VEGETABLE TANNINS USES AND ANTIOXIDANT PROPERTY

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Abstract

Vegetable tannins are phenolic compounds of great economic and ecological interest. They are found in roots, wood, bark, leaves, fruits, seeds and sap of different plant species. The tannins composition and their chemical characteristics that allow a series of reactions to modify their chemical structure give possibilities for a wide range of uses for them. Historic used as tanning agents, recently, the interest for different applications of tannins has grown and several studies evaluated either they can be successfully used or not as flocculants, dispersants, antioxidants, biocides, etc. The antioxidant properties of tannins are well known and they could be used with this purpose in foods, beverages, fuels, lipids systems, polymers, etc, replacing synthetic and other less available plant extracts. However, their antioxidant properties are scarcely studied. The antioxidant property of acacia (*Acacia mearnsii*), chestnut (*Castanea sativa*) and quebracho (*Schinopsis sp.*) of commercial tannins extracts were tested. The commercial tannins were also characterized by their ash content, by TG/DTA analyses, by Folin-Ciocalteau method for the determination of Total Phenolic Content, and against DPPH (1,1-diphenyl-2-picryl-hydracyl) radical for antioxidant activity. The results showed that plant tannins reach a maximum of 87% DPPH radical inhibition at a concentration of 10 mg L^{-1}.

Keywords: tannins, antioxidant, chromium (VI)

1 Introduction

Vegetable tannins are phenolic compounds found in the roots, wood, bark, leaves, fruits, and seeds of different plant species where they are synthesized as secondary metabolites with protective function against insects, fungi, or bacteria. Chemically, tannins are complex polyphenolic substances and, according to their structures, they are divided into two major groups: hydrolysable tannins and condensed tannins. The main part of the composition comes from the structure of the monomeric units: catechin and catechin-based compounds (flavonols, galoflavanols, and flavanol gallate) for condensed tannins; gallic acid and derivatives (ellagic, hexahydroxydiphenic acids) for hydrolysable tannins (Das et al., 2020).

The content and species of tannins vary from one plant to another and also from one part of the plant to another. Hydrolyzable tannins have low availability and limited production worldwide (they account for less than 10% of the world production of commercial tannins), therefore they have a high price, which makes them less attractive. On the other hand, condensed tannins are equivalent to more than 90% of the world production (García et al., 2016).

1.1 Vegetable tannins applications

Tannins extracts are compounds of great economic and ecological interest since the diversity of structures found in the different tannins allows a variety of uses for them. Brief research in recent literature demonstrates the growing interest in vegetable tannins uses. A search in the Scopus database by the words "vegetable and tannins" found 1885 documents in a forty years period (Figure 1). The studies cover several applications in different fields, many studies cover improvement of already commercial uses and others are in the research stage.

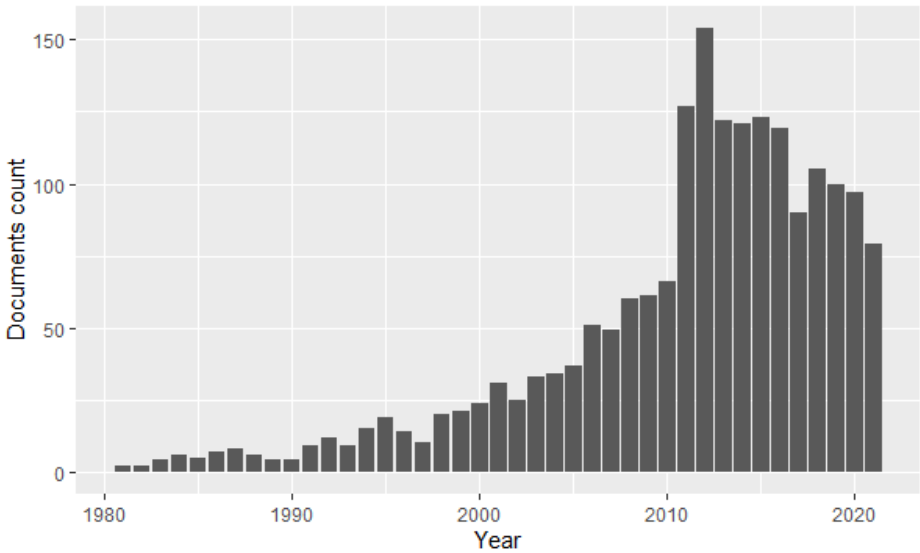

Figure 1. Publications with the terms 'vegetable' and 'tannins' in the Scopus Database

