducted in a tube containing absorbent layers of stationary phase, which separates the mixture into its constituent components. The target compounds are then collected from the eluent stream in different vessels. Flow rates, from nanoliters per minute for research, to high flow rates, for industrial scale applications, are used at PLC. The amount of pure substance to be recovered within a certain time determines the size of the separation column, and therefore the capacity of the purification system (Schulemberg et al., 2019). The most commonly used stationary phase is silica, but other solids are also used, such as: activated charcoal, alumina, calcium carbonate, calcium phosphate, magnesium carbonate, magnesium citrate, magnesium oxide, magnesium silicate, potassium carbonate, sodium carbonate, starch and sucrose. For the mobile phase the main used solvents are: acetone, benzene, chloroform, cyclohexane, diethyl ether, ethyl acetate, isopropanol, methanol, m-xylene, n-hexane, n-pentane, n-propyl chloride, propyl nitrile and water. The choice of the mobile phase is generally based on the polarity, the viscosity, the compatibility with the detector and the volatility (Miller, 2009).

Water with 0.05% of trifluoroacetic acid and acetonitrile were used by Marchal et al. (2015) as mobile phase to isolate lignans from *Quercus petraea* extractives, where the following compounds were found: lyoniresinol, 5-methoxyisolariciresinol, quercoresinol, lyoniside, nudiposide, lyoniresinol 9'-O-β-glucopyranoside, lyoniresinol 9-O-gallate 9'-O-β-xylopyranoside, 5,5'-dimethoxysecoisolariciresinol and ssioriside. In the same way, Cretin et al. (2015) used PLC with water and acetonitrile as mobile phases to isolate (+)-lyoniresinol and (-)-lyoniresinol from the extracts of *Quercus petraea* heartwood, obtaining yields of 44.6% and 43.5%, respectively.

3.4. Countercurrent chromatography (CCC)

The countercurrent chromatography (CCC) is based on the liquid/liquid partition, since its stationary phase is liquid (Miller, 2009). The process for the separation is maintained by the centrifugal force inside the column, which allows the mobile liquid phase to extend through the system, being the stationary and mobile phases immiscible. The substances are separated according to their distribution coefficients, expressed as the relationship between their concentration in the stationary and mobile phases (Del Rio et al., 2015). The fastest form of CCC is called high speed countercurrent chromatography (HSCCC), being a technique capable of producing a high-quality sample separation (Regalado et al., 2011). CCC presents some advantages over conventional column chromatography, such as the absence of a solid support, in which a part of the mobile phase may be permanently retained. Another advantage is the use of a two-phase solvent system consisting of two mutually immiscible solvents, what makes it possible to apply a large number of combinations to find the pair with the best results (Ito, 2005).

Regalado et al. (2011) used HSCCC for the separation of phenolic compounds from a rum aged in oak barrels. The solvent system used was n-hexane-ethyl acetate-methanol-water (1:1:1:1 v:v:v:v), with 0.1% trifluoroacetic acid. Thus, 6 fractions were obtained which were later submitted to high-performance liquid chromatography (HPLC) resulting in the isolation of 48 phenolic compounds from wood (Table 2). The results obtained by Del Rio et al. (2015) show that HSCCC, using hexane-acetonitrile-methanol as a solvent system, allows the separation and purification of lapachol derivatives obtained from the heartwood of *Tabebuia sp.*

4. Application of wood extracts as a wood preservative

Generally, chemical preservatives are used to protect the wood against termites and fungi. However, this can lead to significant environmental problems, since there is a risk of leaching of the used substances, resulting in contamination of both, water and soil (Nagawa et al., 2015). This way, several researches have been carried out to find alternative preservatives from natural resources, being one of the main options the wood extractives (Brocco et al., 2017, 2020; Eller et al., 2020; Kadir and Babar, 2020; Kadir and Hale, 2019; Wu et al., 2020; Zulfiqar et al., 2020). Wood extractives promote the natural durability of wood by bringing to it the ability to resist the biological degradation, since they have antioxidant, antifungal, and insecticidal properties (Mbakidi-Ngouaby et al., 2018; Onuorah, 2000). This protective capacity of wood extractives is due to some of its components that are effective against bacteria, fungi, beetles, and termites. Some of these important compounds are tannins, flavonoids, terpenoids, and sesquiterpenes (Eller et al., 2018; Feraydoni and Hosseinihashemi, 2012; Islam et al., 2009; Kadir and Hale, 2019). In insects some of the effects caused by wood extracts are repellency, antioviposition, sterility, reduced fecundity and loss of flight ability, which allows the extractives to inhibit the action of xylophagous insects (Islam et al., 2009).

