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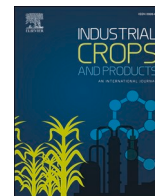
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Bark tannins: Extraction methods, characterization, and reactivity

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ABSTRACT

Tannins are compounds that occur naturally in a wide range of plants. They play an essential role in regulating plant growth and protecting plants from predators. Tannins are classified into two types: hydrolyzable and condensed. Among the many methods used to extract tannin are solvent extraction, solid-liquid extraction, and supercritical fluid extraction, depending on the target application. Supercritical fluid extraction is widely considered superior because it preserves tannin integrity while minimizing solvent use, but the steep operational costs and sophisticated equipment requirements constitute limitations. Tannins have extensive industrial uses, including leather tanning and the production of pharmaceuticals, processed food, adhesives, wine, biomaterials, and biobased polymers. Recently, tannins have gained attention as bioactive agents, with potential applications for advanced technologies such as 3D printing, biomedical devices, and therapeutic interventions for various diseases. This paper provides a detailed description of tannin extraction methods, with a focus on efficiency factors such as temperature, solvent selection, and plant material preparation. Extracted tannins are characterized based on their chemical properties, which are determinant for assessing the application potential in diverse fields. The sustainability and environmental benefits of tannins position them as valuable compounds for use in innovative industrial and environmental solutions.

1. Introduction

Historically, tannins were used for leather tanning. The term “tannin” was coined by Seguin in 1796 (Das et al., 2020). Tannins are polyphenolic compounds that are mainly derived from renewable plant sources (Shirmohammadli et al., 2018). They play important biological roles: besides defending plants against herbivores, pathogens, and environmental stressors, they may also act as allelopathic agents in soil processes (Palo and Robbins, 1991).

Chemically, tannins are divided into hydrolyzable tannins (based on gallic or ellagic acid esters) and condensed tannins (proanthocyanidins), which are oligomers or polymers of flavan-3-ols (Kalia and Avérous, 2016). These molecules are characterized by a high content of aromatic hydroxyl groups, conferring antioxidant, chelating, and antimicrobial properties (Maisetta et al., 2019). Their versatility has attracted growing interest for numerous industrial applications beyond traditional leather tanning, including wood adhesives, resins, foams, hydrogels, coatings,

and metal chelation agents (Braghiroli et al., 2019; Pizzi, 2019).

Tannins are also gaining traction in the food and pharmaceutical industries due to their bioactivity, biodegradability (María Fraga-Corral et al., 2021), and ability to interact with proteins and polysaccharides (V. Dhawale et al., 2022).

New methods are being developed to sustainably recover and purify these compounds. They include ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), pressurized hot water extraction (PHWE) (Usman et al., 2023), and deep eutectic solvents (DES) (Ruesgas-Ramón et al., 2017).

Despite these advances, several challenges persist, notably, the need for pretreatment, solvent compatibility issues, and the risk of co-extracting undesirable compounds (Pizzi et al., 2024b).

Recently, agro-industrial residues and by-products have been valorized as sources of tannins and other bioactives to provide promising sustainable alternatives to synthetic additives. Notably, bioactive compounds such as mangiferin, a C-glucosyl xanthone, which are naturally

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present in *Mangifera indica* and its by-products, offer a broad range of biological activities, including antioxidant, antidiabetic, anti-inflammatory, and cardioprotective effects (Castro-Muñoz et al., 2024). Similarly, carminic acid, a red pigment added to yogurts, juices, and baked goods, exhibits antioxidant and hepatoprotective effects (Ferreira-Suarez et al., 2024). In addition, arabinoxylans, which are hemicellulosic polysaccharides with prebiotic, antioxidant, and gelling properties (Hernández-Pinto et al., 2024), have been recovered using alkaline, enzymatic, hydrothermal, or subcritical water extraction, with yields ranging from 8 to over 70 % depending on the source and method (Hernández-Pinto et al., 2024). Furthermore, fermentation-based biotransformation is increasingly used to enhance the bioavailability and activity of phenolic compounds to produce cosmetic and nutraceutical applications, as described in further detail below (Section 4.2.8).

Thus, the development of new and sustainable extraction methods has a critical role to play in the efficient recovery of high value-added biomolecules. For instance, deep eutectic solvents (DES), which are green solvents formed by combining a quaternary ammonium salt with a hydrogen bond donor (Smith et al., 2014), and ultrasound-assisted extraction (UAE) have been used to obtain significantly increased extraction yields with minimal use of toxic organic solvents. These methods have been used to extract medicinally beneficial compounds such as bioactive terpene lactones (called ginkgolides) from *Ginkgo biloba* (Castro-Muñoz, 2025a) and gingerols, which have strong anti-inflammatory and antioxidant effects, from *Zingiber officinale* (Garza-Cadena et al., 2023). Similarly, supercritical CO₂ extraction (SFE) is often combined with polar co-solvents like ethanol, providing a clean, selective, and efficient alternative for isolating thermolabile compounds, with extraction efficiencies that can surpass those of conventional methods. When integrated into biorefinery strategies, these methods can improve biopolymer extraction rates while reducing environmental impacts (Castro-Muñoz, 2025a; Garza-Cadena et al., 2023).

Importantly, these new approaches draw on underexploited plant biomasses, tend to include reused extraction water, minimize the use of toxic solvents, and reduce energy expenditures, thereby aligning with zero-waste strategies and circular bioeconomy frameworks.

2. Tannins

2.1. Definition of tannins

In 1796, A. Seguin described tannins as substances present in plant extracts that are used to convert animal hides into stable leather (Bele et al., 2010). Tannins are now recognized as a distinct class of polyphenolic compounds characterized by their unique ability to interact with and precipitate alkaloids, gelatin, and various other proteins. In

other words, although all tannins are polyphenols, not all polyphenols can be classified as tannins (Soares et al., 2020). Tannins are water-soluble compounds that typically exhibit molecular weights ranging from 500 to 3000 Daltons (Haslam, 2001).

2.2. Types of tannin and their chemical structure

Tannins are natural polyphenols classified into two main categories: hydrolyzable and condensed tannins. Hydrolyzable tannins (Fig. 1A) are a combination of phenols, including gallic and ellagic acids, sugar esters, and mono or digallic acids (Kalia and Avérous, 2016). Hydrolyzable tannins belong to two main families: gallotannins hydrolyze to produce gallic acid and its derivatives, and ellagitannins hydrolyze to produce ellagic acid (Kalia and Avérous, 2016). Hydrolyzable tannins consist of esters of gallic acid (gallotannins) or ellagic acid (ellagitannins) linked to a sugar unit. Their core, typically glucose, can be readily hydrolyzed into monomeric products by acids or enzymes (Bhat et al., 1998).

Condensed tannins (Fig. 1B), also called proanthocyanidins, are polymeric flavan-3-ols (Fig. 2A) primarily composed of flavonoid units such as catechins and/or epicatechins (Fig. 2B) (Kalia and Avérous, 2016). Unlike hydrolyzable tannins, they have a stable, non-hydrolyzable structure due to strong interflavanic linkages, rendering them resistant to enzymatic and acidic degradation (Bhat et al., 1998; Poncet-Legrand et al., 2003).

Catechins and epicatechins have different stereochemical structures. Catechins adopt a trans configuration (2R,3S) and epicatechins a cis configuration (2R,3R) (Anitha et al., 2020; Bhat et al., 1998). Condensed tannins exhibit significant antioxidant activity, primarily due to their capacity to neutralize reactive oxygen species and chelate transition metal ions. These mechanisms contribute to attenuate oxidative stress and preserve cellular integrity, for a potential role in redox homeostasis and biomolecular protection (Anitha et al., 2020).

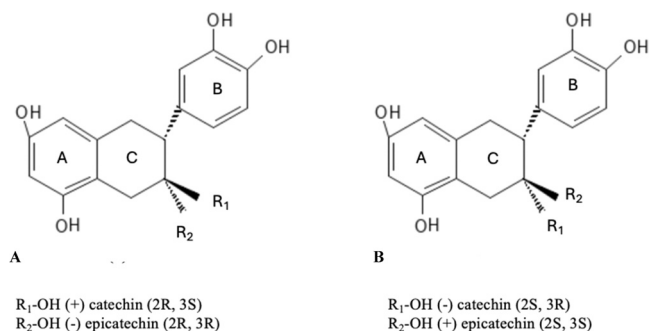


Fig. 2. Molecular structures of stereoisomers (A) (+) catechin, (-) epicatechin, and (B) (-) catechin, (+) epicatechin.

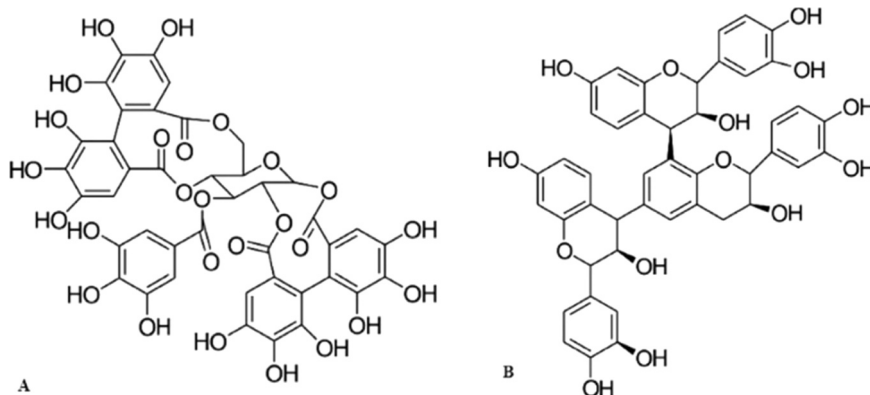


Fig. 1. Examples of molecular structures: (A) hydrolyzable tannin, and (B) condensed tannin.

Tannins with distinct structural characteristics may exhibit diverse functional properties that are suitable for different industrial applications. Consequently, it is essential to develop and optimize extraction methods for these natural phenolics to enhance their utility for specific industrial purposes.

3. Sample pretreatment before extraction

The first essential step in tannin extraction is to dry the biomass material in order to increase the extraction yield. Thus, the appropriate drying temperature for the plants is a critical factor from both an economic and ecological perspective (Rocha et al., 2011). The main drying processes are lyophilization, air drying, oven drying, and sun drying.

Various drying methods and types of equipment are used, depending on the sample characteristics. For example, lyophilization, or freeze drying, is widely used to preserve temperature-sensitive compounds. It was demonstrated a highly effective method for preserving hydrolyzable tannins in birch leaves (Salminen, 2003), where leaves freeze-dried for 48 h retained tannin content with minimal degradation. Additionally, storing leaves at -20°C for up to three months before freeze-drying resulted in only slight changes in specific compounds such as 1,2,3,4,6-penta-O-galloylglucose and isostrictinin (Salminen, 2003). Long-term storage after drying indicated that -20°C was the most effective temperature for preserving tannin stability over a year, whereas storage at 4°C or room temperature ($\sim 22^{\circ}\text{C}$) led to a decrease in tannins and showed evidence of catabolism, with increased levels of compounds such as isostrictinin and 2,3-(S)-HHDP-glucose (Salminen, 2003). These results underscore the importance of combining lyophilization with cold storage at -20°C for optimal tannin preservation.