Among the industrial uses of tannins, the most traditional and common application is leather tanning. It is common for this application that the tannins undergo a sulfitation process, to facilitate the extraction process, whitening of the extract and improvement in the tanning properties (Grasel et al., 2016).

The second most important application is being adhesives for wood products, where the tannins are the more interesting alternative to synthetic adhesives. The growing interest in this application is due that tannin-based adhesives are less prone to emitting formaldehyde, a carcinogenic compound. Further, studies are also focusing on the tannins ability to react with hexamine, used as a hardener, which can produce an adhesive not prone to decompose to formaldehyde at all (Shirmohammadli et al., 2018).

The use of tannins in water treatments is also widespread. In this application, vegetable tannins represent natural alternatives to inorganic metal-based flocculants/coagulants and the

use of tannins as flocculant agents is known since 1970, however, for a long time, this use was neglected. The interest for tannins as flocculants/coagulants began to grow again near 2009 due to the concerns for sustainability and the replacement of chemicals by bio-based alternatives (Pizzi, 2019).

Among other industrial applications for tannins extracts are their use as wood preservatives, corrosion inhibitors and foams. Table 1 present a resume of the important property related to each application.

An important characteristic of tannins is related to the OH groups that confer antioxidant and anti-radical properties to these compounds. Phenolic groups donate hydrogen, which results in the formation of stable free radicals due to the occurrence of resonance in the aromatic ring and, therefore, hinders free radical oxidation chain reactions (Varatharajan & Pushparani, 2018). These antioxidants properties are relevant in many fields.

In the beverage industry, tannins are added in the winemaking process to improve several properties, such as colour stability, odour and mouth-feel, flavour and also promote protections against oxidation and proteic material precipitation. The tannins are naturally present in grapes and were added in the ageing process in oak barrels. However, they can be added directly reducing the ageing time and also compensating low tannin levels in some wines. Tannins used in winemaking are known as "enological tannins" (Laghi et al., 2010).

Their application as food additives is also of great interest. Tannins are used in animal food due to their antioxidants abilities and researches findings relating their use with the improvement of animal digestion. The use in foods and beverages also beneficiate from the know bactericide ability of tannins (Fraga-Corral et al., 2020).

The antibacterial property, together with the antioxidant abilities raise the interest for medicinal and farmaceutical use of tannins where many applications, such as antiviral, anti-fungal and anti-cancer, are under research (Fraga-Corral et al., 2020; Pizzi, 2019).

Especially interesting is the ability of tannins to prevent Cr (VI) formation, which may be also related to the antioxidants properties (Fuck et al., 2011; Ozkan et al., 2015).

Table 1. Properties related to each application of vegetable tannins extracts

Application	Relevant property
Tanning agent	Ability to complex proteins: tannin polyphenols can create crosslinking with collagen by numerous hydrogen bonds. At first, hydroxyl groups (OH) bind with the active collagen centres, and then continue to fill the interfibrillar spaces in leather.
Adhesive	Cross-linking of hydroxyl groups of tannins with formaldehyde and others compounds.
Flocculant	Adjacent phenolic hydroxyls display a specific affinity to metal ions providing the ability to chelate them.
Corrosion inhibitor	The phenol groups oxides and then complex with the metal by ortho-diphenol complexation, protecting from external oxidation.
Antioxidant	OH groups stabilize free radicals and then inhibit further damage these may otherwise cause.
Bactericide	Tannins react with proteins irreversibly, thus complexing within bacterial membranes, neutralizing their activity.