The durability transference is a technique that uses extractives taken from naturally resistant materials to treat and thus preserve the susceptible wood. In this method, extractives may come from a material that is a by-product or waste, what increases the sustainability of the process (Eller et al., 2020). For the impregnation in low natural durability wood, the extractives can be diluted in different concentrations in the same solvents used for its extraction, what allows to evaluate the correlation between the concentration used in the treatment and the wood resistance against xylophagous agents (Kadir and Babar, 2020; Kadir and Hale, 2019). There are two main methods used for the impregnation of wood with extractives. The first method is the impregnation using only vacuum, whereby impregnation is carried out in a single step (Eller et al., 2010, 2018; Kadir and Babar, 2020; Kadir and Hale, 2019; Sablík et al., 2016). The second method consists of vacuum/pressure impregnation of the wood. Thus vacuum allows the removal of air and moisture from the wood cells, while pressure allows the complete impregnation of wood cell, which is the cell wall and the lumen (Brocco et al., 2020; Eller et al., 2020; Hassan et al., 2020; Onuorah, 2000; Velasquez Gil et al., 2019).

Wu et al. (2020) found that the level of inhibition of extractives on fungi, not only depends on the solvent used for extraction and on the extractives concentration, but also on the fungus species. The authors found significant differences in the inhibitory capacity of the wood extractives of *Fokienia hodginsii* (Dunn) A. Henry & H.H. Thomas against *Trametes versicolor* (L.) Lloyd and *Gloeophyllum trabeum* (Pers.) Murrill fungi (Table 3). Another factor that determines the efficiency of extracts against xylophagous agents is the wood specie. This was confirmed by Velasquez Gil et al. (2019) when they used extracts of *Handroanthus serratifolius* (Vahl) S.O. Grose, *Centrolobium paraense* Tul. and *Tectona grandis* L.f. heartwood to preserve *Pinus caribaea* Morelet wood against the action of fungi. In this work they demonstrated that *H. serratifolius* extracts were the most efficient in protecting the wood, followed by *C. paraense* extracts. The retention of extractives in impregnation-treated wood is also an important factor for the action of extractives against xylophagous agents. This is in agreement with the findings of Onuorah (2000) after impregnating the heartwood extracts of *Erythrophleum suaveolens* (Guill. & Perr.) Brenan and *Milicia excelsa* (Welw.) C.C. Berg in the sapwood of *Ceiba pentandra* (L.) Gaertn. at different pressure levels. The author found that the ability of the extractives to inhibit the fungi *Lenzites trabea* (Pers.) Fr. and *Polyporous versicolor* (L.) Fr. was linked to the retention rate (kg/m^3) of the extractives in the sapwood, which depends on the selected impregnation. This is in line with the evidences provided by Kadir and Hale (2019) and Brocco et al. (2017), who demonstrated that the use of a higher extractive concentration in the impregnation resulted in higher treatment efficacy against fungi.

In a recent study, the oil and burgundy solid extracted from *Juniperus virginiana* wood were used together with an amylose inclusion complex (AIC) by Eller et al. (2020) for the protection of the southern yellow pine wood against termites and fungi from white rot and brown rot. The authors found that the treatment of wood with a mixture of extracts and AIC led to more than 70% reduction in mass loss by termites and

Table 3
Application of extractives as preservatives for low natural durability wood.

