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TANNINS MEDICAL / PHARMACOLOGICAL AND RELATED APPLICATIONS: A CRITICAL REVIEW

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ABSTRACT

Tannins are natural phenolic compounds that are widespread and almost ubiquitous in the vegetal world. They can be found in fruit, wood and bark of trees, and many types of wild herbs and plants or from sustainable agriculture and forestry. Their traditional use for leather has been doubled for centuries in the popular medical and pharmacological lore by their use to cure or alleviate a variety of infections and diseases. This has indicated to modern researchers that these materials possess a great potential for the use in modern medicine and pharmacy. This review, after an introduction on tannins their structures and their basic chemistry describe what modern resarchers have been able to glean and demonstrate of their real, identified and quantified effects on various diseases, from their bactericidal, anticancer and antiinflammatory actions to the many other effects that putsnow in more close focus in pharmacology.

Keywords: tannins, tannin types, structure, chemistry, sources, industrial extraction, medicinal uses, pharmacology uses, anticancer, virucidal, antimicrobial, antioxidants

1. INTRODUCTION

The name “tannin” comes from the use of these materials from hides tanning to produce leather. They are light brown to reddish-brown to dark brown spray-dried amorphous powders. They are natural astringent materials commonly found in many parts of plants where they participate in defending the plant from fungal, bacterial and insects attack as well as to the plant survival in periods of drought (Pizzi and Cameron 1986). It is these materials capacity of complexing carbohydrates and in particular of complexing irreversibly proteins that contribute to their defense mechanism of plants. These materials have been found to be of use in human health as well as having a positive influence in veterinary science for ruminants wellbeing, mainly to reduce the production of methane in the rumen due to the potentially deadly fermentation of high protein feeds, alfalfa being the best example, that can be reduced by adding tannin in the diet. The critical reverse of the medal in veterinary science is the total blocking of the absorption of nutritives in ruminants due to an excess of tannins (van Hoven 1984). Tannins are mainly classified in hydrolysable and condensed types. To the first one belong gallotannins and ellagitannins, also known under the common place misnomer of “tannic acid”. Polyflavonoid tannins belong to the second class, these being rarely hydrolysable (Pizzi 1994).

1.1. Major Sources

There are many sources of tannins. They are sourced from numerous trees and small bushes rich in tannins. Among commercial tannins extracted industrially for their economic value for

leather tanning and other applications are black wattle or black mimosa bark *Acacia mearnsii* (black mimosa/wattle) bark extract, *Schinopsis balansae* or *lorentzii* (quebracho) wood extract, *Quercus spp.* (Oak) bark extract, *Castanea sativa* (chestnut) wood. To a lesser extent commercial tannins are also extracted from *Acacia catechù*, and *Uncaria gambir* (Cube Gambier), *Acacia Mangium*, *Rhus coriaria* (sumach), *Terminalia* and *Phyllantus* tree species (myrabolans), *Caesalpina coraria* (divi-divi), *Caesalpinia spinosa* (Tara), *Algarobilla chilena*, the wood of mangroves, numerous varieties of pines bark and firs such as *Pinus radiata* and *Pinus nigra*. There are of course an enormous number and variety of other plants from which tannins can be extracted.

The geographical areas of commercial tannin extracts industrial production are several: (i) mimosa tannin in South Africa, Brazil, India, Tanzania, Zimbabwe. (ii) Quebracho tannin in Argentina. (iii) Mangrove and Cube Gambier in Indonesia. (iv) Chestnut tannin in Europe. Pine bark tannins in New Zealand, Turkey and Chile. Several small producers do exist also in several other geographical areas such as in Poland for oak tannins and France for a grape pip tannins.