Research studies in the Laboratory for Leather and Environmental Studies (LACOURO) covered a few of these uses and brought new knowledge for each specific application. A resume of the findings, among others in the literature, is shown in Table 2.

Table 2. Recent studies in vegetable tannins applications

Application	Objective	Conclusions	Reference
Tanning agent	Study relevant chemical parameters and the correlation with their cross-sectional penetration in the hide	Myrobalan and tara tannins presented low penetration potential while acacia, quebracho, and chestnut went through the skin. The properties associated with the penetration in the hide were the higher tannin content and lower insoluble content present in the material.	(Auad et al., 2019)
Wastewater treatment	Reduce aluminium salts in the coagulation-flocculation process	The values of colour removal, turbidity and total organic carbon were statistically equal to the application with and without tannin. Therefore, is possible to use aluminium sulphate with tannins in order to reduce the	(Anjos, 2016)

		dosages of aluminium sulphate.	
	Recovery of microalgae biomass growth in the wastewater	There was a positive influence in the use of vegetable tannins for the sedimentation of microalgae throughout the neutralization of the impingent charges.	(Fontoura, 2017)
Adhesive	Development of a cornstarch-tannin-sugar adhesive with zero formaldehyde for interior particleboard	Tannin-based resin satisfied the exigencies of panels for interior fittings used in dry medium (P2) according to European norms EN 312 (2010)	(Oktay et al., 2021)
Corrosion inhibitor	Tannins as corrosion inhibitor for AA1200 aluminium alloys	Results show that natural tannin is an efficient inhibitor coated for aluminium Alloy 1200	(Nardeli et al., 2019)
Antioxidant (prevent biofuel degradation)	Evaluate tannin-based antioxidant in the preservation of biodiesel	The protection of oxidation by tannin antioxidant was superior to the synthetic TBHQ (terc-butil-hidroquinona)	(Schaumlöffel et al., 2021)
Antioxidant (preventing Cr (VI) formation)	Evaluate the effects of tannins in Cr(VI) formation in leather	The use of 2.5 and 5.0% of any type of vegetable tannin prevented Cr(VI) formation in all leather samples	(Ozkan et al., 2015)
	Evaluate the Cr(VI) formation in leather in different retanning conditions in leather	The retanning with vegetable tannin has a beneficial effect by preventing the oxidation of chromium	(Fuck et al., 2011)

An important aspect to be considered in the broad applications of vegetable tannins is their biodegradability. Their anti-microbiologic qualities may cause problems at end of live cycles where the tannins-based products may undergo microbiological degradation. The work of Agustini et al. (2018) demonstrates that chromium sludge was a more suitable medium for anaerobic digestion than vegetable tannin sludge. They identified it in all parameters studied (BOD_5, TOC, VSS, NT, IC, pH). Another study evaluated the influence of the concentration of tannin, the forms of culture and the type of tannin used in the cultivation of *Aspergillus niger* in solid-state and submerged culture cultivations (Spier & Gutterres, 2018). The consumption of tannins in solid-state and submerged cultivation was similar at 13% and 15%, respectively proving the fungistatic action of the phenolic compounds present in the tannins on *Aspergillus niger*. However, the same study presents an alternative treatment in which fungal strains of the genus Penicillium were more effective reaching 97.3% consumption of acacia tannin.

With this overview, it can be seen that many different factors are relatable to tannins application, thus, the objective of this study was to evaluate some tannins properties, relating them to new applications under research, especially tannins use as antioxidants.

2 Materials and methods

2.1 Materials

The tannins evaluated were the commercial tannins extracts of acacia (Tanac S.A.), quebracho (Silvateam S.A.), and chestnut (Silvateam S.A.).