Raw material	Solvent	Impregnation parameters	Results	Ref.	Raw material	Solvent	Impregnation parameters	Results	Ref.
Fokienia hodginsii heartwood	AtOAc, MeOH, Chl, ACTN and PET	Impregnation of *Pinus massoniana* Lamb. Conc.: 2–10%	Inhibitory effects against the *Trametes versicolor* and *Gloeophyllum trabeum* fungi amplified with increasing concentration	Wu et al. (2020)	*Cinnamomum* sp., *Eugenia griffithii* Duthie, *Canarium littorale* Blume, *Cynometra malaccensis* Meeuwen and *Scorodocarpus borneensis* (Baill.) Becc.	EtOH, MeOH, ACTN and TO-IMS	VI of *Hevea brasiliensis* Conc.: 0.5–4%	Reduction of weight loss due to the termite *Coptotermes gestroi* (Wasmann) Efficiency increases with the concentration	Kadir and Babar (2020)
Juniperus virginiana	Liquid CO_2 and EtOH	VI of Southern pine	Reduction in the mass loss by the termite *Reticulitermes flavipes* (Kollar) and the fungus *Gloeophyllum trabeum*	Eller et al. (2010)	*Tectona grandis* heartwood	Water and EtOH	VPI of *Tectona grandis* sapwood Conc.: 0.125–8%	Complete inhibition of *Postia placenta* (Fr.) M. J. Larsen & Lombard, *Trametes versicolor, Chaetomium globosum* Kunze, *Gloeophyllum trabeum* and *Neolentinus lepideus* (Fr.) Redhead & Ginns fungi (4%−8%)	Brocco et al. (2017)
Handroanthus serratifolius, Centrolobium paraense and Tectona grandis heartwood	EtOH	VPI of *Pinus caribaea* Conc.: 0.2–2%	Effective against *Gloeophyllum trabeum* and *Trametes versicolor* fungi (1% and 2%) *H. serratifolius* extract was the most efficient	Velasquez Gil et al. (2019)	Heartwood and bark of *Ziziphus mauritiana* Lam.	HX, water, AtOAc and PET	Immersion impregnation of *Populus deltoides* W. Bartram ex Marshall Conc.: 10–30%	Increase in the resistance of wood against underground termites	Zulfiqar et al. (2020)
Juniperus virginiana heartwood	Supercritical CO_2 and MeOH	VPI of Southern yellow pine Conc.: 5%	> 70% reduction in mass loss by the termite *Reticulitermes flavipes* 30–80% reduction in mass loss by wood decay fungi	Eller et al. (2020)	*Erythrophleum suaveolens* and *Milicia excelsa* heartwood	MeOH	VPI of *Ceiba pentandra* sapwood	Effective against the *Lenzites trabea* and *Polyporous versicolor* fungi	Onuorah (2000)
Juniperus virginiana heartwood	Supercritical CO_2	VI of Yellow poplar Conc.: 5%	Reduction of mass loss by termites and white rot and brown rot fungi High termite mortality	Eller et al. (2018)	*Tectona grandis, Pinus roxburghii* (Sargent), *Dalbergia sissoo* Roxb. ex DC. *and Cedrus deodara* heartwood	EtOH-TO	VPI of *Pinus* sp. and *Populus* sp. Mixture: 20% linseed oil and 0.43% extracts	Protection against the termite *Heterotermes indicola* (Wasmann)	Hassan et al. (2020)
Tectona grandis heartwood	Water, EtOH and EtOH-water	VPI of *Pinus* sp. and *Tectona grandis* sapwood Conc.: 4%	Faster dead of *Nasutitermes corniger* (Motschulsky) termites Reduction in wood mass loss	Brocco et al. (2020)	*Neobalanocarpus heimii* (King) P.S. Ashton, *Shorea curtisii* Dyer ex King, *Cotylelobium lanceolatum* Craib and *Madhuca utilis* (Ridl.) H.J. Lam	Water	VI of *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. Conc.: 2–8%	Reduction of mass loss in response to *Trametes versicolor*, *Lentinus sajor-caju* (Fr.) Fr. and *Coniophora puteana* (Schumach.) P. Karst. fungi	Kadir and Hale (2019)
Robinia pseudoacacia heartwood	MeOH-water	VI of *Fagus sylvatica* Conc.: 10%	Reduction in the wood mass loss due to the fungus *Trametes versicolor* (from 43.6% to 12.7%)	Sablík et al. (2016)					