In contrast, air drying leaves at room temperature (about 22°C) in a fume hood for 4 days resulted in less effective preservation of hydrolyzable tannins compared to freeze-drying, as the enzymatic activities and chemical changes during air drying led to tannin degradation and altered compound balances (Salminen, 2003).

Oven drying in a ventilated drying oven for 24 h at 40°C (Bimakk et al., 2011) allowed fast and uniform drying, but led to thermal damage and significant changes in the volatile composition of certain plants, with varying effects depending on the species and the specific compounds present (Manan, 2023).

Sun drying is conducted at varying temperatures, depending on environmental conditions, generally using drying trays or nets placed in the shade to prevent degradation by direct sunlight (Rocha et al., 2011). The significant drawbacks to these methods include the inability to handle large volumes generated by mechanical harvesters and failure to meet the stringent quality standards required for certain plants (Rocha et al., 2011). Additionally, high ambient temperatures and high humidity during the harvest season promote the growth of insects and mold on crops (Rocha et al., 2011).

Drying significantly decreases the moisture content of samples, which increases their brittleness and reduces their tendency to adhere in the milling apparatus, and hence facilitates grinding. The aim is to produce uniformly sized particles, thereby increasing the overall surface area and improving the tannin extraction efficiency.

Grinding is another critical operation in the extraction process. Although various methods and types of equipment are used to optimize the efficiency, dry grinding is considered the most effective and practical (Makkar, 2003). In any case, for optimal sample preparation prior to tannin extraction, the grinding method and equipment must be suitable for the plant part and its specific properties (Belwal et al., 2018).

Typically conducted with vibrating or rotary screens, sieving ensures uniform processing of large material volumes (Abilleira et al., 2021). For example, to extract tannin from yellow birch bark, the material was first dried and crushed, then sieved to achieve uniform particle size (Abilleira et al., 2021). Tannin extraction from dry biomass produced higher yields compared to wet biomass (Sealy-Fisher and Pizzi, 1992). Although extraction from wet biomass was effective, it produced lower yields due

to tannin dilution by the moisture content in the bark (Sealy-Fisher and Pizzi, 1992). Table 1 shows the clear influence of moisture content on tannin yield for a same species, such as *Pinus radiata*. At 8.3 % moisture content, the yield reaches 12 %, whereas at a higher moisture content of 12 %, the yield drops to 10.1 %. This comparison of pretreatments for extraction under otherwise identical conditions highlights the significant effect of moisture content on the extraction efficiency. Based on the presented data, a moisture content below 10 % appears to be a critical threshold for optimal tannin yield.

In sum, when preparing samples for tannin extraction, the drying, grinding, and sieving methods must be specifically adapted to the plant part and its properties to ensure maximum tannin extraction and preservation.

4. Tannin extraction

Tannin concentrations vary across species according to several factors, including biological origin, genetic variability, age, and position within the tree (e.g., inner or outer wood) (Das et al., 2020; Puech et al., 1999). Additionally, the heterogeneous nature of tannins complicates the extraction process, presenting a significant challenge for their valorization (Das et al., 2020). Tannin extraction does not follow a single protocol, and methods may vary considerably, resulting in extracts with various impurities, such as minerals, stilbenes, and sugars (Das et al., 2020), further influencing the properties of the powder. For example, the presence of carbohydrates in tannins reduces their antifungal effectiveness (Zhu et al., 2019). The amount of impurities is also influenced by processing parameters such as particle size, temperature, pressure, duration, solvent type, and solid/solvent ratio (Magangana et al., 2020). Of these, the temperature and the solid/solvent ratio are the primary factors influencing the carbohydrate content of extracted tannins (Osman et al., 2024). Consequently, tannin extraction must be conducted with care and with precisely controlled operating parameters (Shirmohammadli et al., 2018) to ensure optimal yield and quality of the obtained extracts. Thus, before proceeding to the extraction stage, a preparation phase is necessary, including sample pretreatment.

The composition of tannin varies significantly across plant species. Table 2 presents the different extraction efficiencies for different species, likely due to variations in chemical structure, cell wall composition, and tannin distribution. The extraction methods include solid-liquid extraction (SLE), supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), and ultrasonic-assisted extraction (UAE). Certain species, such as *Pinus ocarpa*, produce high tannin yields under various extraction conditions. For example, extraction with water + 5 % Na_2CO_3 at 100°C for 2 h resulted in a 35.7 % yield, whereas using water alone under the same conditions yielded only 24.8 % (Vieira et al., 2024). Similarly, for *Pinus pinaster*, extraction with water + 5 % NaOH produced a 31.3 % yield, indicating that this species is particularly rich in tannins (Chupin et al., 2013). However, this result should be interpreted with caution: while the yield appears high, the extract also contained a significant proportion of non-tannin compounds such as carbohydrates and lignins, as evidenced by the low Stiasny number ($\sim 18\%$). This suggests that the extraction method used (Chupin et al., 2013) compromised the purity and functional quality of the tannins, hence limiting its industrial relevance.

Other species produced lower tannin extraction yields. Thus, for *Picea mariana*, extraction with distilled water at 100°C for 1 h yielded 9.96 % (Diouf et al., 2009). Increasing the extraction time to 1.5 h slightly improved the yield to 19.4 %, demonstrating that the time can directly influence extraction efficiency (Francezon et al., 2017). The best extraction conditions and the highest-yield species are highlighted in Table 2. Of these, *Pinus ocarpa* stands out with the highest yield at 35.7 % when extracted with water and 5 % Na_2CO_3 at 100°C for 2 h under a reflux system (Vieira et al., 2024), indicating particularly effective tannin extraction for this species under these specific conditions. Another high-yield species is *Acacia dealbata*, at 29.5 % obtained

Table 1
Sample size and moisture content before extraction.

Plant (Plant part)	Moisture (%)	Sample pretreatment conditions	Grinding method	Particle size after grinding	Tannin yield (%w/w) ^a	Reference
<i>Eucalyptus globulus</i> (leaves)	12.5	Air-dried, crushed	Cut into 2 cm ² pieces, air-dried for 48 h, and milled to < 1 mm using an Ultra Centrifugal Mill (ZM 100, Retsch)	0.5–1.0 mm 30 % size < 0.25 mm, 55 % 0.25–1 mm in size, 15 % 1–2 mm in height	15	(Tomasi et al., 2023)
<i>Picea abies</i> (Bark)	10.44	Washed with water, dried at room temperature under normal weathering conditions, and crushed	-	-	30.46	(Talmaciu et al., 2016)
<i>Pinus radiata</i> (Bark)	8.30	Harvested (age 23), crushed, sieved (1–2 mm in size), dried at room temperature, and then stored in sealed bags until used for experiments	Ground in a two-knife mill (UNTHA, RS30–4S, Austria, and AMIS Maschinen-Vertriebs GmbH, Germany)	1–2 mm	12	(Aspé and Fernández, 2011)
<i>Pinus radiata</i> (Bark)	12	Harvested, crushed, sieved (20 ± 5 mm size), dried at room temperature, and stored at 23 ± 1 °C in a room	Ground with a mill (Condux, Hamilton, Canada)	20 mm	10.1	(Ramos et al., 2013)
<i>Larix europaea</i> (Bark)	8.12–10.28	Harvested, crushed, and sieved (particles 0.5–0.8 mm in size)	-	0.5–0.8 mm	< 2	(Ravber et al., 2015)

^a % w/w: Extraction yield is expressed in grams of extract per 100 g of dry material.

by Soxhlet extraction with water at 100 °C for 3 h (Abilleira et al., 2021). Although this yield is slightly lower than that for *Pinus ocarpa*, Soxhlet extraction remains a very effective method under relatively simple conditions. Additionally, *Carya illinoensis*, extracted with supercritical CO₂ at 50 °C and 200 bar, yielded 29 % (do Prado et al., 2014). Overall, *Pinus ocarpa* showed the highest yield, closely followed by *Acacia dealbata* and *Carya illinoensis*, all of which achieved high yields under optimized extraction conditions.

At the same time, species like *Punica granatum* yielded significantly lower amounts of tannins (0.21–0.67 %) when extracted with supercritical CO₂ at 50 °C and 300 bar (Cavalcanti et al., 2012). In sum, tannin composition varies widely among plant species, and these differences must be carefully considered in order to select the most suitable species and conditions for tannin extraction for specific industrial applications.

4.1. Extraction parameters

The key influential factors for tannin extraction efficiency include temperature, solvent type and concentration, extraction time, and extraction method. In the case of tree bark, bark age and sample height in the tree are additional factors. All these parameters are critical for optimizing the yield and preserving the integrity of the extracted compounds, as described next.

Temperature significantly impacts the yield (Naima et al., 2015), as shown in Table 2. Extraction temperatures typically range from 80 to 100 °C (Chupin et al., 2013; Vieira et al., 2024), with tannins showing greater solubility at higher temperatures, thereby enhancing extraction efficiency (de Hoyos-Martínez et al., 2019). The higher energy of the solvent molecules accelerates the diffusion of tannins from the solid matrix into the liquid solvent, reducing extraction time and improving process efficiency (de Hoyos-Martínez et al., 2019). However, while higher temperatures are beneficial, they must be carefully selected to prevent thermal degradation of tannins, which are sensitive to excessive heat (de Hoyos-Martínez et al., 2019). Due to their complex aromatic structure, tannins have high thermal stability. Thus, according to thermogravimetric analysis (TGA), the thermal degradation of purified condensed tannins begins at around 200 °C. In comparison, crude extracts with higher polysaccharide content exhibited earlier degradation onset, at around 150 °C (Gaugler and Grigsby, 2009).

A temperature of 90 °C is commonly used for solid-liquid extraction (SLE) methods (Kemppainen et al., 2014), depending on the applied

solvent, as temperatures near the solvent's boiling point optimize extraction efficiency. Temperatures of 80–100 °C are often used to extract polyphenols (Chupin et al., 2013; Vieira et al., 2024), although different extraction methods have different temperature and pressure requirements based on the solvent's properties and the chemical nature of the targeted compounds. Table 2 summarizes the main extraction methods along with the applied temperature and pressure ranges.

