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Characterization of Commercial Hydrolyzable and Condensed Tannins by Fourier Transform Infrared Spectroscopy

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Abstract

This study uses Fourier transform infrared spectroscopy to characterize and differentiate four major commercial tannin extracts (quebracho colorado, mimosa, tara, and chestnut) to establish a robust quality control framework for their industrial utilization in bio-based materials and green chemistry applications. While all tannins exhibited common O–H and aromatic C=C–C stretching bands, specific spectral regions allowed for clear discrimination without time-consuming wet-chemical assays. Condensed tannins (quebracho and mimosa) showed an intense C–O ether linkage peak (1240-1285 cm^{-1}), absent in hydrolyzable extracts. Conversely, hydrolyzable tannins (tara and chestnut) were distinctly identified by a prominent ester carbonyl C=O band (1705–1735 cm^{-1}). Further differentiation revealed gallotannin-specific markers in tara (e.g., 1090, 870, 755 cm^{-1}) that were notably weak or absent in chestnut, corroborating chestnut's predominant composition of ellagitannins. This systematically indexed FTIR database provides a rapid, non-destructive, and reliable screening tool for the classification, authentication, and standardization of diverse commercial tannin sources, streamlining their selection and processing as high-value technological inputs in sustainable formulations.

Keywords

characterization, condensed tannins, FTIR spectroscopy, hydrolyzable tannins, tannins

1 Introduction

1.1 Definition and classification of tannins

The definition of substances known as tannins has evolved over time, with different authors proposing varied interpretations. However, generally speaking, they can be defined as secondary polyphenolic metabolites of higher plants with immense structural diversity and variable molecular masses (ranging from 300 to 20,000 Da). They are typically water-soluble and possess the property of irreversibly binding to proteins, leading to their precipitation [1–4]. The primary role of these biomolecules in plants is to provide protection against insects, fungi, and bacteria [1, 5]. While some authors propose more categories, tannins can broadly be classified into two main groups: hydrolyzable tannins and condensed tannins [1, 2, 6–9].

1.1.1 Hydrolyzable tannins

Hydrolyzable tannins contain hydrolyzable ester linkages between their constituent molecules. This means they can be hydrolytically fractionated into their components, for example, under acidic or basic conditions, or by enzymes called tannases [2–4, 8]. Hydrolyzable tannins can be further divided into gallotannins and ellagitannins [2, 4, 7].

Gallotannins are the simplest hydrolyzable tannins. They form when gallic acid units (3,4,5-trihydroxybenzoic acid) esterify the hydroxyl groups of a polyol residue (Fig. 1) [2, 4, 10, 11]. This polyol is commonly D-glucose, although other types of polyols, such as quinic acid in tara tannins, can also be present [1]. The polyol's hydroxyl groups can be partially or fully substituted with gallic acid units. Additional gallic acid units can then attach *via* a depside linkage (an ester linkage between monocyclic aromatic units) [7, 12]. If substitution is partial, the remaining hydroxyl groups may or may not be substituted with various other residues [2, 7, 11].

Ellagitannins are formed from gallotannins through the oxidative coupling of at least two gallic acid units [1, 2, 4, 10]. This joining of two gallic acid units *via* their aromatic

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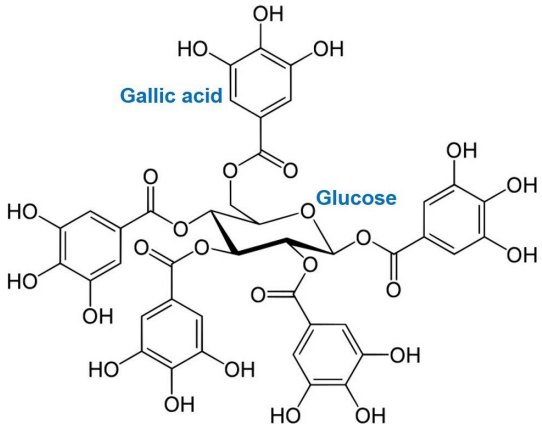

Fig. 1 Typical structure of gallotannins, characterized by a central polyhydroxy residue, typically *β*-D-glucopyranose, with its hydroxyl groups fully or partially esterified with gallic acid units. The compound shown, pentagalloylglucose, serves as the starting point for many complex tannin structures.

carbon atoms leads to the formation of an axially chiral hexahydroxydiphenoyl (HHDP) moiety, which is the characteristic structural element of ellagitannins (Fig. 2) [2, 7, 11]. The hydrolysis of ellagitannins yields ellagic acid due to the spontaneous lactonization or intramolecular esterification of the HHDP residues [4, 7, 13]. Ellagitannins can be further subdivided into structures containing open glucose cores and closed (cyclic) glucose cores [13].

Both gallotannins and ellagitannins encompass a large number of positional isomers and stereoisomers [13].

1.1.2 Condensed tannins

Condensed tannins are relatively resistant to hydrolytic degradation because their constituent monomers are connected by C–C linkages [2, 3]. Chemically, they

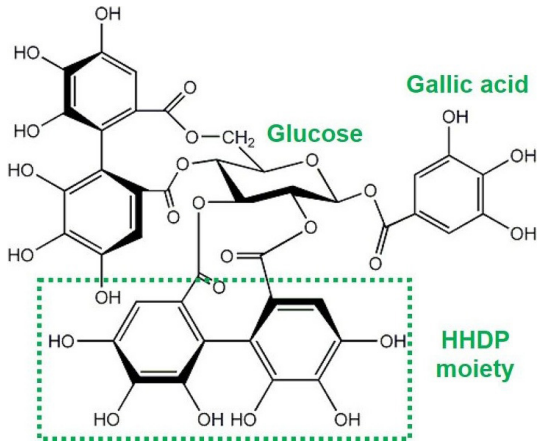

Fig. 2 Typical structure of ellagitannins, analogous to that of gallotannins, but featuring the linkage of at least two gallic acid units via their aromatic carbon atoms, leading to the formation of the characteristic axially chiral HHDP structural element.

are flavonoid derivatives known as proanthocyanidins, formed by the polymerization of flavan-3-ol units [2, 4, 5, 10, 14]. Each repeating flavan-3-ol unit features three rings designated A, C, and B (Fig. 3), with a systematic numbering of the carbon atoms [4, 15]. The phenolic A and B rings exhibit different reactivities [16]. A rings act as highly reactive nucleophiles, while B rings provide antioxidant properties and excellent sites for complex formation with metals and other biopolymers [11, 17, 18].