2. CHEMISTRY OF TANNINS

2.1. Tannins structures

Tannins are natural polyphenols. Condensed tannins are flavonoid units oligomers (Fengel and Wegener 1984; Tang et al 1991; Haslam 1966; Roux 1992) while hydrolysable tannins are also recognized to-day as oligomers of carbohydrates-polyphenol units (Pasch and Pizzi 2002; Radebe et al 2013a) although incorrectly thought in the past to be non-oligomeric (Haslam 1966; Roux 1992; Tang et al 1991).

2.2. Hydrolysable Tannins

Hydrolysable tannins extracts have several main constituents. Originally the two positional isomers of equal 935 mass castalagin (I, Fig. 1, 934 g/mol) and vescalagin, in about 14% and 16% weight proportion in the extract were considered to be the two main constituents of ellagitannins (Fengel and Wegener 1989; Tang et al 1991) with a lower proportion, around 6%-7% of the positional isomers castalin and vescalin (II, Fig. 1, 632 g/mol) (Fengel and Wegener 1989; Tang et al 1991), 6% gallic acid, and 3% pentagalloyl glucose monomer. The point to note is that the tannin structure is formed by carbohydrates which are covalently bonded with multiple links to the phenolic part of the extract, thus the carbohydrates are a dominant part of the tannin.

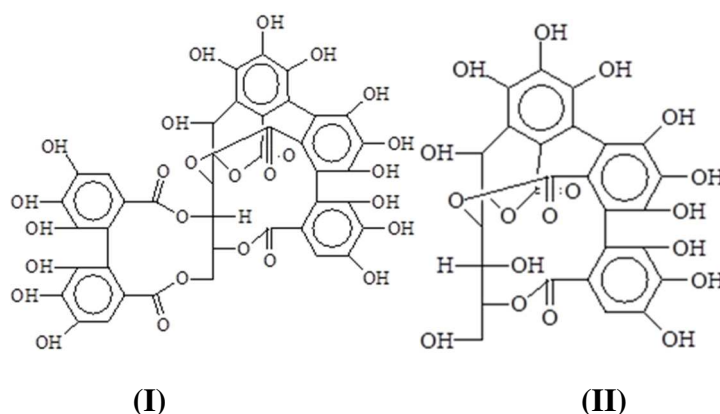


Figure 1. Castalagin (I) (934 g/mol) and vescalin (II) (632 g/mol) structures present in chestnut wood hydrolysable tannin extract

However, castalagin, vescalagin, castalin and vescalin have been found eventually to be rearrangements of the real tannin units occurring mainly during the extraction. Several other tannins of this class are a mix of gallic and ellagic acids, of gallic and digallic acids esters of a sugar, and ellagic acid. However, at 25.4% (or higher) by weight the main constituents was shown to be an unknown compound the structure of which was not determined due to its very high molecular weight (Tang et al 1991). More recently, this main constituent, or better mix of closely related constituents, has been identified for the hydrolysable chestnut wood tannin extract (Pasch and Pizzi 2002). The real structure of this tannin as it is in its natural state before extraction, and conserved in this major fraction of the extract, has been shown to be a series of oligomers formed by the repeating structural unit pentagalloyl glucose shown in Fig. 2. This confirmed that castalagin, vescalagin, vescalin and castalin are just hydrolysis rearrangements of the tannin real native structure (Pasch and Pizzi 2002).

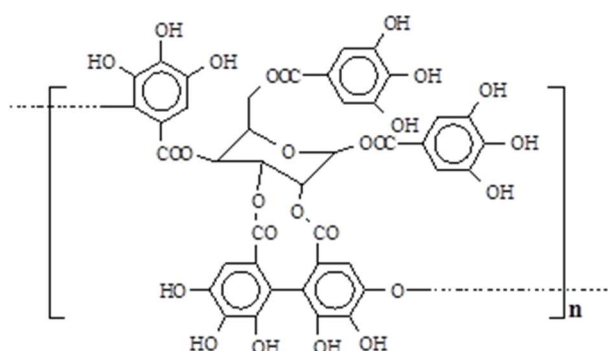


Figure 2. Schematic representation of the pentagalloyl glucose repeating unit of chestnut wood hydrolysable tannin extract oligomers