A major challenge in industrial applications is to determine and apply the optimal extraction temperature. Increased extraction temperatures can lead to relative decreases in the proportions of polyphenolics in the extract, mainly due to increased co-extraction of carbohydrates, which dilutes the polyphenolic fraction. However, this effect may vary depending on the extraction system, experimental conditions, plant matrix, and polyphenol types involved. Thus, it is a balance between the percentage yield of the total extract and the percentage of polyphenolics in the extract. Moreover, excessively high temperatures may induce self-condensation reactions of certain polyphenolic compounds, leading to the formation of insoluble or poorly soluble extracts, or precipitation and rearrangements into insoluble precipitates called phlobaphenes (Sealy-Fisher and Pizzi, 1992) or procatechuic acid (Navarrete et al., 2011) for some tannin polyphenols. Therefore, each extraction method offers unique advantages and disadvantages based on the operational parameters and target application. Solid-liquid extraction offers a versatile choice, with a balance between efficiency and compound stability. Microwave-assisted extraction accelerates the process while protecting compounds from degradation. Ultrasound extraction is particularly effective in maintaining supercritical CO₂ conditions, thereby enhancing efficiency. Lastly, supercritical fluid extraction stands out for its ability to maximize solubility at high pressures without compromising the integrity of sensitive compounds. The choice of method ultimately depends on the desired balance between efficiency, compound preservation, and operational requirements. A detailed discussion of these methods is presented in the next section.

The solvent type and concentration are further critical influential factors for extraction efficiency. As presented in Table 2, one of the most commonly used solvents for tannin extraction is water, often combined with additives such as Na₂CO₃, NaOH, Na₂SO₃, and NaHSO₃ to enhance extraction performance. For example, a 5 % solution of Na₂CO₃ or NaOH is frequently used for effective tannin extraction (Chupin et al., 2013; Vieira et al., 2024). Other studies have shown that mixtures of specific

Table 2
Extraction of tannins from different species using different methods and parameters.

Method	Raw material			Extraction conditions			Process parameters			References
	Part	Species	Size	Solvent type	Equipment	Temperature (°C)	Time (h)	Pressure (bar)	Yield (% w/w) ¹	
SLE	Bark	<i>Acacia dealbata</i>	< 0.5 mm 0.5–1 mm and > 1 mm	Water	Soxhlet Capped Erlenmeyer flasks, orbital shaker with temperature control	100	3	1.013	29.5	(Abilleira et al., 2021)
	Bark	<i>Pinus radiata</i>	1–2 mm	Acetone 70 %	Agitation vessel, heating source	82	3	1.013	12	(Aspé and Fernández, 2011)
	Bark	<i>Coriaria nepalensis</i>	< 1 mm	NaOH	Glass beaker (1 L), reflux system, mechanical stirrer and heating system	82	1.05	1	16.02	(Guo et al., 2020)
	Bark	<i>Eucalyptus globulus</i>	0.4 mm 45–212 µm (8 %) 212–600 µm (35 %) 600µm–1.18 mm (43 %)	NaOH, Na ₂ SO ₃ , and Na ₂ CO ₃		70	-	1.013	27.1	(Amari et al., 2021)
	Upper and aerial parts	<i>Phyllanthus niruri</i>	1.18–3.35 mm (14 %)	Distilled water	Soxhlet Boiling flask under reflux, Büchner funnel	100	3	-	26.2	(Markom et al., 2007)
	Bark	<i>Pinus ocarpa</i>	-	water + 5 % Na ₂ CO ₃	Boiling flask under reflux, Büchner funnel	100	2	1.013	35.7	(Vieira et al., 2024)
		<i>Pinus ocarpa</i>	-	water + 1 % Na ₂ CO ₃	Boiling flask under reflux, Büchner funnel	100	2	1.013	30.9	(Vieira et al., 2024)
		<i>Pinus ocarpa</i>	-	water + 5 % NaHSO ₃	Boiling flask under reflux, Büchner funnel	100	2	1.013	26.7	(Vieira et al., 2024)
		<i>Pinus ocarpa</i>	-	Water	Boiling flask under reflux, Büchner funnel	100	2	1.013	24.8	(Vieira et al., 2024)
		<i>Pinus ocarpa</i>	-	water + 1 % NaHSO ₃	Boiling flask under reflux, Büchner funnel	100	2	1.013	19.9	(Vieira et al., 2024)
	Bark	<i>Picea mariana</i>	0.25–0.5 mm	Distilled water	Reflux extractor, whatman filter, labconco FreeZone 12 L, console freeze drying system	100	2	1	2.1	(Francezon et al., 2017)
		<i>Picea mariana</i>	0.25–0.5 mm	Distilled water	Reflux extractor, whatman filter, labconco FreeZone 12 L, console freeze drying system	80	2	1	16.5	(Francezon et al., 2017)
	<i>Picea mariana</i>	0.25–0.5 mm	Distilled water	Reflux extractor, whatman filter, labconco FreeZone 12 L, console freeze drying system,	80	1.5	1	16.1	(Francezon et al., 2017)	

(continued on next page)

Table 2 (continued)

Method	Raw material			Extraction conditions					Yield (% w/w) ¹	References
	Part	Species	Size	Solvent type	Equipment	Process parameters				
						Temperature (°C)	Time (h)	Pressure (bar)		
		<i>Picea mariana</i>	0.25–0.5 mm	Distilled water	reflux extractor, whatman filter Labconco FreeZone 12 L, console freeze drying system	100	1.5	1	19.4	(Francezon et al., 2017)
		<i>Picea mariana</i>	0.25–0.5 mm	Distilled water	Reflux extractor whatman filter, labconco FreeZone 12 L, console freeze drying system	80	1	1	15.6	(Francezon et al., 2017)
		<i>Picea mariana</i>	0.25–0.5 mm	Distilled water + 5 % NaOH + 0.25 % Na ₂ SO ₃	FreeZone 12 L console freeze drying system	100	1	1	19.3	(Francezon et al., 2017)
Bark		<i>Pinus pinaster</i>	< 1 mm	+ 0.25 % NaHSO ₃ + 1 % NaOH + 0.25 % Na ₂ SO ₃	Agitation vessel heating source whatman filter	80	2	1.013	31.3	(Chupin et al., 2013)
		<i>Pinus pinaster</i>	< 1 mm	+ 0.25 % NaHSO ₃	Agitation vessel heating source whatman filter	80	2	1.013	22.1	(Chupin et al., 2013)
		<i>Pinus pinaster</i>	< 1 mm	Distilled water	Agitation vessel heating source whatman filter	100	3	-	25.8	(Chupin et al., 2013)
Peels		<i>Castanea sativa</i>	Fine powder	Distilled water + 1 % Na ₂ SO ₃	Boiling flasks under reflux Büchner funnel	85	4	-	7.03	(Aires et al., 2016)
Bark		<i>Picea mariana</i>	40–60 mesh < 0.1 mm, 0.1–0.5 mm, 0.5–1 mm, 1–2 mm and > 2 mm	Distilled water	Reflux extractor whatman filter freeze-dryer	100	1	1.013	9.96	(Diouf et al., 2009)
Bark		<i>Pinus pinaster</i>	1–2 mm and > 2 mm	Water + 5 % NaOH	Reactor spray dryer	90	-	1.013	25	(Vázquez et al., 2001)
		<i>Pinus pinaster</i>		Water + 2.5 % NaOH		-	0.5	1.013	16.5	(Vázquez et al., 2001)
		<i>Pinus pinaster</i>		Water + 5 % NaOH		70	-	1.013	18.9	(Vázquez et al., 2001)
Bark		<i>Picea abies</i>	-	Water + 2 % NaHSO ₃ + 0.5 % Na ₂ CO ₃	Reactor	90	2	-	20.9	(Kempainen et al., 2014)
		<i>Picea abies</i>	-	Water + 2 % NaHSO ₃ + 0.5 % Na ₂ CO ₃		60	2	1.013	16.1	(Kempainen et al., 2014)
Bark		<i>Pinus radiata</i>		Acetone (70 %)		82	3	1.013	12	(Aspé and Fernández, 2011)

(continued on next page)

Table 2 (continued)

Method	Raw material			Extraction conditions					Yield (% w/w) ¹	References	
	Part	Species	Size	Solvent type	Equipment	Temperature (°C)	Time (h)	Pressure (bar)			
SFE	Bark	<i>Pinus radiata</i>	20 mm	Acetone (25 %)	Reactor evaporator lyophilized Reactor filter	120	2	1.013	11	(Ramos et al., 2013)	
		<i>Pinus radiata</i>	20 mm	Ethanol (25 %)	evaporator lyophilized Reactor filter	120	2	1.013	10.1	(Ramos et al., 2013)	
		<i>Pinus radiata</i>	20 mm	Acetone (25 %)	evaporator lyophilized	40	2	1.013	3.5	(Ramos et al., 2013)	
	Upper and aerial parts	<i>Pinus radiata</i>	45–212 µm (8 %) 212–600 µm (35 %) 600 µm–1.18 mm (43 %)	Ethanol (25 %)			40	2	1.013	3	(Ramos et al., 2013)
		Supercritical fluid extraction unit supercritical CO ₂ pump extraction Cell	50	-	300	0.21–0.67	(Cavalcanti et al., 2012)				
								Leaves	<i>Punica granatum</i>	24–48 mesh	CO ₂
	Shells	<i>Carya illinoensis</i>	60 mesh	CO ₂		50	-		200	29	(Tomasi et al., 2023)
		Leaves	<i>Eucalyptus globulus</i>	-	Water	Microwave oven rotary evaporator freeze dryer Ultrasonic bath	141	15 s	1	15	(Aspé and Fernández, 2011)
	Bark		<i>Pinus radiata</i>	1–2 mm	Acetone (70 %)	freeze dryer Ultrasonic bath	20	180 s	1	10.4	(Aspé and Fernández, 2011)
Bark		<i>Pinus radiata</i>	1–2 mm	Acetone (70 %)	capped flasks	25	360 s	1	9.5	(Aspé and Fernández, 2011)	

¹ % w/w: Extraction yield is expressed in grams of extract per 100 g of dry material.

concentrations of additives such as 5 % Na₂CO₃ or NaOH combined with lower percentages of Na₂SO₃ and NaHSO₃ can effectively extract tannins from barks (Chupin et al., 2013; Vieira et al., 2024). Water-ethanol mixtures are often preferred for their efficiency in extracting polyphenols, although methanol, acetone, and deep eutectic solvents are also used, depending on the end product (Ruesgas-Ramón et al., 2017).