The structural variability of proanthocyanidins is primarily observed in a well-defined variation in their hydroxylation pattern [19]. Differences in this pattern mainly occur at position 5 of the A ring and position 5' of the B ring of the flavan-3-ol unit (Fig. 3). Thus, condensed tannins feature four principal structural units, distinguished by the presence of –OH or –H groups at these positions (Fig. 3). This, in turn, allows for the subdivision of proanthocyanidins into four major subgroups [4, 7, 8, 11, 20, 21]:

- **Procyanidin-type tannins**, based on catechin (–OH at 5, –H at 5').
- **Prodelphinidin-type tannins**, with gallocatechin as their structural unit (–OH at 5, –OH at 5').
- **Profisetinidin-type tannins**, composed of fisetinidol monomeric units (–H at 5, –H at 5').
- **Prorobinetinidin-type tannins**, containing robinetinidol subunits (–H at 5, –OH at 5').

The condensed tannins industrially extracted are commonly of the profisetinidin and prorobinetinidin types [16].

From a biosynthetic perspective, condensed tannins are formed through the successive condensation of flavan-3-ol monomeric units, primarily *via* C-4→C-8 linkages in procyanidins and prodelphinidins, and C-4→C-6 linkages in profisetinidins and prorobinetinidins. They achieve a degree of polymerization ranging from two to over fifty units (Fig. 4) [2, 3, 7, 10, 11, 14, 19, 22]. Both the coupling pattern between units and their substitution pattern

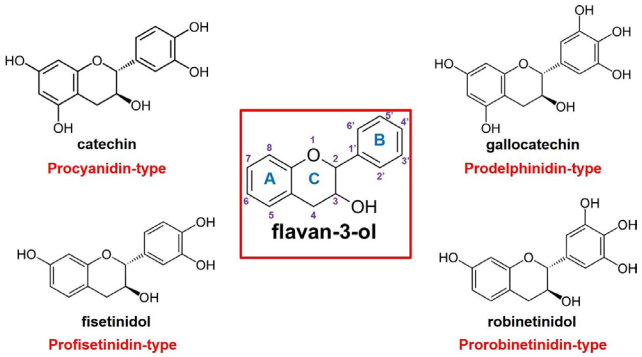

Fig. 3 Flavan-3-ol structural units of different types of condensed tannins

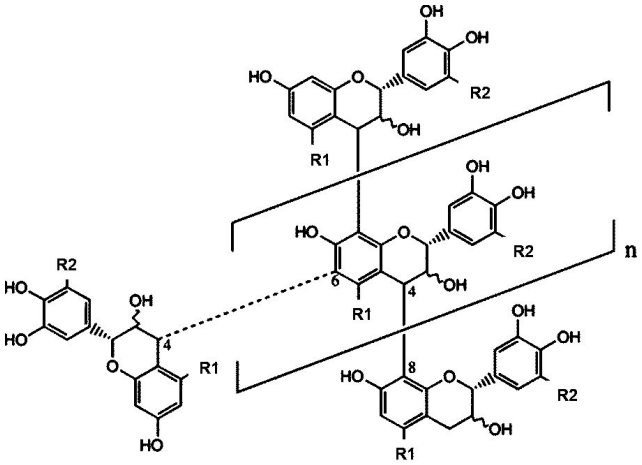

Fig. 4 Model structure for condensed tannins

can vary widely [2, 7]. In some tannins, the –OH group at position 3 of the C ring is esterified with gallic acid [14]. Furthermore, the presence of asymmetric centers in the monomeric units leads to a variety of stereoisomers [3].

1.2 Tannin extraction

Tannin extraction procedures are highly varied. Generally, tannins are extracted from plant materials (bark, wood, stems, leaves) using hot water or mixtures of water and organic solvents (e.g., acetone, methanol, ethyl acetate) [6]. Tannin extraction is more efficient with smaller plant material particle sizes, as this increases the surface area in contact with the solvent. Therefore, plant material is typically crushed, ground, or chipped before any extraction is performed [6, 8].

The hot water extraction method is the most popular among various procedures, both industrially and in the laboratory [4, 6, 11]. The method's simplicity and lower cost are the main reasons for its popularity. The use of hot pressurized water allows for reduced handling time and solvent consumption [7, 11]. For some tannins, such as those from quebracho colorado, small percentages of sulfite or sodium metabisulfite can be added to improve the solubility of high molecular weight oligomers [8, 23]. Tannin sulfitation, performed either during or after extraction, is one of the oldest and most valuable reactions in condensed tannin chemistry. This process yields tannins with reduced viscosity and increased cold-water solubility, thereby enhancing their ease of handling [23].

Industrial extraction typically yields a solution containing between 4% and 5% by weight of tannins, with an extraction efficiency of around 60–65% [11]. After clarification by decantation, the tannin solution is concentrated by evaporation to reach the desired concentration, usually between 40% and 50% by weight [11]. This operation is performed under vacuum to limit tannin oxidation. The concentrated solution can either be stored after adding a stabilizing agent, or it can be further processed. Most commonly, this involves reducing it to a dry powder (90–96% dry mass) through spray drying [11]. This technique involves atomizing the liquid raw material into tiny droplets, which then come into contact with hot air in a drying chamber.