ACTN: Acetone; AtOAc: Ethyl acetate; Chl: Chloroform; Conc.: Concentration; EtOH: Ethanol; HX: Hexane; IMS: Industrial methylated spirit; MeOH: Methanol; PET: Petroleum ether; TO: Toluene; VI: Vacuum impregnation; VPI: Vacuum/pressure impregnation.

between 30% and 80% reduction in mass loss by decay fungi of wood, being the mixture more efficient than each extract used separately. In other study, Brocco et al. (2020) proved that woods of *Pinus* sp. and sapwood of *Tectona grandis*, when treated with extractives of *T. grandis* heartwood, became more resistant against the termites *Nasutitermes corniger*. Extractives promoted a decrease in the loss of mass and time for the death of termites, with the extractives obtained by ethanol extraction being more efficient than those obtained by water extraction. The results obtained by Zulfiqar et al. (2020) show that the extractives of heartwood and bark of *Ziziphus mauritiana* are capable of increasing the resistance of *Populus deltoides* wood against underground termites.

Eller et al. (2018) utilized the oil extracted from the heartwood of *Juniperus virginiana* via supercritical CO_2 to treat yellow poplar wood. The oil was diluted in ethanol and in amylose inclusion complex/-polyvinyl alcohol and then impregnated into the wood. The results showed that both treatments made the wood more resistant against the termite *Reticulitermes flavipes* and the fungi *Gloeophyllum trabeum*, *Postia placenta*, *Trametes versicolor*, and *Irpex lacteus* (Fr.) Fr. It was verified by decreased wood mass loss and increased termite mortality. On the other hand, Hassan et al. (2020) obtained the extractives from *Tectona grandis*, *Pinus roxburghii*, *Dalbergia sissoo*, and *Cedrus deodara* heartwood by a Soxhlet extraction with ethanol/toluene, and then mixed each of the extractives with linseed oil to treat *Pinus* sp. and *Populus* sp. wood by vacuum and pressure against the termite *Heterotermes indicola*. The authors found that the mixture had a better effect against the termite than either the extractives or linseed oil used separately.

A study about the biological activities of *Robinia pseudoacacia* conducted by Sablík et al. (2016) showed that the heartwood extractives obtained through methanol-water extraction have a good antifungal capacity. The authors found that samples of *Fagus sylvatica* L. wood treated with the extractives of *R. pseudoacacia* showed a mass loss of 12.7% when subjected to the fungus *Trametes versicolor* for 6 weeks, while untreated samples showed a mass loss of 43.6%.

5. Conclusions

There are numerous studies that have evaluated the use of wood and forest residues as a source of bioactive molecules. Therefore, this article provides an in-depth review of the different works published in this field. The first step in obtaining the molecules under study is the extraction. Currently, conventional methods are still the most widely employed, especially on an industrial scale. However, the disadvantages of these methods, together with the good results obtained with the alternative technologies described in this work, suggest that these methods will acquire a greater importance in the area. Hence, it is undoubtedly necessary to move one step further and explore the possibility of scaling up these technologies. The use of new green solvents will also be boosted in the near future due to the great concern for environmental pollution, as well as for avoiding the risks derived from the use of organic solvents.

The different purification techniques are becoming very relevant, thanks to the market opportunities that are emerging due to the properties demonstrated by the different compounds that constitute wood extracts. These processes are often very expensive, so the future holds the prospect of more economically and environmentally sustainable processes. This will require, on the one hand, the reduction of additional purification, for example by using more selective solvents in extractions; on the other hand, the optimization of these processes, especially when they are used on an industrial scale.

The applications of bioactive wood molecules are diverse and include the replacement of synthetic preservatives for wood with low natural durability. This fact, together with the latest trends towards the use of materials from renewable sources, makes wood extracts a natural material with great potential. Furthermore, the application is not necessarily limited to wood, since good results have also been reported for the use of wood extracts as repellents or insecticides.

Overall, wood extracts are confirmed as wood protective agents. However, there are many factors that influence both the extraction and the separation of the compounds, which is why it is considered necessary therefore to continue to investigate this field.

Declaration of Competing Interest

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

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