Deep eutectic solvents (DES) are increasingly used for tannin extraction due to their ecofriendliness and efficiency (Ruesgas-Ramón et al., 2017). Among these, choline glycerol chloride has demonstrated the highest effectiveness (Molnar et al., 2024a). However, despite their potential, DES are largely confined to laboratory-scale studies, and their implementation at industrial scale remains under development. Additionally, the solvent choline chloride urea (1:2) has been demonstrated particularly effective for extracting hydrolyzable tannins, gallic acid, and ellagic acid from *Alchemilla vulgaris* L. (Kovač et al., 2022), while a mixture of acetic acid and glycerol achieved high efficiency when combined with ultrasonic and enzyme-assisted extraction methods for tannin recovery from tea leaves (Vo et al., 2023). Because these solvents are adapted to specific tannin properties, they ensure a more environmentally friendly and often more efficient extraction compared to traditional organic solvents. Table 1 presents the different types of solvents used to extract tannins from various plant species along with the associated equipment and process parameters. The extraction time is

another critical factor that varies significantly based on the method, process parameters, and type of plant material. Each extraction method presents distinct advantages and limitations in terms of efficiency, energy cost, and environmental impact. For instance, conventional SLE typically requires extraction times ranging from 0.5 to 48 h, whereas MAE provides exceptional efficiency, with extraction times between 1.04 and 18 min (de Hoyos-Martínez et al., 2019), depending on the microwave power and other extraction parameters.

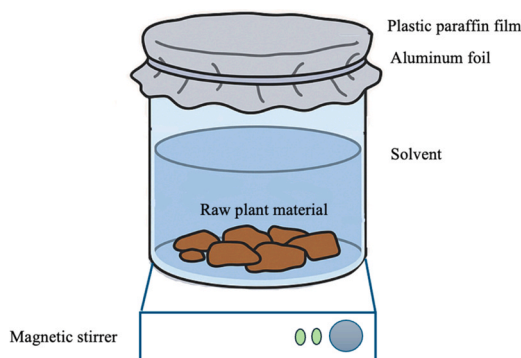


Fig. 3. Schematic representation of the maceration process.

4.2. Extraction methods

As mentioned above, tannin extraction can be conducted with various traditional methods that are tailored to the specific tannin source (e.g., wood, bark, leaves, or fruit) and its intended application. The following sections provide an overview of the most commonly used extraction methods.

4.2.1. Maceration

Maceration at room temperature is a traditional extraction method in which the bio-sourced material is soaked in a solvent (Seidel, 2005) to isolate bioactive compounds such as simple polyphenols, condensed tannins, and hydrolyzable tannins (Cvetanović et al., 2020). This simple method remains highly effective for tannin extraction and is still widely used (Safdar et al., 2017).

Raw materials such as tree bark are immersed in a suitable solvent at room temperature (Naima et al., 2015). The process is conducted in a glass crystallizer covered with aluminum foil to protect light-sensitive compounds and sealed with a plastic paraffin film to prevent the solvent from evaporating (Fig. 3). Moderate mechanical agitation facilitates optimal solvent-plant material interaction to promote the extraction of the desired compounds (Naima et al., 2015).

Maceration efficiency is influenced by several factors, with solvent selection being a critical parameter. Commonly used solvents, including water, ethanol, and methanol, exhibit distinct physicochemical properties that impact their effectiveness in extracting specific compounds. For instance, 80 % methanol was demonstrated particularly effective for extracting tannins from 10-year-old bark. The age of the plant material also plays a significant role, as younger barks (3 years) yielded 3.197 mg Cya/g of tannin while older barks (10 years) yielded 27.96 mg Cya/g of tannin. These yields, expressed as milligrams of cyanidin equivalents per gram of bark using the acid-butanol assay for condensed tannins, indicate that bark maturity can significantly influence both the concentration and type of extracted compounds. The maceration duration is also critical: extraction times ranged from 2 to 24 h, with significant impacts on the quantity of extracted compounds (Naima et al., 2015).

In addition, as demonstrated by Drovou et al. (2015), a five-day maceration of *Parkia biglobosa* shells in water without additives resulted in the extraction of tannins still linked to long carbohydrate chains, with up to 22 sugar residues attached to the flavonoid core. In contrast, tannins obtained by semi-industrial extraction at high temperatures (70–74°C) in the presence of bisulfite additives consisted mainly of

low-molecular-weight polyphenolic oligomers, with most carbohydrate linkages hydrolyzed. This indicates that maceration, with its mild conditions, preserves more complex natural structures, while industrial extraction tends to simplify the tannin structure by breaking glycosidic bonds (Drovou et al., 2015). Therefore, the extraction method must be carefully selected to target complex bioactive forms or simplified oligomeric tannins as well as the intended application of the final product (Drovou et al., 2015).

Overall, maceration remains the preferred method for extracting bioactive compounds due to its simplicity, adaptability, and ability to meet diverse extraction requirements.

4.2.2. Hot water extraction

The use of hot water alone is an environmentally sustainable approach to tannin extraction from barks, and is often preferable to chemical solvent-based methods due to its low cost, efficiency, and environmental friendliness (de Hoyos-Martínez et al., 2019).

For example, in Faye et al., 2021 (Faye et al., 2021), bark was initially dried at 105°C to stabilize its moisture content and then fed into a reactor containing distilled water at pH 5.72. The extraction temperature was 95°C and duration varied from 0.5 to 6 h, depending on the experimental needs. The biomass was added gradually under constant stirring to ensure uniform extraction. After extraction, the solution containing the tannins was centrifuged to separate the soluble compounds. The solid residues were subsequently washed with hot water and centrifuged again (Faye et al., 2021). This method was optimized to maximize the tannin yield while minimizing water and energy consumption. Several innovative strategies were also implemented to reduce the environmental impact of the extraction and isolation steps. These improvements, which included additional steps to improve the process efficiency, resulted in not only higher tannin yields but also a more sustainable process. The extraction process lasted for 2 h, and the solution containing the extracted tannins was reused as a solvent to process a new batch of bark. In a repetitive cycle, the solution from each extraction was filtered to remove insoluble particles and then immediately recycled, while still hot, for the next extraction. This approach significantly reduced water and energy consumption, particularly when processing biomass with low tannin content. The extraction solution was recycled multiple times until nearly saturated with solutes, thereby optimizing resource utilization. At the end of the process, all water-soluble components were collected in a single step, ensuring maximum efficiency (Faye et al., 2021).

Hot water extraction refers to a specific type of solid-liquid extraction where only hot water is used as the solvent. In contrast, solid-liquid extraction (SLE) is a broader term that encompasses any extraction process involving a solid matrix and a liquid solvent regardless of the solvent type, as described in the next paragraph.

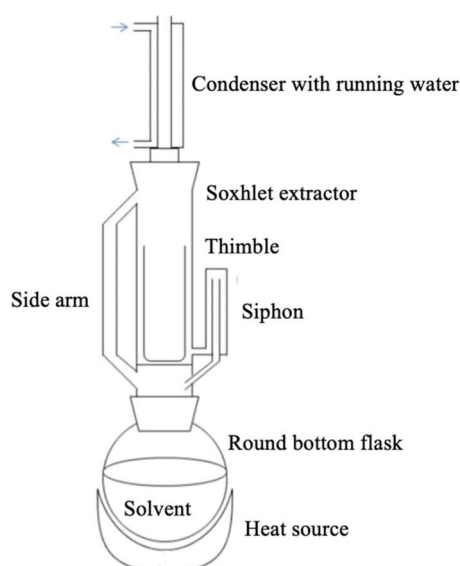


Fig. 4. Schematic representation of a Soxhlet extractor.

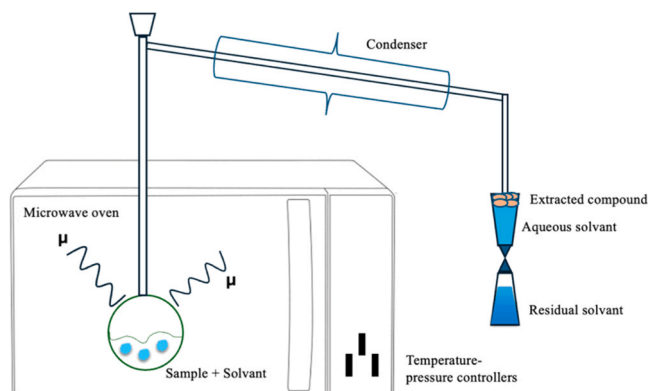


Fig. 5. Schematic representation of the microwave-assisted extraction process.

4.2.3. Solid-liquid extraction (SLE)

SLE is a classic, effective, reliable, and easy-to-implement method that uses organic or aqueous solvents [46], whereby the polyphenolic compounds are transferred from a solid matrix, such as leaves, bark or other plant parts, to a liquid solvent (de Hoyos-Martínez et al., 2019; Luque de Castro and García-Ayuso, 1998). As depicted in Fig. 4, the tannins are dissolved in an appropriate solvent, usually water, methanol, or deep eutectic solvents (Kovač et al., 2022), and the liquid extract is then separated from the solid matrix. The extraction efficiency depends on factors such as particle size, temperature, agitation, and solvent choice (Abilleira et al., 2021). This method enables continuous and thorough extraction of target compounds, ensuring high efficiency (Daso and Okonkwo, 2015). Compared to more advanced methods like SFE and MAE, SLE is generally more cost-effective, as it requires lower investments in equipment and solvents (Tierney et al., 2013). Table 2 shows the yields obtained by this method.

4.2.4. Supercritical fluid extraction (SFE)

SFE with CO₂ is a highly effective method for solubilizing and extracting tannins. However, despite its superior selectivity and efficiency (Markom et al., 2007), its application is limited by the high cost of specialized equipment (Friedrich and Pryde, 1984). In order to maintain CO₂ in its supercritical state, pressures and temperatures must be precisely controlled, requiring advanced technical expertise. Additionally, the high temperatures and pressures may sometimes affect the chemical structure of heat-sensitive compounds, potentially reducing their quality (Arumugham et al., 2021). As determined by thermogravimetric analysis (TGA), thermal degradation is usually observed at above 150°C (Gaugler and Grigsby, 2009), depending on the structure and purity of the tannins. Because SFE with CO₂ is typically conducted at temperatures ranging from 31 to 80 or 90°C, well below the degradation threshold, the chemical integrity of tannins is preserved during the extraction process. Therefore, despite the need for higher pressure, the moderate temperatures used in SFE ensure that the structural and functional properties of tannins remain intact.

In this method, the critical point is defined as the maximum temperature and pressure of a pure substance in vapor-liquid equilibrium (Ajila et al., 2011). Beyond this point, a fluid shares properties between a gas and a liquid, such as the typical density of liquids with the penetrating power of gases (Azmir et al., 2013). The most widely used supercritical fluid is carbon dioxide (CO₂), due to its desirable properties, which include non-toxicity, non-flammability, non-corrosiveness, availability, and low critical temperature and pressure (31°C and 74 bars) (Azmir et al., 2013).

The main advantages of this extraction method are the use of moderate temperatures and a non-toxic, easily removable solvent (CO₂). These conditions create an oxygen-free environment that prevents subsequent oxidation reactions. However, a major limitation is the high investment cost, due to the high pressures required for the extraction process (Perrut, 2000a). Additionally, the non-polar nature of CO₂

reduces its ability to solubilize tannins, resulting in low extraction efficiency for polar compounds. To improve the solubility, co-solvents such as methanol, ethanol, acetone, or water must be added.