Extracted tannin always contains various impurities, including minerals, stilbenes, and sugars [6]. The quantity of these impurities is influenced by several operational parameters, including particle size, temperature, pressure, extraction time, solvent type, and the solid-to-solvent ratio [6, 8]. Industrial hot-water extraction without sulfitation may co-extract non-phenolic carbohydrates, such as soluble oligosaccharides and polysaccharides, which can interact with tannins and influence the physicochemical properties of commercial extracts [9].

Among the different types of tannins, hydrolyzable tannins have limited natural sources compared to condensed tannins, leading the latter to dominate the global market [6]. According to Pizzi [18], the total annual global extraction of tannins exceeds 220,000 tons, with 90% corresponding to condensed tannins.

1.3 Main commercial tannins: sources and production

Condensed tannins are highly abundant in quebracho wood and in the barks of legumes (*Acacia* sp., *Lotus* sp., and *Sericea* sp.), conifers (*Tsuga* sp. and *Pinus* sp.), birches (*Betula* sp.), gambier (*Uncaria* sp.), and mangrove species (*Rhizophora* sp.) [6, 15, 18, 24]. This section details two significant commercial sources of condensed tannins: quebracho heartwood extract and mimosa (*Acacia mearnsii*) bark extract.

On the other hand, hydrolyzable tannins have a more limited distribution, comprising only a few dicotyledonous species. They are found in chestnut (*Castanea* sp.), myrobalans (*Terminalia* and *Phyllantus* sp.), *Caesalpinia* sp., oak (*Quercus* sp.), eucalyptus (*Eucalyptus* sp.), and *Myrica* sp. extracts [6, 8, 15, 24]. The most commonly used commercial hydrolyzable tannins are those extracted from tara pods and chestnut wood [11], which will also be analyzed in greater depth in this section.

1.3.1 Quebracho colorado tannins

Quebracho colorado is the common name for two different tree species from the Anacardiaceae family, *Schinopsis balansae* (quebracho colorado chaqueño) and *Schinopsis*

lorentzii (quebracho colorado santiagueño), both native to the Gran Chaco region of Argentina, Paraguay, and Bolivia [24]. Wild quebracho colorado forests have been exploited for over a century as a significant source of vegetable tannins and highly durable, robust wood [24]. Quebracho colorado tannins represent a complex mixture of polyphenols obtained by extraction from the tree's heartwood, the innermost and most resilient portion of the trunk [25]. To obtain the hot-water-soluble quebracho extract (also known as natural, common, or ordinary extract), the bark and sapwood are removed from the logs. The remaining heartwood is then chipped and subjected to direct extraction with boiling water [24]. This process induces the hydrolysis of heterocyclic rings, resulting in the formation of condensation products termed phlobaphenes or red tannins. These products are soluble in hot water but exhibit limited solubility in cold water [4, 26, 27].

To produce the widely recognized and utilized cold-water-soluble quebracho extracts (also termed sulfited extracts), it is necessary to convert these phlobaphenes into fully soluble tannins *via* a sulfitation process. This can be achieved by treating the hot-water-soluble extract with bisulfite, or by directly extracting the wood chips with a boiling aqueous bisulfite solution [24]. Subsequently, the liquid extracts are concentrated by evaporation. Finally, the concentrated viscous liquid undergoes spray drying to yield the powdered extracts.

Quebracho extracts typically comprise 95% tannins and 5% soluble sugars on a dry weight basis [24]. The tannins derived from quebracho heartwood are predominantly profisetinidin-type condensed tannins. Here, leucofisetinidin, a flavan-3,4-diol, serves as the precursor for the fisetinidol-type structural unit [27, 28]. According to Venter et al. [24], all oligomers feature catechin as the initiating unit and fisetinidol-type extending units. For instance, Fig. 5 illustrates a fisetinidol-catechin-fisetinidol trimer isolated from *S. balansae*. Venter et al. proposes

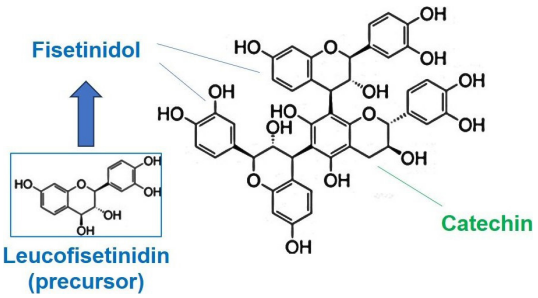

Fig. 5 Trimer isolated from *S. balansae*. The trimer is angular, with one fisetinidol-type unit at the C-8 position and another at the C-6 position of the catechin.

that this trimer functions as the sole intermediate in the formation of all higher oligomers [24, 29].

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis of commercial sulfited quebracho tannin extract, conducted by Pasch, demonstrated the presence of high molecular weight oligomers with low water solubility, such as heptamers, octamers, nonamers, and decamers [16]. Conversely, electrospray ionization mass spectrometry (ESI-MS) analysis of hot-water-soluble quebracho extract, performed by Venter, revealed the presence of 33% dimers, 37% trimers, 21% tetramers, and 8% pentamers, with larger oligomers present in small quantities [24, 29]. Venter et al. attributes the relatively scarce formation of pentamers and higher oligomers to the low reactivity of the fisetinidol A ring [24]. Furthermore, the absence of hydroxyl (OH) groups at position 5 on the fisetinidol-type units imparts a notable stability to the oligomer against acid hydrolysis of the interflavanoid bond [19, 24].

1.3.2 Black wattle or mimosa tannins

Acacia mearnsii, commonly known as black wattle or mimosa, is a fast-growing tree belonging to the legume family. Its bark contains large quantities of tannins, reaching up to 30% of its dry weight [30]. It's primarily distributed across southern Australia, where it's native, and southern Africa, where it was introduced in the late 19th century [29]. Subsequently, it has spread to other temperate and subtropical regions worldwide [31]. The main commercial plantations (primarily for wood, charcoal, and tannin production) are located in East and Southern Africa, Asia (India, China, and Vietnam), and southern Brazil [32, 33].