The classification of the hydrolysable tannins in gallotannins and ellagitannins derives from the hydrolysis products obtained: gallic acid and glucose for the former and ellagic acid (two aromatic rings) and glucose for the latter. There are variations on this base theme, thus, gallo-tannins of tara (gallic acid plus quinic acid, and glucose) and caffeic-tannins (quinic acid esterified with caffeic acids and glucose), also occur (Giovando et al 2013; Radebe et al 2013a) (Figure 3).

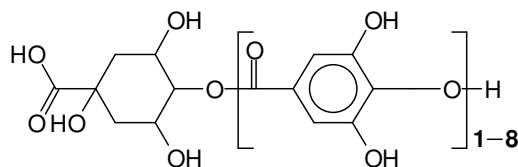


Figure 3. Example of the structures of tara tannin and caffe-tannins (quinic acid esterified with caffeic acids plus glucose compounds).

Several commercial tannins are hydrolysable tannins such chestnut (*Castanea sativa*) wood, myrabolans (*Terminalia* and *Phyllanthus* tree species), divi-divi (*Caesalpinia coriaria*), tara, algarobilla, valonea, oak and others.

2.2.1. Gallotannins

Hydrolysable gallotannins are often incorrectly named “tannic acid”, this really being a mix of very similar compounds, such as penta-(digalloyl)-glucose and tetra-(digalloyl)-glucose or tri-(digalloyl)-di-(galloyl)-glucose, and similar others (Pizzi et al 2009; Giovando et al 2013; Radebe et al 2013a).

2.2.2. Ellagitannins

Oxidative coupling reactions of galloyl fragments leads to the presence of additional structures in ellagitannins (Pizzi et al 2009), a progressive enzymatic oxidation of gallotannins being the cause of the biosynthetic pathway generating them. First a pentagalloylglucose unit is oxidized to form an ellagitannin unit. This then dimerizes by specific oxidation, enzyme-catalyzed, of the ellagitannin unit with the galloyl group of another tannin unit. In this way a dimer presenting a valoneyl group is formed, this reactions cycle continuing to yield oligomers of progressively higher molecular weight. Chestnut wood tannin extract is an example of ellagitannin.

2.3. Condensed (Polyflavonoid) Tannins

More than 90% of the total world industrial extraction of tannins (>220,000 tons per year) at present is composed of polyflavonoid tannins (Pizzi 1983). The limit is only imposed by the number of extraction factories in operation because the potential for their worldwide extraction from a variety of sources is of the order of millions of tons. These tannins are widely distributed in nature. The wood and bark of a number of tree species are particularly rich sources due to their high concentration. Other abundant sources as Waste grape skins in wineries are also an interesting source of these tannins. To this class of tannins belong *Acacia* (wattle or mimosa bark extract), *Schinopsis* (quebracho wood extract), *Tsuga* (hemlock bark extract), and *Rhus* (sumach extract) and some pine barks tannins which are commercially and extensively exploited.

The flavonoid monomer units of these tannins is shown in Figure 4. These units are either linked C4 to C6 or C4 to C8 (Fig. 5), this depending from the tannin type

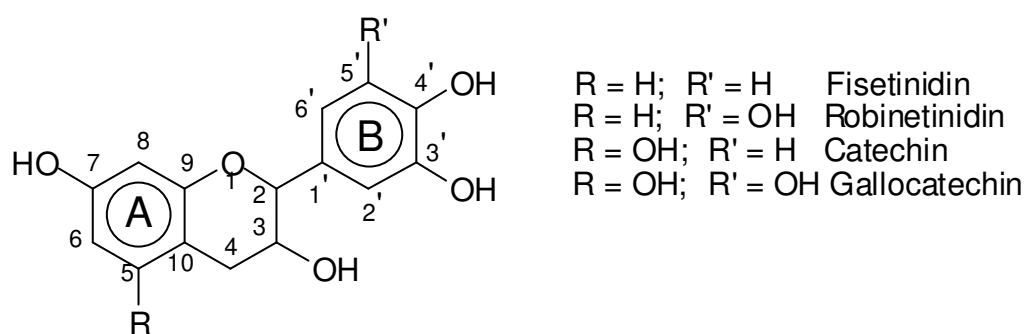


Figure 4. Examples of the types of flavonoid repeating units present in condensed tannin oligomers.