4.2.5. Microwave-assisted extraction (MAE)

This method (Fig. 5) combines traditional solvents with microwave heating (Dahmoune et al., 2014). The microwave radiation is absorbed by the solid matrix, where it is converted into heat, which facilitates the diffusion of the solvent into the bio-sourced matrix. Additionally, polar solvents can be added to induce dipole rotation and ion migration, thereby increasing the solvent penetration and improving the extraction yield (Ciuperca (Apreutesei) et al., 2023).

The advantages of MAE include rapid extraction times, moderate extraction temperatures, minimal solvent use, and improved efficiency, leading to higher yields (Ciuperca (Apreutesei) et al., 2023). On the other hand, it requires high initial investments and maintenance costs for the equipment, works better with polar solvents, and is less effective when non-polar or volatile solvents are used. Additionally, large samples or aggregated small particles may not heat uniformly, and a post-extraction cooling period is needed (Ciuperca (Apreutesei) et al., 2023).

As presented in Table 2, MAE is distinguished by its high efficiency in a very short time, with 15 % yield in just 15 s (Tomasi et al., 2023). Moreover, when the solvent is water, which is non-toxic and

Table 3

Summary of extraction performance for different methods.

Extraction method	Advantages	Limitations	References
Maceration	Simple, easy to perform, low-cost Classic, effective, reliable	Lengthy, low efficiency	(Tambun et al., 2021)
SLE and hot water extraction	Easy to implement, cost-effective Selectivity, non-toxic CO ₂ solvent, moderate time	Less technologically advanced, longer extraction times	(Abilleira et al., 2021; Daso and Okonkwo, 2015; de Hoyos-Martínez et al., 2019)
SFE		High cost, requires co-solvents for polar compounds	(Guo et al., 2011; Perrut, 2000b) (Ciuperca (Apreutesei) et al., 2023; Dahmoune et al., 2014; Xiao et al., 2012)
MAE	Fast, improved yields, lower solvent use	High initial costs, limited yield with non-polar solvents Non-uniform ultrasonic intensity, potential degradation of sensitive compounds	(de Hoyos-Martínez et al., 2019; Ghitescu et al., 2015; Pastor et al., 1997)
UAE	Fast, lower solvent use		

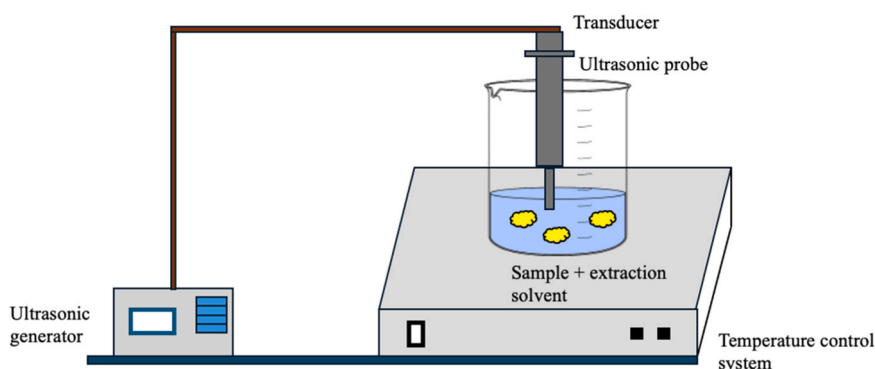


Fig. 6. Schematic representation of the ultrasound-assisted extraction process.

environmentally sustainable, this method is more eco-friendly. The extraction effectiveness is determined by several factors, including the choice of solvent, particle size, ratio of solvent to solid, temperature, and extraction time.

4.2.6. Ultrasound-assisted extraction (UAE)

Ultrasound-assisted extraction (UAE) (Fig. 6) operates by propagating sonication waves within a sound intensity range of 5–1000 W/cm² at frequencies exceeding 20 kHz (Rifna et al., 2023). Higher sonication power enhances the tannin extraction efficiency. However, exceeding the critical threshold, typically observed at around 0.28 W/cm³ depending on the material, can produce excessive heat and induce chemical degradation of the tannins. Although the increase in temperature is not as great as in other methods, it can still affect the quality of the tannin extracts and reduce their effectiveness (Esclapez et al., 2011). Notably, this method is characterized by its rapid extraction kinetics, typically requiring no more than 30 min (de Hoyos-Martínez et al., 2019). The main advantages of this method are speedy extraction, simplicity, and low cost. However, the lack of uniform ultrasonic intensity distribution across the sample presents a limitation (de Hoyos-Martínez et al., 2019) that lowers the effectiveness, particularly at industrial scale.

This method was shown to perform better than traditional Soxhlet extraction. Besides reducing the consumption of organic solvents, it shortened the extraction time while achieving higher yields for larger sample quantities: 2–10 g samples were processed in just 6 min, faster than other presented methods (Pastor et al., 1997). As demonstrated by (Ghiteșcu et al., 2015), optimizing UAE of polyphenols from black spruce bark offers a promising approach to enhance the yield of bioactive compounds, unlocking further opportunities for large-scale applications.

4.2.7. Current industrial extraction methods

Hot water extraction is currently the predominant industrial method for tannin recovery. Generally, 2–3 % sodium metabisulphite is used to stabilize the polyphenolic compounds, and occasionally 0.5 % sodium hydrogen carbonate is added to buffer the pH and improve the solubility (Sealy-Fisher and Pizzi, 1992). Various extraction methods have been assessed for efficiency, environmental sustainability, cost-effectiveness, and processing time. The comparative strengths and weaknesses underscore the impact of method selection on extract quality and its alignment with industrial and green chemistry objectives. Ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) appear to be particularly advantageous for tannin extraction. UAE combines rapid extraction with lower solvent use, whereas MAE improves the yield and reduces energy consumption. Both methods adhere to green chemistry principles in terms of solvent reduction, energy efficiency, and greater selectivity. Nevertheless, the inadequate scalability for industrial applications and the expensive equipment required have discouraged widespread adoption. Meanwhile, although hot water extraction is favored by industries due to its simplicity and robustness, it is less selective and may necessitate longer processing times.

The extraction methods were compared to identify their advantages and limitations, including cost, time, and the use of environmentally friendly solvents. Table 3 summarizes the advantages and limitations.

As mentioned above, for optimal tannin recovery, the plant species and parts must be selected with care. In addition, as presented in Table 2, the choice of extraction method has a major impact on the yield, efficiency, and operational feasibility. Whereas solid–liquid extraction (SLE) offers the advantages of low cost, simplicity, and ease of implementation, it usually requires longer extraction times (typically 1–4 h) and relatively high temperatures (up to 100°C) to achieve satisfactory yields, which can range from 2.1 % to 35.7 %, depending on the species, solvent composition, and particle size. In contrast, more recent methods such as microwave-assisted extraction (MAE) and ultrasound-assisted

extraction (UAE) have shorter reaction times (as brief as 15–360 s) while producing relatively high yields (around 9–15 %). These methods also reduce the risk of thermally degraded tannins and improve the extraction efficiency, especially when using optimized solvent systems. However, industrial-scale adoption is limited due to the steep initial investment, sophisticated equipment, and high maintenance costs for ultrasonic baths and microwave reactors. Supercritical fluid extraction (SFE), albeit promising in terms of selectivity and environmental impact, showed highly variable results, ranging from only 0.21–0.67 % for *Punica granatum* leaves to 29 % for *Carya illinoensis* shells, underscoring the importance of species-specific optimization as well as the limited solubility of some tannins in CO₂ under standard conditions.

Overall, SLE remains the most commonly used method thanks to its accessibility and scalability. At the same time, advanced methods such as MAE and UAE offer compelling alternatives for improving the yield and extraction speed. Importantly, most of the studies listed in Table 2 were conducted at laboratory scale, with limited data available on upscaling to pilot or industrial reactors. Future studies should attempt to optimize promising tannin extraction methods at larger scale in order to promote commercial applications.

4.2.8. Fermentation for enhanced tannin extraction

Fermentation is an emerging biotechnological strategy to improve the extraction and transformation of bioactive compounds from plant-based by-products (Siddiqui et al., 2023). For tannin-rich biomass such as bark, fermentation can be applied as a complementary pre-treatment to conventional extraction methods (Maria Fraga-Corral et al., 2021). Microorganisms used in fermentation such as *Aspergillus niger*, *Lactobacillus plantarum*, and *Saccharomyces cerevisiae* produce enzymes such as tannase, cellulase, and xylanase, which enhance the degradation of the lignocellulosic structure of the biomass (Garza-Cadena et al., 2023; Siddiqui et al., 2023). This enzymatic activity facilitates the release of tightly bound polyphenolic compounds, including hydrolyzable and condensed tannins, thereby increasing the extraction yield and improving the accessibility of functional ingredients (Bhat et al., 1998; Cirkovic Velickovic and Stanic-Vucinic, 2018; Siddiqui et al., 2023).

In addition, fermentation can transform the chemical structure of tannins to improve the solubility, stability, and bioactivity. According to Siddiqui et al. (2023), microbial fermentation contributes to convert underutilized plant materials such as apple pomace and sugarcane bagasse into enriched feedstock by generating novel bioactive metabolites. These include antioxidant compounds, peptides, and organic acids, with potential applications in food processing, cosmetics, pharmaceuticals, and biomaterials. For example, fermentation can depolymerize complex tannins by releasing bound phenolics and breaking them down into smaller, more bioavailable molecules, although it can also reduce tannin's astringent characteristics, and the astringent capacity is particularly desirable for cosmetic, food, pharmaceutical, and nutraceutical applications (Siddiqui et al., 2023). In sum, fermentation pre-treatment enables recovering high value-added compounds without the need for toxic solvents (Siddiqui et al., 2023).

4.2.9. Constraints and considerations in tannin extraction

Tannin is a valuable biopolymer with multiple applications across industrial, scientific, and environmental fields. However, its extraction from lignocellulosic biomass such as bark presents multiple challenges that must be addressed to ensure efficient recovery, purity, and bioactivity of the final extracts (Şen et al., 2023). These challenges include the complex structure of the plant matrix, particularly in barks, where tannins are often closely associated with cell wall components such as lignin, cellulose, and hemicellulose (Kemppainen, 2015). These interactions significantly hinder tannin solubilization, reduce extraction efficiency, and frequently lead to the co-extraction of non-phenolic impurities, including sugars, organic acids, and other polyphenolic compounds that may dilute the biological activity of the final product (Das et al., 2020; Zhu et al., 2019).