This tree possesses a remarkable ability to adapt to different climatic conditions and a high reproduction rate, both by seeds and resprouting. It also forms symbiotic associations with nitrogen-fixing bacteria and exhibits high biomass production, giving it a competitive advantage regarding space, light, water, and nutrients. For these reasons, *Acacia mearnsii* is considered a renewable and sustainable source of tannins [34]. However, its capacity to rapidly colonize vast areas of land also makes it an invasive species [31].

Mimosa extract is obtained from the bark of *Acacia mearnsii* trees cultivated on a large scale in commercial plantations [35]. It takes 7 to 10 years for the tree to grow sufficiently for harvesting and bark removal, which is cut and stripped under controlled conditions [36]. For every ton of bark extracted, black wattle generates almost 5 tons of heartwood, which is used in multiple applications, including firewood, charcoal, construction timber, posts,

and furniture [31]. Harvesting and extraction are seasonal and largely depend on rainfall patterns in the country where it is cultivated [36].

The tannins are extracted from the bark with hot water, using autoclaves and applying a countercurrent principle [34, 36]. The liquid extract is concentrated by evaporation, and subsequently, the concentrated viscous liquid is spray-dried to obtain the powdered mimosa extract. This powder typically contains 70–80% tannins and 5% moisture, while the remainder consists of hydrocolloid gums, sugars, and small molecules [30, 34–36].

MALDI-TOF analysis of commercial mimosa tannin extract, performed by Pasch, revealed that these tannins are predominantly of the prorobinetinidin type and exhibit a highly branched structure [16]. Subsequently, an ESI-MS study by Venter et al. determined that the oligomers are composed of an initiating unit, which can be catechin or gallocatechin, linked to extending units that, while possibly fisetinidol-type, are predominantly robinetinidol-type [29, 35]. According to Martinez [37], the approximate percentages of the four flavan-3-ol structural units present in mimosa tannins are: 56% robinetinidol, 24% fisetinidol, 20% gallocatechin, and 20% catechin.

The biogenesis of these tannins occurs through the condensation between catechin or gallocatechin and the extending units in their flavan-3,4-diol precursor state (robinetinidol-4α-ol and leucofisetinidin) [35]. The formation of interflavanoid linkages occurs preferentially first through the sterically less hindered and more nucleophilic C-8 position of the catechin or gallocatechin initiating units (Fig. 6) [35]. According to Venter et al.'s findings [35], the proanthocyanidin dimers from acacia extract consist of 42% robinetinidol-catechin, 39% robinetinidol-gallocatechin, 12% fisetinidol-catechin, and 7% fisetinidol-gallocatechin, confirming their predominant prorobinetinidin nature. The second flavan-3-ol extending unit is added at the vacant C-6 position of the initiating unit's A ring, leading to trimers with angular structures [29, 35]. The most abundant trimers are robinetinidol-catechin-robinetinidol (represented in Fig. 6), constituting 32%, and robinetinidol-gallocatechin-robinetinidol, at 27% [35]. The third and subsequent extending units are added to the less reactive A rings of the fisetinidol or robinetinidol units [35]. As with quebracho, the characteristic absence of 5-hydroxyl groups in the chain-extending units of acacia tannins imparts stability to the interflavanoid linkage against acid hydrolysis [35].

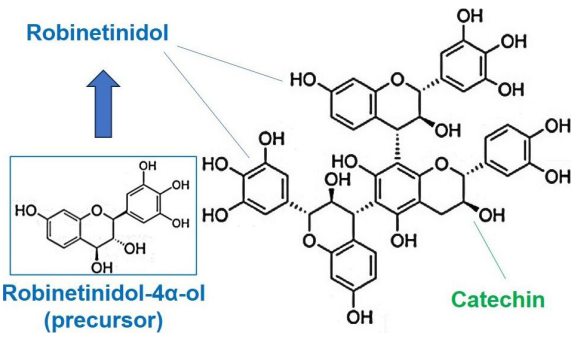

Fig. 6 Most abundant trimer found among Acacia mearnsii proanthocyanidins. The trimer is angular, with one robinetinidol-type unit at the C-8 position and another at the C-6 position of the catechin.

MALDI-TOF analysis by Pasch revealed the presence of oligomers in the extract, reaching a maximum of octamers [16]. Conversely, MALDI-TOF analysis of a commercial tannin dissolved in neutral aqueous medium indicated that it ranges from monomers to hexamers [38]. Regarding the relative abundance of different oligomers, Venter et al. ESI-MS study showed that mimosa extract contains approximately 9% monomers, 42% dimers, 40% trimers, 9% tetramers, and 1% pentamers and higher oligomers [33, 35]. Therefore, about half of the tannins in the extract consist of trimeric and higher oligomers.

Mimosa extract is cold-water soluble and does not require sulfitation for complete solubilization, unlike the relatively poor solubility of hot-water-soluble quebracho extract. This is attributed to the presence of robinetinidol extending units in mimosa tannins, which possess an additional aromatic hydroxyl group compared to fisetinidol (Fig. 3), favoring the formation of hydrogen bonds with water and thus increasing their solubility [24]. Nevertheless, sulfitation is sometimes employed to improve tanning properties.

1.3.3 Tara tannins

Tara (*Caesalpinia spinosa*) is a tree from the legume family native to South America. It is widely distributed, from Venezuela to northern Chile, but is especially abundant in the arid coastal areas and the Andean region of Peru [39–41]. Peru accounts for 80% of the world's tara production, exporting approximately 10,000 tons annually [39, 40, 42]. Its high content of gums and tannins gives it significant economic value, leading to various applications in the food industry, leather tanning, paint manufacturing, adhesive development, and the medical-pharmacological research field [39, 41–46]. Tara infusions have traditionally been used in Peruvian folk medicine to treat fever, tonsil inflammation, colds, and stomach problems [40, 46].