The tannin oligomers of quebracho and mimosa tannins are composed of 2 to 10-11 of these units linked together (Pizzi 1983; Pasch et al 2001). Their mean degree of polymerization is

4 to 5, while it is of 6 to 7 for the soluble fraction of pine tannin extract (Radebe et al 2013b).

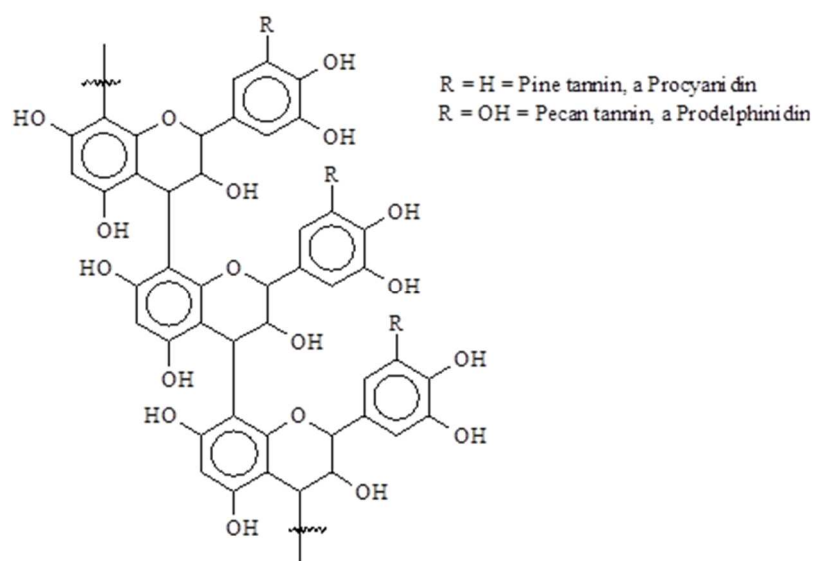


Figure 5. Example of condensed tannin oligomer in which the units are all linked C4-C8. This configuration is characteristic of predominantly procyanidin tannins, thus formed by catechin, epicatechin and gallocatechin (equally called delphinidin) (cf. Figure 4)

Commercial tannin extracts often contain substantial amounts of other constituents, the polyphenolic content being in general guaranteed by the manufacturer to be between 75% and 80% by weight. These additional compounds tend to impact and alter noticeably the viscosity of water solutions of the tannin extracts as in the majority they are hemicelluloses oligomeric fragments, thus short variable length carbohydrates chains. Simple carbohydrates (hexoses, pentoses, and disaccharides) and short carbohydrate chains have also been found linked to the flavonoid units themselves (Figure 6) (Abdalla et al 2014; Drovou et al 2015).