Tannins vary in terms of heterogeneity (i.e., structural diversity) and chemical reactivity. These variations may affect both process standardization and cross-study comparison, especially when the extraction method is not tailored for a specific tannin type or targeted application. This precludes the use of a single, consistent extraction process. Thus, unless the extraction process is designed for a particular tannin type or its intended use, the results may be inconsistent. For example, hydrolyzable tannins are relatively easy to solubilize but prone to hydrolytic degradation (Bhat et al., 1998), while condensed tannins (proanthocyanidins) are highly polymerized and resistant to hydrolysis, thermal processing, and enzymatic attack (Bule et al., 2020). Moreover, the extraction yield and purity of tannins vary widely depending on factors such as the plant source and part, solvent type, temperature, and extraction time. Inappropriate control of these parameters often leads to co-extraction of undesired compounds (e.g., carbohydrates or hemicellulose fragments), which may impact tannin purity and properties (Fraga-Corral et al., 2020; Pizzi et al., 2024a).

Raw plant materials often require pretreatments such as drying, grinding, and removal of interfering substances to enhance the extraction efficiency and selectivity. Pretreatments can mitigate tannin degradation and polymerization during extraction. However, this extra step increases the process complexity and costs and introduces the requirement to optimize the biomass source (Ding et al., 2017; Pizzi et al., 2024a). While advanced methods like UAE, MAE, and supercritical CO₂ extraction offer excellent yields at laboratory scale, they are often limited by high capital investment and complex equipment upscaling. For example, supercritical CO₂ extraction involves co-solvents and high pressure (Pizzi et al., 2024a). Although solid-liquid extraction remains the most scalable method, it is less selective and may require longer processing times and chemical additives (Guo et al., 2011; Imamoglu, 2024; Markom et al., 2010; Vo et al., 2023). Extraction methods involving heating or alkaline solvents may cause tannin degradation, internal rearrangements, or self-condensation, whereby insoluble precipitates form that reduce extract quality. Moreover, extracts often contain impurities that affect the biological activity, and consequently the applicability to pharmaceutical and nutraceutical industries. Therefore, post-extraction purification and adequate biological property assessments are required to ensure functional efficacy (Ding et al., 2017).

To overcome these limitations, pretreatment steps are essential. Drying is necessary to reduce moisture content, enhance grinding efficiency, and stabilize tannins prior to extraction. Freeze-drying (lyophilization) is particularly effective for preserving hydrolyzable tannins, while oven drying at mild temperatures may be suitable for more thermally stable condensed tannins (Salminen, 2003). Grinding and sieving increase the surface area and promote solvent penetration, especially when targeting small particle sizes (< 1 mm), as demonstrated by Abilleira et al. (2021) and Sealy-Fisher and Pizzi (1992). Indeed, dried biomass with low moisture (< 10 %) leads to higher tannin yields compared to fresh samples (Abilleira et al., 2021; Sealy-Fisher and Pizzi, 1992). In addition to physical pretreatments, biological and enzymatic approaches are emerging as powerful tools. As highlighted by Siddiqui et al. (2023), microbial fermentation using strains such as *Aspergillus niger*, *Lactobacillus plantarum*, and *Saccharomyces cerevisiae* can be applied as biopretreatment to degrade lignocellulosic barriers and release bound phenolics (Siddiqui et al., 2023). Enzymes such as tannase, cellulase, and xylanase facilitate matrix breakdown and may also induce partial depolymerization of complex tannins, thereby improving their bioavailability and functional performance (Garza-Cadena et al., 2023). Nevertheless, fermentation effectiveness depends on multiple variables, including strain selection, substrate composition, process duration, pH, and aeration, all of which require optimization and cost-benefit analysis before industrial application (Wijeyekoon et al., 2021). Furthermore, a critical but often overlooked consideration is that the extraction yield is not always correlated with biological activity. Extracts with high yield but poor selectivity may be rich in inert

carbohydrates or degraded polyphenols. Thus, it is essential to conduct a post-extraction assessment of the bioactivity. Antioxidant assays, antimicrobial tests, and enzyme inhibition studies should be integrated into extraction protocols to ensure the relevance of extracted tannins and to diversify their applications across food, cosmetic, pharmaceutical, and biomaterial industries (Pizzi, 2021).

5. Tannin purification and characterization methods

5.1. Tannin purification methods

Once the tannins are extracted, one or more purification steps are generally performed to obtain a more purified and potentially more effective product. Recently, interest has grown in membrane filtration systems, using porous membranes under applied pressure to separate specific components from a liquid (Conidi et al., 2018) and hence extract the desired compounds (Cravotto et al., 2022). The purification steps vary depending on the extraction method and the desired characteristics of the end product. After tannin extraction using MAE, the purification steps are ultrafiltration (UF), diafiltration (DF), and nanofiltration (NF) (Aimone et al., 2023). The post-extraction solution is subjected to ultrafiltration using a membrane with a cut-off of 1000 Da. This concentrates the tannins by retaining larger molecules while allowing smaller ones, such as gallic and ellagic acids, to pass through the membrane. Diafiltration, or membrane-based separation, removes more small molecules from the retentate solution to further concentrate the tannin-rich fraction. Fresh water is added to improve the separation of the compounds. Two different tests can be performed to assess the impact of the amount of water used: single diafiltration and triple diafiltration. The goal is to maximize the concentration of tannins in the retentate. Subsequently, nanofiltration, a pressure-driven membrane filtration process with a separation capability between that for ultrafiltration and for reverse osmosis, is performed to concentrate the remaining metabolites and recover the water, which can be reused in the process. After this triple diafiltration, the obtained retentate fraction is considerably enriched with tannins to reach almost 100 % tannin concentration. The resulting permeate is much more diluted, as the tannins are effectively separated from the other compounds (Aimone et al., 2023).

Membrane technologies offer numerous advantages, including lower energy and chemical consumption, operational flexibility, ease of control, and relatively low operating and maintenance costs. They also function at low temperatures, with the option to incorporate a cooling system (Conidi et al., 2015). They are well-suited for scale-up and can be effectively applied in large-scale operations (de Santana Magalhães et al., 2019; Oriez et al., 2019). Despite their advantages, however, membrane processes have limitations as well, including membrane fouling, high initial costs, limited lifespan, and sensitivity to operating conditions. Furthermore, scaling up requires technical expertise (Conidi et al., 2015; de Santana Magalhães et al., 2019; Oriez et al., 2019).

These purification steps are indispensable for increasing the purity and concentration of tannins for industrial purposes such as the production of dietary supplements, cosmetics, and additives for the leather and wood glue industry.

5.2. Tannin characterization methods

To analyze and optimize the properties of extracted molecules, a range of advanced analytical characterization methods are used, as described in the next paragraphs. Table 4 presents the main analytical methods along with their application fields and specific analysis conditions.

Gel permeation chromatography (GPC) is used to determine the molecular size and distribution of extracted tannins, providing essential information about their molecular weight and polydispersity index (Guo et al., 2024).

Reversed-phase high-performance liquid chromatography (HPLC-

Table 4
Analytical methods for tannin characterization.

Method	Instrument/Method setup	Experimental parameters	Main identification results	References
MALDI-TOF MS	KRATOS Kompact MALDI 4, UV laser at 337 nm (nitrogen laser) matrix: 2,5-Dihydroxybenzoic acid (2,5-DHB) 20 kV acceleration voltage	Linear and reflectron mode, positive ion detection ($[M+H]^+$, $[M+Na]^+$), mass range: 500–500,000 Da high vacuum ($\sim 10^{-6}$ mbar)	Determination of polymer molecular weights, measurement of molecular weight distribution (polydispersity), detection of sodium and potassium adducts	(Pasch et al., 2001)
	KRATOS Kompact MALDI 4, pulsed nitrogen laser (337 nm), positive ion mode, delayed extraction (200–800 ns), linear flight path, 20 kV acceleration	Maritime pine tannin (4 mg/mL in acetone), matrix 2,5-DHB (10 mg/mL), NaCl for ionization, dried droplet method, 0.5–1 μ L spotted	Oligomers of catechin, epicatechin, epigallocatechin, epicatechin gallate; up to 20–21 repeating units, discovery of new repeating units (264 Da), procyanidin-type structures confirmed	(Navarrete et al., 2010)
	Bruker UltrafleXtreme, positive reflector mode, pulsed nitrogen laser (337 nm)	<i>Pinus brutia</i> bark extract (4 mg/mL in acetone), matrix 2,5-DHB (10 mg/mL), NaCl added; 0.5–1 μ L spotted and dried on target	Flavonoid monomers and hexamer peaks (~ 1750 Da), predominance of procyanidins; presence of taxifolin, minor prodelphinidins; C4–C6 and C4–C8 linkages	(Ucar et al., 2013)
	MALDI-TOF mass spectrometer	10 mg of tannin dissolved in 2 mL of acetone, matrix: 2,5-dihydroxybenzoic acid and NaCl	Tannin oligomers detected \rightarrow monomers, dimers, trimers (288 Da spacing), A-type linkages, polymerization patterns	(Mewoli et al., 2023)
GPC	PLgel column (7.8 \times 300 mm Waters) NaNO ₃ solution 0.1 mol·L ⁻¹ mobile phase	Volume of on-target solution: 0.5 μ L 5 mg/mL tannin extract in acetone, matrices: 2,5-dihydroxybenzoic (10 mg/mL) added NaCl to accelerate ion formation	Monomers and oligomers identified \rightarrow catechin, galocatechin, fisetinidin (MW: 208.25–306 Da), structural composition analyzed	(Njom et al., 2024)
	ACE C18-PFP reversed-phase column (150 \times 4.6 mm, 3 μ m particle size), detection at 280 nm (DAD), excitation at 280 nm, emission at 320 nm (FLD)	Sample: 20 mg/mL in methanol, matrix: 20 mg/mL acid 2,5-dihydroxybenzoic (DHB) analysis mode: positive ionization	Molecular composition and polymerization degree of tannins characterized	(Jiang et al., 2020)
HPLC - RP	RP-HPLC-Purospher STAR Hibar, HR RP18 (150 mm \times 2.1 mm, 3 μ m), UV-visible detection (240–600 nm)	Flow rate 0.8 mL/min, injected volume 50 μ L	Molecular weight: 433–1375 Da, polydispersity index (PDI) 1.001–1.044	(Guo et al., 2024)
	RP-HPLC - C18 Symmetry (4.6 \times 250 mm)	Phloroglucinolysis reaction reagents: phloroglucinol 100 g/L in methanol with 0.2 M hydrochloric acid and 20 g/L ascorbic acid	Identification and quantification of proanthocyanidin cleavage products	(Wimalasiri et al., 2024)
	FTIR spectrometer	50°C for 20 min, flow rate 0.8 mL/min	Condensed tannins detected, key peaks: (-)-epicatechin, (+)-catechin, DPn = 11.4	(Yu et al., 2023)
FTIR	FTIR spectrometer	Phloroglucinolysis reaction reagents: phloroglucinol (75 g/L), ascorbic acid (15 g/L), HCl 0.3 M	Condensed tannins detected, (-)-epicatechin, (+)-catechin, DPn = 11.4	(Jiang et al., 2020)
		temperature: 50°C for 30 min	Key peaks: 3213 cm ⁻¹ (phenolic OH), 1710 cm ⁻¹ (C=O), polyphenolic structure	(Guo et al., 2024)
		mobile phase: formic acid 0.1 % (A) and acidified acetonitrile 0.1 % (B)	Key features: phenolic OH, aromatic rings, ester linkages \rightarrow condensed and hydrolyzable tannins confirmed	(Mewoli et al., 2023)
		flow rate 0.2 mL/min	Key groups: phenolic OH, ester C-O \rightarrow tannin monomers and oligomers (galocatechin gallate, radicinin)	(Fatema-Tuj-Zohra et al., 2023)
13C NMR	13C spectrometer	Mobile phase: 0.1 % (v/v) trifluoroacetic acid (A) and acetonitrile (B)	Condensed and hydrolyzable tannins, key peaks: 1700 cm ⁻¹ , 1608 cm ⁻¹ , 1518 cm ⁻¹ \rightarrow flavanols, aromatic rings	(Njom et al., 2024)
		gradient: 10–30 % B (0–30 min), 30–10 % B (30–35 min),	Condensed and hydrolyzable tannins, key peaks: 1700 cm ⁻¹ (C=O), 1608 and 1559 cm ⁻¹ (C=C), 1518 cm ⁻¹ (aromatic C-O) \rightarrow flavanols and catechins confirmed	(Hayat et al., 2020)
		temperature: 25°C	Flavonoid structures, polymerization degree, linkages \rightarrow relevant for industrial applications	(Pizzi et al., 2024b)
13C NMR	13C spectrometer	flow rate: 1 mL/min	Ester carbonyl groups (168.14–165.94 ppm) \rightarrow hydrolyzable tannins confirmed, molecular structure analyzed	(Guo et al., 2024)
		Scanning range: 400–4000 cm ⁻¹	Tannin structure: flavonoid units, carbon linkages, polymerization degree \rightarrow industrial relevance	(Pizzi et al., 2024b)
		resolution: 4 cm ⁻¹		
		number of scans: 32		
		Scanning range: 500–4000 cm ⁻¹ , resolution: 4 cm ⁻¹		
		Scanning range (scans): 600–4000 cm ⁻¹		
		Scanning range (scans): 4000–600 cm ⁻¹		
		Scanning range (scans): 8300–350 cm ⁻¹ resolution: 0.5 cm ⁻¹		
		3 repetitions		
1H NMR	1H spectrometer	Phenolic hydroxyl signals: ~ 9.36 ppm		
		Carbon signals of the polyol structure: 61.63–75.62 ppm		
		Ester carbonyl group signals: 165.94–168.14 ppm		
		C6': 120–121 ppm		
		C5' and C2': 115–117 ppm		
		C9: ~ 155 ppm		