Tara tannins are obtained from its fruits, which are flattened, indehiscent pods ranging from yellow to orange-red [39]. Each tara tree can yield an average of 20 to 40 kg of pods annually [47]. Commercial tara powder is obtained

by crushing the seedless pod, resulting in a fine, light-yellow powder (100–200 mesh, particle size of 75–150 μm) with a tannin concentration ranging between 40% and 60% w/w [40, 41, 43]. Subsequently, the tannin-rich tara extract is obtained through a process that includes water extraction at 65–70 °C, filtration, concentration, and atomization [41]. Since its extraction does not involve deforestation, tara tannins can be widely used in industry as a sustainable and ecological raw material [48].

Tara tannins are of the hydrolyzable type and are characterized by being predominantly gallotannins [39, 40, 43, 49, 50]. Their structure differs from other gallotannins because, instead of a central glucose residue, they feature a central quinic acid unit, to which gallic acid units are linked *via* ester bonds (Fig. 7) [39–41, 48–50]. The gallic acid residues can not only be attached to each of the four hydroxyls carried by the quinic acid but can also form aryl esters with one or more additional gallic acid residues through what are known as depside bonds [41, 49, 50].

1.3.4 Chestnut tannins

Chestnut (*Castanea sativa*) is a deciduous tree belonging to the Fagaceae family. It is native to Southern Europe and Asia Minor, particularly the Mediterranean region [51]. It's widely cultivated for both its wood and its edible fruits, chestnuts.

The first step in the commercial production of chestnut tannins involves producing chips or shavings from the tree trunks. The chestnut chips are then placed in autoclaves filled with boiling water at 100 °C [51]. This generates a dark, thick liquid, which then undergoes several purification processes before being ready for commercialization. First, the solution is cooled to room temperature to precipitate impurities present in the plant material. Then, to facilitate storage, packaging, and shipping, the solution is spray-dried to obtain a powder. The chestnut tannin obtained in this manner has a dark brown color, reminiscent of powdered instant barley coffee.

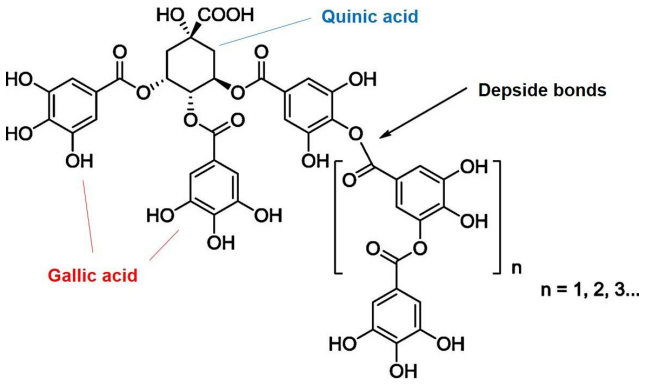

Fig. 7 Structure of tara tannins, based on galloylated quinic acid

Chestnut tannins are of the hydrolyzable type, with most of them being ellagitannins [52]. The predominant ellagitannins are the vescalagin and castalagin diastereoisomers, along with their oligomeric derivatives [13, 53]. These compounds possess an open glucose core to which both an HHDP group and a nonahydroxytriphenoyl (NHTP) group are esterified [13, 53] (Fig. 8).

According to Pizzi, castalagin and vescalagin account for approximately 14.2% and 16.2% by mass of the commercial chestnut tannin extract, respectively [18, 29]. Ellagitannins with a cyclic glucose core, such as the casuarictin/potentillin isomers (1-O-galloyl-bis-HHDP-glucose) [13], are also found, and their structure is shown in Fig. 2. Chestnut extracts also contain gallotannins like pentagalloylglucose [13], whose structure is represented in Fig. 1.

1.4 Fourier transform infrared spectroscopy for tannin characterization

The remarkable structural diversity of tannins, arising from differences in hydroxylation patterns, interflavonoid linkages, degree of polymerization, and the presence of galloyl or hexahydroxydiphenoyl groups, is reflected in their spectroscopic behavior. Recent studies have demonstrated that Fourier transform infrared (FTIR) spectroscopy provides characteristic fingerprints that can be directly related to the molecular architecture of complex polyphenolic systems, allowing the discrimination of different tannin classes and subclasses [9, 54]. Since these families exhibit distinct physical and chemical properties and are employed industrially according to the requirements of specific applications, the correct identification and classification of tannin extracts are of fundamental importance for their appropriate utilization. Owing to its rapidity, minimal sample preparation, and non-destructive nature, FTIR spectroscopy has emerged as

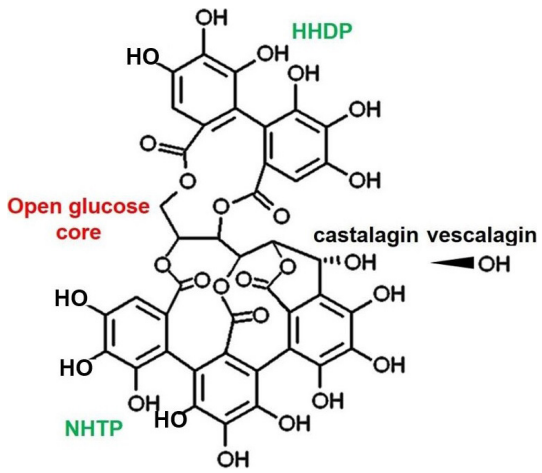

Fig. 8 Chemical structures of diastereoisomers castalagin and vescalagin

a valuable analytical tool for the characterization, classification, authentication, and quality control of commercial tannin extracts and other plant-derived polyphenolic materials.

2 Materials and methods

2.1 Commercial tannin sources and physicochemical characterization

As a source of quebracho colorado tannins, the commercial Tupasol ATO cold-water-soluble extract (Silvateam, Argentina) was used [55, 56]. This product is a dry spray-dried powder of quebracho colorado wood extract (a mixture of *S. balansae*/*lorentzii*), treated with sodium bisulfite to convert phlobaphenes into soluble tannins.