2.2 Tannins characterization

The determination of total phenolic content (TPC) was carried out by the Folin-Ciocalteau method. For the TPC determination, a 0.1 mL aliquot of tannin extracts stock solution (160 ppm) was mixed with 0.4 mL of ethanol solution (10% v/v in distilled water) and 2.5 mL of the Folin-Ciocalteau reagent (10% v/v in distilled water). After 3 min, 2.0 mL of 7.5% (m/v) Na_2CO_3 solution was added and the mixture remained at rest from 1h, protected from light. The absorbance was measured at 760 nm in the UV–Vis spectrophotometer (Thermo Scientific Visible Spectrophotometer – GENESYS 30). The TPC was determined using a standard curve of gallic acid and the results were expressed in gallic acid equivalent per gram of sample (mg EAG/g).

The thermal characterization of the compounds was performed in a thermogravimetric analyzer (TA Instruments - SDT Q600) within the 25–900 °C range and a 10 °C/min heating rate, under a nitrogen atmosphere.

Ash content was determined by TAPPI T211 standard method.

2.3 Antioxidant activity analysis

Antioxidant activity was determined by the 2,2-diphenyl-1-picryl-hydrazine (DPPH) radical scavenger method. Briefly, 3.7 mL of DPPH radical solution in methanol (40 mg L−1) were mixed with 0.3 mL of tannin extracts and TBHQ samples, with concentrations varying from 67 to 200 mg/L. The samples remained at rest in the dark for 30 min. Subsequently, the absorbance was measured at 517 nm (Thermo Scientific Visible Spectrophotometer – GENESYS 30). The percentage of DPPH radical activity inhibition (percentage of antioxidant activity) was calculated by Eq. (1),

$$(\%)\ inhibition\ = \frac{(A0 - At)}{A0} \times 100 \qquad (1)$$

where A0 is the absorbance of the pure DPPH solution and At the absorbance of DPPH in the presence of a sample.

3 Results

3.1 Tannins characterization

The total phenol content found (presented in Table 3) evidenced a large amount of phenols present in the vegetable tannins, especially when compared to other plant extracts (Schaumlöffel et al., 2021). Thus, applications that depend on this structure, such as antioxidant and corrosion inhibitors, can benefit from the use of vegetable tannins.

Table 3. Ash and total phenolic content for the different tannins.

Tannin	TPC (mgEAG g^{-1})	Ash content (%)
Acacia	645.2 ± 21.5	3,13 ± 0,16
Chestnut	511.6 ± 115.2	1,40 ± 0,18
Quebracho	660.2 ± 44.8	8,35 ± 0,25

The ash content is related to the salt plants absorb while growing and to the salts added in the sulfitation process (Weiler et al., 2019). Although due to the origin of ashes their content may vary from a tannin sample to another, the ash values found (Table 3) are similar to those in other studies (Zanetti et al., 2021). This amount can be considered small in the case of applications that require full combustion at the end of its use, as is the case of the combustion of wastewater sludge to energy production. However, in applications where the final product specification requires low ashes, such as the use as an antioxidant in oils, food and beverages, the ash content should be considered when the tannins are added in great quantity, especially the quebracho tannin.

The thermal analyses (Figure 2) demonstrate that the tannins lost about 10% of weight in 143, 134, 157 °C for acacia, chestnut and quebracho, respectively. Near 80% of weight still present for of acacia and quebracho at 250 ºC and for chestnut at 295 °C. These are indications that applications that require exposition to higher temperatures require evaluation of thermal behaviour.