(continued on next page)

Table 4 (continued)

Method	Instrument/Method setup	Experimental parameters	Main identification results	References
³¹ P NMR	³¹ P NMR spectrometer	Carbohydrates and oligomers: 60–90 ppm, C4-C8 interflavonoid bonds: intense peak, predominant in procyanidin-like tannins, C4 non-reactive site: 27–28 ppm, C5 and C7 sites: 156–157 ppm Phosphorylation with 2-Chloro–4,4,5,5-tetramethyl–1,3,2-dioxaphospholane, assignment of signals depending on the specific environments of the –OH groups Degradation temperature: 95–110 °C (peak), 250–270 °C (second peak)	Phosphorylated tannins analyzed, hydroxyl groups quantified → reactivity and industrial use (resins, adhesives) Moisture loss and breakdown of structural components	(Pizzi et al., 2024b) (Pizzi et al., 2024b)
	Thermogravimetric analyzer	20 ± 1 mg fiber sample temperature: 25–600 °C heating rate: 20 K/min 10 mg sticky mixture temperature: 20–900 °C heating rate: 5 °C/min	Thermal stability, weight loss, degradation behavior → composition and thermal properties analyzed Tannin resins: thermal degradation (204–305 °C), complete decomposition (>344 °C)	(Pizzi et al., 2024b)
TGA/DTG			Total phenolic content quantified → FC method more accurate, especially with sugar interference	(Njom et al., 2024)
DSC	Differential scanning calorimeter	Temperature range: 20–250 °C	Viscoelastic properties characterized → glass transition temperature (Tg) and modulus of elasticity	(Pizzi et al., 2024b)
	Dynamic thermomechanical analyzer	Temperature: 20–200 °C, heating rate: 10 °C/min	Viscoelastic properties, thermal stability, curing behavior → performance under temperature variations	(Pizzi et al., 2024b)
DTMA		Temperature: 25–250 °C, heating rate: 10 °C/min	Tannin resin MOE: 3825 MPa → thermomechanical properties	(Mewoli et al., 2023)
Folin-Denis (FD) and Folin-Ciocalteu (FC) methods	UV-Vis spectrophotometer	Absorbance at λ = 760 nm, precipitation of polyphenols with polyvinylpyrrolidone Water-soluble tannins, viscosity tuned in various protic and aprotic solvents	Thermal behavior evaluated → glass transition temperature (Tg) and mechanical property changes with temperature Tannin solubility analyzed → impact on processing, performance, and industrial applications (adhesives, bio-based materials)	(Pizzi et al., 2024b)
Solubility	Solubility testing apparatus or viscometer	45 %–50 % tannin solution in water with 8 % paraformaldehyde powder prorobinetinidin/profisetinidin tannins: 110–150 s procyanidin/prodelphinidin tannins: 35–50 s	Determination of liquid-to-gel transition time for tannin-based resins Tannin resin gel time varies with pH → shorter at higher pH (FAH: 300 s, FAV: 440 s at pH 10.4–11)	(Pizzi et al., 2024b)
Gelling time	Gelling time measurement setup	temperature: 100 °C, pH adjustment with 33 % NaOH solution		(Mewoli et al., 2023)

RP) is used to identify and quantify proanthocyanidin cleavage products, enabling a detailed characterization of tannins following acid catalysis. This method also helps identify key compounds such as procyanidins, (-)-epicatechin, and (+)-catechin while determining their degree of polymerization (Jiang et al., 2020; Wimalasiri et al., 2024; Yu et al., 2023).

Fourier transform infrared spectroscopy (FTIR) is used for structural identification. It differentiates between hydrolyzable and condensed tannins by detecting characteristic molecular vibrations of phenolic hydroxyl and ester linkages (Fatema-Tuj-Zohra et al., 2023; Guo et al., 2024; Mewoli et al., 2023; Njom et al., 2024).

Nuclear magnetic resonance (NMR) spectroscopy further refines this characterization. ¹H NMR detects the presence of phenolic hydroxyl groups (Pizzi et al., 2024b), and ¹³C NMR characterizes the properties of polyols and ester groups and the degree of polymerization (Guo et al., 2024). Additionally, ³¹P NMR quantifies aliphatic and aromatic hydroxyl groups, providing valuable data for synthesizing highly reactive tannin-based polymers (Pizzi et al., 2024b).

To assess the thermal behavior of tannins, thermogravimetric analysis (TGA) is used to monitor sample degradation as a function of temperature, revealing differences in thermal stability based on extract purity (Pizzi et al., 2024b). Folin-Denis (FD) and Folin-Ciocalteu (FC)

biochemical analyses are widely used to estimate polyphenol content. These methods eliminate interference from non-phenolic compounds to ensure accurate quantification of total phenolic content by spectrophotometry (Pizzi et al., 2024b). Another essential factor for tannin applications is solubility, which determines their suitability for the production of polymeric materials such as adhesives and foams (Pizzi et al., 2024b). Optimal industrial processing necessitates a thorough understanding of how tannins dissolve in different solvents. The gelling time must also be measured to characterize the condensed tannin reactivity, which affects the suitability of tannins for resin manufacturing (Pizzi et al., 2024b).

Finally, UV spectroscopy and MALDI-TF spectrometry are powerful tools for quantifying tannins and identifying oligomeric structures. These methods enable precise characterization of tannin monomers, dimers, and trimers while providing insights into the polymerization degree (Hayat et al., 2020; Jiang et al., 2020; Mewoli et al., 2023; Yu et al., 2023). UV spectrophotometry has been demonstrated a particularly reliable and accessible method for quantifying total phenolic content in both condensed and hydrolyzable tannins. Antoine et al. (2003); (2004) demonstrated that UV absorbance at 280 nm correlates well with phenolic concentration, showing linear relationships in narrow concentration ranges and exponential correlations over broader

ranges. The authors demonstrated that this method not only simplifies routine analysis, it also outperforms traditional titration methods, such as the Divergan method, by capturing low-molecular-weight phenolic compounds, monomers, and dimers that are otherwise undetectable in tanning-specific assays. As such, UV spectrophotometry provides a broader and more accurate assessment of the phenolic profile, enabling applications beyond leather tanning, including biomaterials, pharmaceuticals, and food products.

Table 4 presents various analytical methods used to characterize key properties of tannins. Molecular weight and polydispersity, determined by GPC and MALDI-ToF, influence solubility, chemical reactivity, and performance in industrial applications. The monomer composition, assessed by HPLC and MALDI-ToF, helps identify the tannin types and predict their antioxidant potential and reactivity. FTIR and NMR reveal the presence of functional groups such as hydroxyl and carbonyl groups, which largely determine the chemical behavior of tannins, particularly in resin and adhesive formulations. Thermal analysis methods (TGA, DSC, DMTA) provide information on the thermal stability, glass transition temperature, and mechanical performance of tannin-based materials. Differential scanning calorimetry (DSC) further examines thermal transitions in tannin-based polymers, particularly in curing reactions with formaldehyde. To complement these analyses, dynamic mechanical thermal analysis (DMTA) provides critical information on the viscoelastic properties of tannin adhesives to assess the response to thermal stress, the elasticity modulus, and overall performance compared to synthetic adhesives (Pizzi et al., 2024b).

Importantly, solubility, viscosity, pH, and gelling time must be measured to assess tannin behavior during formulation and curing under different conditions. Lastly, the total phenolic content, typically measured by colorimetry, is a meaningful indicator of antioxidant activity and overall extract quality.

6. Chemical reactivity

Due to their complex structure and the presence of multiple functional groups, tannins present a wide range of chemical reactivities. The hydrophobic nature of their aromatic rings facilitates π - π stacking interactions, enabling them to effectively associate with various aromatic molecules (Watrelet and Norton, 2020). In parallel, the numerous hydroxyl groups confer hydrophilicity and high reactivity, allowing the formation of hydrogen bonds, where oxygen atoms act as acceptors while hydrogen atoms act as donors. These hydroxyl groups are also involved in metal chelation, leading to the formation of stable complexes with different metal ions (Watrelet and Norton, 2020). Additionally, tannins are prone to hydrolysis, condensation, or substitution reactions with ammonia and acetylations, resulting in modifications of their chemical and biological properties. Furthermore, the nucleophilic sites present in their structure allow reactions with aldehydes, leading to the formation of new compounds with a wide range of potential applications (Watrelet and Norton, 2020).