For mimosa tannins, the commercial Weibull cold-water-soluble extract from TANAC company, Montenegro, Brazil, was employed [57]. This powdered extract is obtained through hot water extraction, followed by concentration and spray drying. According to the manufacturer, the raw materials used to produce these extracts come from renewable sources and are responsibly managed.

As a raw material for tara tannins, the commercial T40 extract from Indunor, a company now part of the multinational Silvateam, was used [58]. According to the supplier, this product is obtained by extracting tara powder using a mixture of non-toxic solvents, followed by several purification and concentration operations. Finally, the concentrated liquid was atomized, resulting in a fine yellowish powder that is highly soluble in cold water.

For chestnut tannins, the chestnut extract N2 provided by Indunor-Silvateam was used [59]. According to the company, chestnut trees are not felled; instead, coppicing techniques are utilized, which leverage mature wood and allow for more robust subsequent growth. Furthermore, the amount of wood used annually represents only a small percentage of the forest regeneration rate, ensuring that this is a completely sustainable activity [59].

The tannin, moisture, and ash contents, together with the water solubility of the commercial extracts, were obtained from the technical data sheets supplied by the respective manufacturers [55–59].

For pH and conductivity measurements, a 0.10% (w/v) aqueous solution of each commercial tannin extract was prepared by dissolving 0.1 g tannin in 100 mL ultrapure water. Ultrapure water was obtained using an OSMOION purification system (APEMA, Buenos Aires, Argentina), delivering water with an electrical conductivity less than 1.0 μS/cm. Complete dissolution was achieved by mechanical stirring at 5000 rpm for 1 h. The pH of each solution was measured using a PHS-3E pH meter (Arcano, China). Electrical conductivity was determined using a conductivity cell equipped with two platinized platinum electrodes connected to a conductivity meter (Model 170, ATI Orion, Germany) operating at a measurement frequency of 1000 Hz. All measurements were performed at room temperature (20 ± 2 °C).

The total polyphenol content of the extracts was determined by the Folin-Denis method. The Folin-Denis reagent was prepared by dissolving 10.0 g sodium tungstate ($Na_2WO_4{\cdot}2H_2O$) (ACS reagent, Sigma-Aldrich, Argentina), 2.0 g phosphomolybdic acid [$H_3PMo_{12}O_{40}{\cdot}H_2O$] (p.a., Biopack, Argentina), and 5.0 mL 85% H_3PO_4 (ACS reagent, Anedra, Argentina) in 80 mL ultrapure water, followed by heating under reflux for 6 h. After cooling, the final volume was adjusted to 100 mL. Tannic acid was used as the calibration standard, and standard solutions containing 1–10 mg/L tannic acid were prepared by dilution from a 1000 mg/L stock solution. For analysis, 0.20% (w/v) solutions of each commercial tannin extract were prepared and subsequently diluted tenfold to obtain concentrations within the calibration range. For the colorimetric reaction, 1.0 mL sample or standard solution was mixed with 2.0 mL saturated Na_2CO_3 solution and 2.0 mL Folin-Denis reagent in a 25 mL volumetric flask, and the volume was adjusted with ultrapure water. After 30 min of reaction, absorbance was measured at 750 nm using a UV–Vis spectrophotometer (UV-2600, Shimadzu, Japan). The total polyphenol content was finally expressed as milligrams of tannic acid equivalents per gram of commercial extract (mg TAE/g).

2.2 Fourier transform infrared spectroscopy analysis

All four commercial tannins were characterized using attenuated total reflection–Fourier transform infrared (ATR-FTIR) spectroscopy. FTIR analyses were carried out using an FTIR spectrometer (Spectrum One, PerkinElmer, USA) equipped with a plug-and-play Universal ATR accessory with a diamond/ZnSe crystal. Spectra were recorded in the 4000–700 cm^{-1} range at a resolution of 4 cm^{-1}, averaging 32 scans per sample.

3 Results and discussion

3.1 Commercial tannin sources and physicochemical characterization

The tannin, moisture, and ash contents of the commercial extracts were provided by their respective manufacturers, and the corresponding values are summarized in Table 1. All extracts exhibited tannin contents between 70 and 85% (w/w), moisture contents ranging from 5 to 8% (w/w), and total ash contents between 0.6 and 1.0% (w/w). Among

Table 1 Characteristics of commercial tannins (Q: quebracho; M: mimosa; T: tara; C: chestnut)

Tannin	Q	M	T	C
Tannin content (% w/w)	72	72	85	76
Moisture content (% w/w)	8	5	7	8
Water-insoluble matter (% w/w, 25 °C)	0.2	0	0	0
Total ash content (% w/w)	0.6	0.6	1.0	0.7
pH of a 0.10% w/v aqueous solution	4.8	4.6	3.3	3.6
Electrical conductivity of a 0.10% w/v aqueous solution (μS/cm)	33.7	76.9	165.1	90.8
Total polyphenol content (mg TAE/g)	476	497	594	568

the extracts studied, only quebracho contained a small fraction of water-insoluble compounds at 25 °C.

The pH and electrical conductivity values of the 0.10% (w/v) aqueous solutions are also presented in Table 1. All commercial tannin extracts produced distinctly acidic solutions, with the lowest pH values observed for tara and chestnut extracts. This acidity can be attributed to the presence of phenolic groups in all tannins, sulfonic groups introduced during the sulfitation process in mimosa and quebracho extracts, and carboxylic groups associated with tara tannins or with the partial hydrolysis products of hydrolysable tannins. Although the differences in ash content among the extracts were relatively small, tara exhibited the highest value, which was reflected in its higher electrical conductivity due to the increased concentration of mineral salts.

The total polyphenol content, expressed as milligrams of tannic acid equivalents per gram of commercial extract (mg TAE/g), is likewise summarized in Table 1. The values ranged from approximately 475 to 600 mg TAE/g, with the highest polyphenol contents corresponding to the extracts with the highest tannin contents.

3.2 Fourier transform infrared spectroscopy analysis

The FTIR spectra in the 4000–700 cm^{-1} range of the four commercial tannins are presented in Fig. 9.