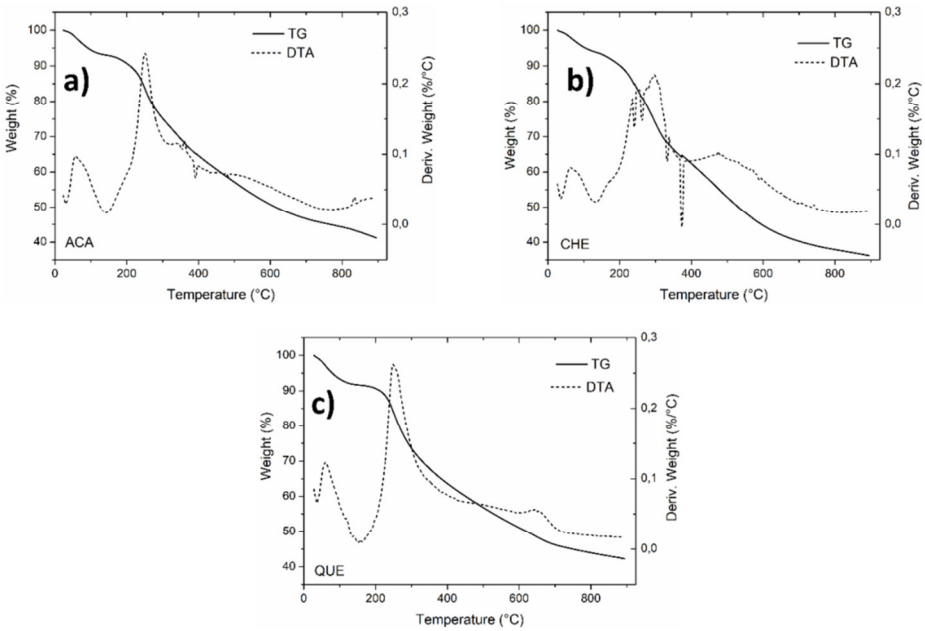

Figure 1. TG/DTA curves for ACA, CHE and QUE tannins

3.2 Antioxidant ability

The antioxidant capacity was evaluated by the DPPH radical scavenger essay and the results are shown in Figure 3. The tannins with a concentration of 10 mg/L were able to inhibit more than 85% of DPPH radical in aqueous medium. Compared with other vegetable extracts like green tea extracts, that inhibited 70% with a concentration of 250 ppm (Bharti & Singh, 2020), tannins effects present superior performance.

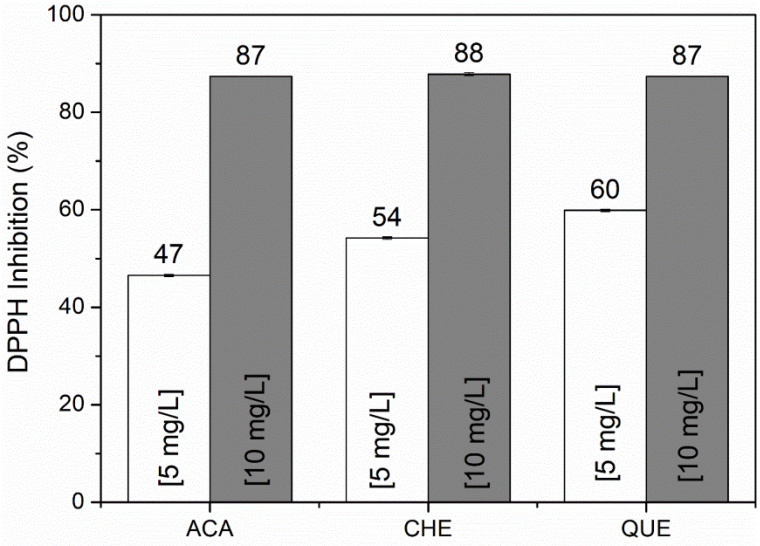

Figure 3. DPPH Scavenging activity of acacia (ACA), chestnut (CHE) and quebracho (QUE) tannins

4 Conclusion

Vegetable tannins are natural compounds, therefore they are been considered as an alternative material for replacing synthetic compounds in many applications, providing more sustainable processes. Their properties and structure make them suitable for a broad range of applications, such as tanning agent, adhesive, flocculant, corrosion inhibitor, antioxidant, food additive and for medicinal uses. The analyses of some tannins properties showed high total phenolic content. The ashes content, on the other hand, were small. The thermal analyses showed that at 250°C 80% of tannins mass were still present and the antioxidant activity results showed that vegetable tannins reach 87% of DPPH inhibition at a concentration of 10 mg/L.

These analyses demonstrate the potential for the use of tannins as antioxidants due to their phenolic content and antioxidant activity. However, further studies are required to scale new research findings in industrial applications, which can increase the competitiveness of tannins productive chains.

5. Acknowledgments

We would like to thank the scholarships provided by Brazilian Federal Agency for the Scholarship (CAPES).

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