Tannins have a strong affinity for binding to proteins through various interaction mechanisms. Covalent bonding can occur through the oxidation of tannins to quinones or semi-quinones, forming stable bonds with amino acids such as lysine and cysteine.

The chemical reactivity of tannins, particularly condensed tannins extracted from mimosa (*Acacia mearnsii*), has been investigated in terms of interactions with proteins, especially collagen used in leather tanning. According to (Pizzi, 2021), these interactions may involve both ionic and covalent bonds, where the proportions vary significantly depending on temperature. At room temperature, only a small number of covalent and ionic linkages are formed between the flavonoid units of the tannin (mainly robinetinidin, followed by fisetinidin, catechin, and gallo catechin) and collagen amino acids such as arginine, aspartic acid, and glutamic acid. However, at 80°C, ionic bonds disappear entirely and are replaced by a much higher number of covalent bonds. These include esterification between the -COOH groups of amino acids and the

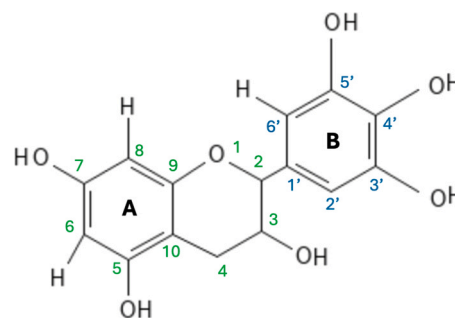


Fig. 7. Chemical reactivity of hydroxyl groups in flavan-3-ol units for metal chelation.

aliphatic -OH group at the C3 position of the flavonoid, as well as amination reactions between the phenolic -OH groups of tannins and the amino groups of the amino acids. Although this increase in covalent cross-linking contributes to the enhanced mechanical stability of the material, it may also promote thermal shrinkage of leather by tightening the tannin-protein matrix. These findings were confirmed by MALDI-ToF mass spectrometry, which identified a wide variety of reaction products, some exceeding 1800 Da in molecular weight, reflecting the structural complexity of tannin-protein networks formed at elevated temperatures (Pizzi, 2021).

Alternatively, non-covalent interactions, including hydrogen bonding and hydrophobic interactions, may also occur. These diverse bonding mechanisms promote the protein-tannin interactions, underscoring the importance of the chemical conditions that govern the nature of tannin-protein interactions (Sekowski et al., 2014). Protein-tannin interactions may also be limited by the interference of other molecules, such as polysaccharides (e.g., gum arabic, pectin, gellan, polygalacturonic acid, and xanthan), which compete with proteins for tannin binding (Sekowski et al., 2014).

These interactions between tannins and polysaccharides are commonly exploited in the beverage industry, notably to improve the quality of wine (Brandão et al., 2017). These non-covalent interactions, influenced by factors such as temperature and ionic strength, act to clarify the wine and reduce the astringency. They may also help people manage their blood sugar by reducing the digestion rate of polysaccharides. Such combinations are currently being studied to produce nutraceuticals, where microencapsulation, for example with pectin, improves the protection and effectiveness of the active compounds (Brandão et al., 2017).

Tannins interact with lipids mainly through hydrophobic interactions, hydrogen bonds, and electrostatic forces, thereby influencing the properties of cell membranes (Karonen, 2022). These interactions can affect cellular processes such as signaling, transport, and membrane stability. In addition, tannins inhibit lipid peroxidation, reducing oxidative stress and protecting lipid-rich foods from oxidation. They also modulate lipid metabolism by reducing lipid absorption in the gut and influencing gene expression and enzyme activity related to lipid metabolism, making them potentially useful for managing metabolic disorders (Karonen, 2022).

Tannins are widely recognized for their ability to chelate metals. Accordingly, they are used to absorb metals, particularly in water and wastewater treatments, and to recover valuable metals from fluids. In the chelation process, the ortho-dihydroxy groups on the catechol ring of tannins (at the 3'-4' positions, see Fig. 7) are the primary phenolic hydroxyl groups involved, enabling catechol-like coordination with metals. Although metal chelation occurs with all classes of tannins, condensed tannins are more likely than hydrolyzable tannins to form complexes with metals due to their higher chemical reactivity (Koopmann et al., 2020).

Condensed tannins demonstrate significant chemical reactivity owing to the presence of ortho-dihydroxyl (o-diphenol) groups on the B-

ring of their flavonoid structure, which provide efficient chelation sites for diverse metal ions. The interactions are affected by both the pH and the molecule structure, which can facilitate the development of stable complexes with various metals. Among the most studied is iron (III), which forms colored complexes with stoichiometries that vary with pH, resulting in green (1:1), blue (1:2), or red (1:3) solutions. Pyrogallol-type ligands, such as those found in black wattle tannins, complicate complexation due to additional vicinal hydroxyl groups, but remain valuable for colorimetric analysis and corrosion inhibition. Germanium (IV) creates colorless, tris-chelated complexes with tannins, exhibiting stability constants comparable to those of simpler ligands, whereas aluminum (III) generates successive complexes whose solubility is contingent upon ligand acidity and stoichiometry—attributes useful for vegetable tanning. Boron (III), commonly called boric acid, creates soluble, colorless complexes used in spectroscopic studies of flavonoids. Divalent metals, including Cu(II), Zn(II), Ni(II), and Co(II), interact similarly with tannins, with stability patterns following the Irving–Williams series, for potential applications in wood preservation and resin cross-linking. Supplementary metals such as Ti(IV), Mo(VI), W(VI), Ba(II), Ga(III), and Zr(IV) generate colored or precipitated complexes that enhance tannin use in leather dyeing and sophisticated analytical methodologies. Despite the polymeric characteristics of tannins, their *o*-diphenol groups exhibit consistent reactivity, providing a strong foundation for metal coordination in both traditional and novel applications (Slabbert, 1992).

Generally, tannins can act as both reducing and stabilizing agents to form metal chelation complexes with a wide range of metal ions. Additionally, the formation of tannin–metal complexes is strongly influenced by the initial pH value (Koopmann et al., 2020).

7. Conclusion

This review explores a variety of processes used for tannin extraction and subsequent characterization, along with a broad range of applications. The key takeaways are summarized as follows.

Tannins, which are naturally occurring polyphenols found in various plant sources, present substantial potential for sustainable use in various industries. Tannin can be extracted by many methods, including microwave-assisted extraction (MAE), ultrasonic-assisted extraction (UAE), solid-liquid extraction (SLE), and supercritical fluid extraction (SFE), each differing in terms of efficiency and cost. Of these, SFE stands out for its ability to preserve the tannin structure while minimizing solvent use, although it requires expensive equipment and involves higher operational costs.

Several factors influence the effectiveness of tannin extraction, including temperature, solvent choice, and plant material preparation. These parameters must be optimized in order to obtain high-quality tannins with desirable properties for target applications. After extraction, tannins can be characterized for their physicochemical properties, which vary depending on the extraction method. These properties render tannins valuable for diverse applications, including food preservation, pharmaceuticals, and biomaterials. Additionally, their bioactive potential has motivated explorations of new applications in advanced fields like 3D printing and biomedical devices.

Despite this promise, some challenges remain regarding the scalability and cost-effectiveness of certain extraction processes. Further research is also needed to harness the full potential of tannins in terms of environmental sustainability, where they could play a major role in improving industrial processes and reducing ecological footprints, among others. In sum, tannins extracted by optimized methods offer eco-friendly and sustainable alternatives to synthetic chemicals and could contribute to consequential innovations across multiple sectors. Future studies should focus on improving extraction methods to maximize yield and purity. They could also explore new applications for tannins in emerging industries such as green chemistry, renewable energy, and environmental remediation.

8. Perspectives

In future research, particular attention should be paid to the development of green extraction strategies using deep eutectic solvents (DES) and ionic liquids (ILs) (Cabezas et al., 2025; Castro-Muñoz, 2025b). These environmentally friendly solvents offer promising alternatives to conventional organic solvents thanks to their biodegradability, tunable polarity, low toxicity, and ability to form specific interactions with polyphenolic compounds such as tannins (Cabezas et al., 2025; Castro-Muñoz, 2025b). Recent studies have demonstrated the applicability of DES to extract heavy metals and antioxidants from complex matrices, and sponge-like ionic liquids have been used in selective purification processes using high-efficiency low-energy systems (LES). Such systems could be adapted to provide more sustainable and efficient selective extraction protocols (Castro-Muñoz, 2025b). Recent studies have also shown that DES can achieve higher extraction yields and better preservation of tannin bioactivities compared to conventional solvents, while recycling solvents and reducing the environmental burden (Ferreira and Sarraguça, 2024; Molnar et al., 2024b). Similarly, ionic liquids demonstrate excellent solvating ability and thermal stability, making them suitable for tailored extraction processes (Ventura et al., 2017). Despite certain challenges that remain to be resolved, including viscosity management, solvent recovery, and cost, these emerging solvent technologies offer strong prospects for more sustainable and scalable tannin extraction methods for industrial applications.

Beyond classical uses in leather tanning, recovered tannin fractions have shown strong potential for applications in the production of sustainable functional materials, and particularly biopolymer-based packaging. Recent studies have demonstrated that tannins can be incorporated into gelatin-chitosan edible films to enhance the antimicrobial and antioxidant properties and improve the mechanical resistance. These bio-based packaging solutions are biodegradable and eco-friendly alternatives to conventional plastics, particularly for preserving highly perishable food products like fish and seafood (Eranda et al., 2024).

In sum, efficient and sustainable tannin extraction calls for a holistic approach that combines appropriate biomass pretreatment, process optimization, and bioactivity assessment. These constraints must be addressed in order to bridge the gap between laboratory research and industrial-scale applications, and to fully exploit the potential of tannin-rich biomass as part of circular bioeconomy strategies to add significant value to agroindustrial byproducts.

CRedit authorship contribution statement

Gisele Amaral-Labat: Writing – review & editing, Validation. **Aziz Bentis:** Writing – review & editing, Validation, Methodology, Investigation, Conceptualization. **Clément Lacoste:** Writing – review & editing, Validation, Supervision. **Antonio Pizzi:** Writing – review & editing, Writing – original draft, Validation. **Emna Ben Abda:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Ahmed Koubaa:** Supervision, Resources. **Braghiroli Flavia:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Flavia Braghiroli reports financial support, administrative support, equipment, drugs, or supplies, and writing assistance were provided by Natural Sciences and Engineering Research Council of Canada (NSERC). If there are other authors, they 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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Data availability

No data was used for the research described in the article.

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