In all commercial tannins, a typical broad band is observed spanning a range of 2900 to 3600 cm^{-1}, attributed to the O–H stretching of the multiple hydroxyl groups present in the extracts [60–62]. This corresponds to an envelope of bands originating from OH substituents in different chemical environments and participating in intermolecular hydrogen-bonding interactions, which are known to broaden and shift the O–H stretching region in polyhydroxylated systems [63–65]. A slight shoulder can also be perceived around 2950 cm^{-1}, which arises from the asymmetric C–H stretching vibrations of aliphatic –CH, $–CH_2$, and $–CH_3$ groups present as carbohydrate

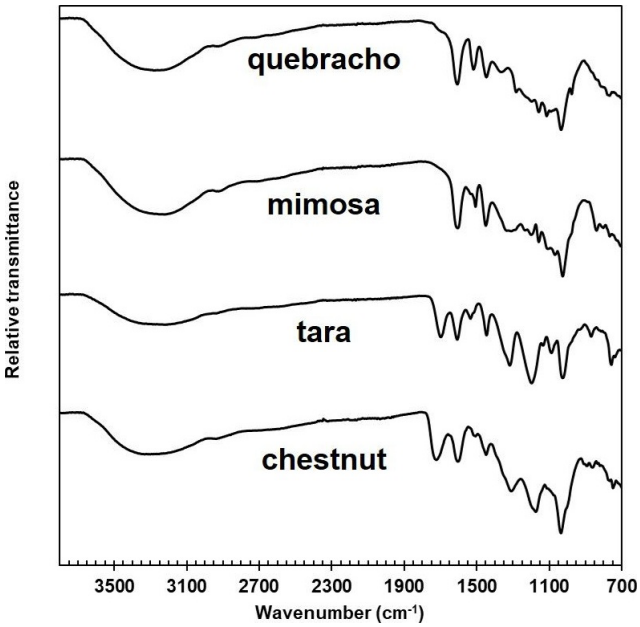

Fig. 9 Representative FTIR spectra comparing commercial quebracho, mimosa, tara, and chestnut tannins

and sugar impurities in the extracts, as well as from the C-rings of the flavan-3-ol units in quebracho colorado and mimosa condensed tannins [60–64].

In Fig. 10, the IR fingerprint region (1800–700 cm^{-1}) is closely examined. This spectral area is rich in data concerning the specific bond types and their complex vibrational modes within the tannin molecule. Its analysis facilitates the fine differentiation of tannin types and their botanical

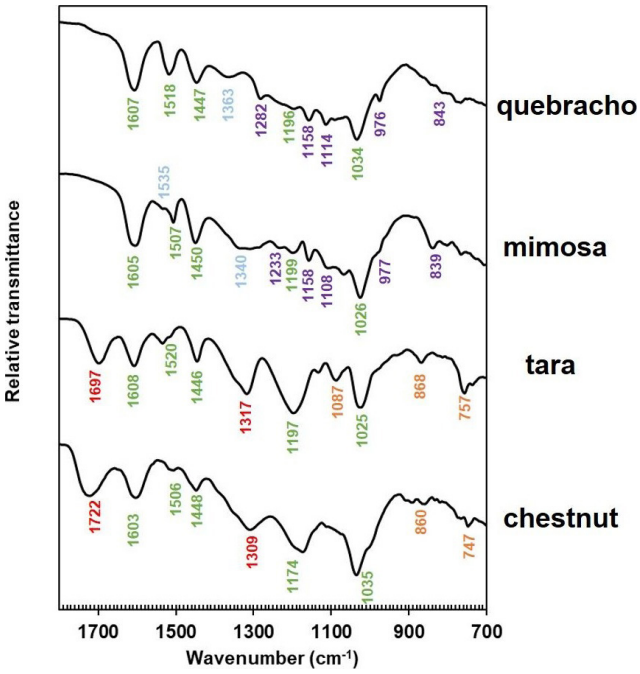

Fig. 10 Characteristic wavenumbers in the fingerprint region of FTIR spectra for commercial tannins, highlighted with the color code from Fig. 11

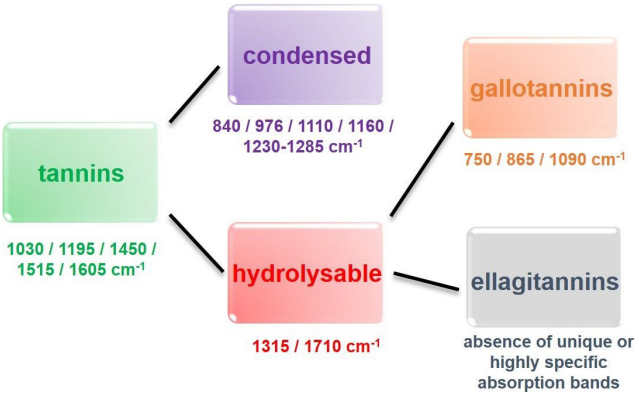

Fig. 11 Characteristic wavenumbers in FTIR spectra for tannin identification

origins, especially when broader functional group regions exhibit comparable features [9]. In this regard, Fig. 11 summarizes the characteristic wavenumbers of both the bands common to all plant tannins and the distinctive bands for condensed tannins, hydrolyzable tannins, and gallotannins.

All tannins exhibit five characteristic absorption bands in their FTIR spectra. Three of these, located around 1605–1620 cm^{-1}, 1505–1520 cm^{-1}, and 1445–1455 cm^{-1}, are associated with the C=C–C stretching vibrations of the aromatic rings [9, 54, 60, 63]. The remaining two bands, in the range of 1175–1200 cm^{-1} and 1030–1040 cm^{-1}, are linked to the symmetric and antisymmetric stretching vibrations of the C–O bonds [60, 63, 66–68]. These bands are highlighted in green across all four spectra in Fig. 10. It's evident that the 1505–1520 cm^{-1} region shows more intense C=C–C stretching signals for quebracho colorado and mimosa condensed tannins compared to the hydrolyzable tannins from tara and chestnut [63, 69]. Conversely, the C–O stretching band around 1175–1200 cm^{-1} is more intense for the hydrolyzable tannins [63].

Condensed tannins exhibit a characteristic intense peak in the 1230 to 1285 cm^{-1} range, which can be assigned to the asymmetric stretching vibration of the C–O ether linkage present in the pyran-derived C-ring structure (Fig. 4). Strong bands are also observed around 1160 cm^{-1} and 1110 cm^{-1}, likely due to the symmetric stretching of the cyclic C–O–C ether function and in-plane bending of aromatic C–H bonds, respectively [54]. Additionally, weak bands are present at 976–977 cm^{-1} and 840–845 cm^{-1}, probably associated with the out-of-plane bending of aromatic C–H bonds [29, 64, 66–68, 70]. In Fig. 10, these bands are highlighted in purple in the spectra of quebracho colorado and mimosa tannins, confirming that these commercial extracts are predominantly composed of condensed tannins. Furthermore, peaks of low intensity are observed at 1363 cm^{-1} for quebracho colorado tannins and 1340 cm^{-1} for mimosa tannins. These may originate from C–O stretching vibrations or from the in-plane deformation of phenolic O–H bonds [54, 64, 69, 70]. Similar FTIR signatures have been reported for condensed tannin extracts obtained from several pine species [71]. In particular, the intense band in the 1230–1285 cm^{-1} region, associated with ether C–O stretching in the flavan-3-ol C-ring, together with bands near 1110 and 1160 cm^{-1}, has also been identified in these extracts and may therefore be considered diagnostic markers of condensed tannin structures [71]. The occurrence and relative intensities of these bands therefore support their use as diagnostic markers for the identification of condensed tannin-rich materials. Moreover, subtle differences in the 1500–1550 cm^{-1} region may reflect variations in the hydroxylation pattern of the flavonoid B-ring, thereby providing additional information for differentiating commercial condensed tannin extracts such as quebracho and mimosa. A small shoulder at approximately 1535 cm^{-1} is observed exclusively in the mimosa spectrum (Fig. 10). Similar signals have been reported for flavonoid systems containing aromatic rings with two hydroxyl substituents, suggesting that this feature may be associated with the predominance of dihydroxylated prorobinetinidin units in mimosa tannins and may therefore provide additional structural information regarding the composition of the extract [9].

Hydrolyzable tannins, on the other hand, display two characteristic intense bands: one around 1700–1725 cm^{-1}, attributed to the stretching vibration of carbonyl groups (C=O bonds) from phenolic esters, and another around 1310–1320 cm^{-1}, which can be assigned to a combination of the symmetric C–O–C stretching vibration of the ester function with the O–H deformation vibration [29, 60, 66–70]. These bands are highlighted in red in the tara and chestnut tannin spectra in Fig. 10, confirming that these commercial extracts are primarily composed of hydrolyzable tannins. The band around 1700–1725 cm^{-1} is crucial for distinguishing hydrolyzable tannins from condensed tannins, as the latter do not naturally contain carbonyl groups [9, 63]. The exact position of this ester carbonyl band may also be influenced by hydrogen-bonding interactions involving neighboring hydroxyl groups, as similar redshifts of ν(C=O) have been reported for model compounds containing well-defined carbonyl and hydroxyl functionalities [65].

Gallotannins exhibit three additional medium to weak intensity bands that can serve as markers for this tannin subclass: a band around 1090 cm^{-1}, attributed to the symmetric C–O–C stretching of the aryl phenolic ester; another near 865 cm^{-1}, due to the out-of-plane bending

of aromatic C–H bonds; and finally, a band around 750 cm^{-1}, originating from the symmetric deformation or ring breathing of the sugar ring [29, 66–68]. Ellagitannins, conversely, do not present characteristic bands. Consequently, they can only be identified if marker bands for hydrolyzable tannins are present and marker bands for gallotannins are absent [64, 67]. The typical gallotannin bands are highlighted in orange in the tara and chestnut tannin spectra in Fig. 10. A high intensity of these bands is observed in the tara extract, confirming the abundant presence of gallotannins, whereas the bands are extremely weak in the case of chestnut tannins, corroborating that chestnut hydrolyzable tannins are predominantly ellagitannins.

4 Conclusions

The presence of a characteristic broad O–H stretching band (2900–3600 cm^{-1}) and specific aromatic C=C–C and C–O stretching vibrations (e.g., 1610–1620, 1505–1520, 1445–1455 cm^{-1} and 1175–1200, 1030–1040 cm^{-1}, respectively) were common across all tannin samples, reflecting their fundamental polyphenolic nature. However, crucial distinguishing features emerged. Condensed tannins displayed an intense peak at 1230–1285 cm^{-1}, indicative of the C–O ether linkage in their pyran rings, which was particularly strong in quebracho colorado and mimosa. Conversely, hydrolyzable tannins were clearly identified by a prominent carbonyl (C=O) stretching band at 1700–1725 cm^{-1}, absent in condensed tannins, alongside a distinct C–O stretch at 1310–1320 cm^{-1}.

Further discrimination within hydrolyzable tannins was achieved by identifying specific marker bands for gallotannins (e.g., 1090, 865, and 750 cm^{-1}). The high intensity of these bands in tara extract confirmed its primary composition as gallotannins. In contrast, their remarkable weakness in chestnut tannin spectra, coupled with the presence of hydrolyzable tannin markers, strongly corroborated that chestnut extracts are predominantly ellagitannins, which lack their own characteristic FTIR absorption bands.

This comprehensive FTIR analysis underscores its power as a rapid and effective technique for the qualitative assessment, classification, and authentication of commercial tannin extracts. The distinct spectral signatures observed provide valuable insights into their predominant chemical structures and botanical origins, which is essential for their diverse industrial applications and for guiding further research into their properties.

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