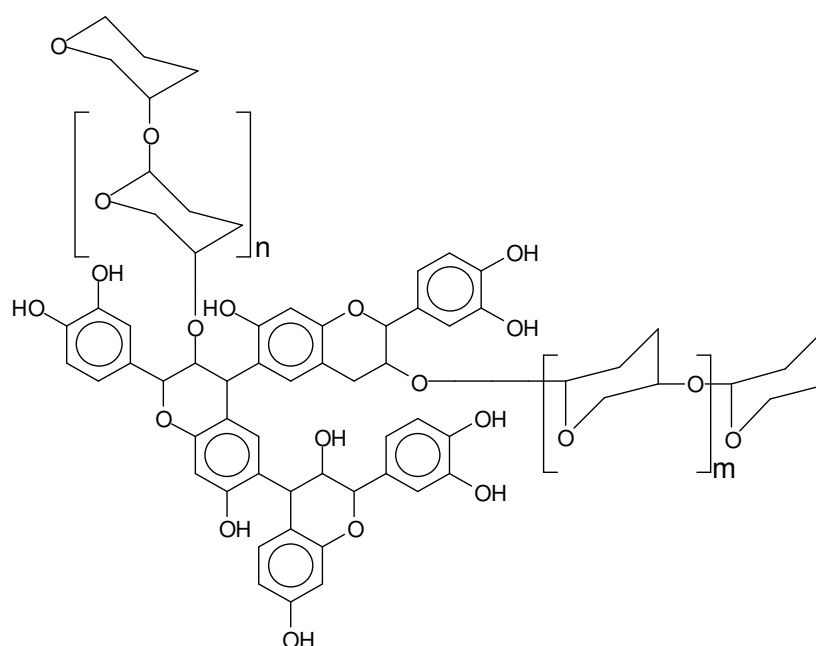


Figure 6. Example of 4-6-linked profisetinidin (fisetinidin trimer) and carbohydrates linkages (Drovou et al 2015). The closed chair carbohydrate units are represented without their numerous-OH groups for ease of visualization.

Low percentages of monoflavonoids (flavan-3,4-diols, flavan-3-ols, dihydroflavonoids, flavanones, chalcones, and coumaran-3-ols) and some glucuronates are also present in the extracts (Roux and Paulus 1961; Drewes and Roux 1963; Roux 1972; Roux et al 1975).

Different couplings of A and B aromatic rings are present in different tannins. Flavonoid units presenting a coupling of resorcinol A rings and pyrogallol B rings are predominant (70% by weight of the polyphenolic part) in mimosa bark tannins. This is doubled by flavonoid units possessing a parallel but secondary coupling of resorcinol A rings and catechol B rings (25% by weight) (Roux 1965). About 5% of the remaining flavonoid units present a phloroglucinol A-ring and a catechol B-ring coupling. This latter units are generally either the lower terminal units of oligomers or the central units of the so-called “angular tannins”, a potential branching point (Pizzi 2009). The extract balance is made up by the so-called “non-tannins” being composed of hydrocolloid hemicelluloses fragments (3% to 6% by weight) strongly influencing extract viscosity, mono and di-saccharides, and traces of amino and imino acids (Roux 1965; Pizzi 1983). Similar A-ring and B-ring couplings exist for quebracho tannin with a predominance of catechol B-rings (*Schinopsis Balansae*, and *Lorentzii*) (King and White 1957; Clark-Lewis and Roux 1959; Roux and Paulus 1961; Roux 1975; Crestini et al 2016) and a lower proportion of phloroglucinol A-rings. These types of couplings occur also in hemlock and Douglas fir tannins. Only phloroglucinol A-rings and catechol or pyrogallol B-rings occur instead couplings in pine tannins and other procyanidins (Porter 1974; Rossouw et al 1980; Hemingway and McGraw 1983). A great minority coupling of phloroglucinol A-ring and phenol-ring has also sometime been reported (Porter 1974; Hemingway and McGraw 1983). The total predominance of the phloroglucinol A-ring in these procyanidin tannins accelerates their reactivity, for instance with aldehydes.

3. PHARMACEUTICAL AND MEDICAL APPLICATIONS

Tannins are known bactericides because they react with proteins irreversibly, thus complexing within bacterial membranes, neutralizing their activity. As a consequence, tannin-based pharmaceuticals to cure intestine infections have long-time been marketed. They have effective anticancer properties. Tannins have also many applications for other pharmaceutical/medical uses but all these are targeted for future use rather than the present.

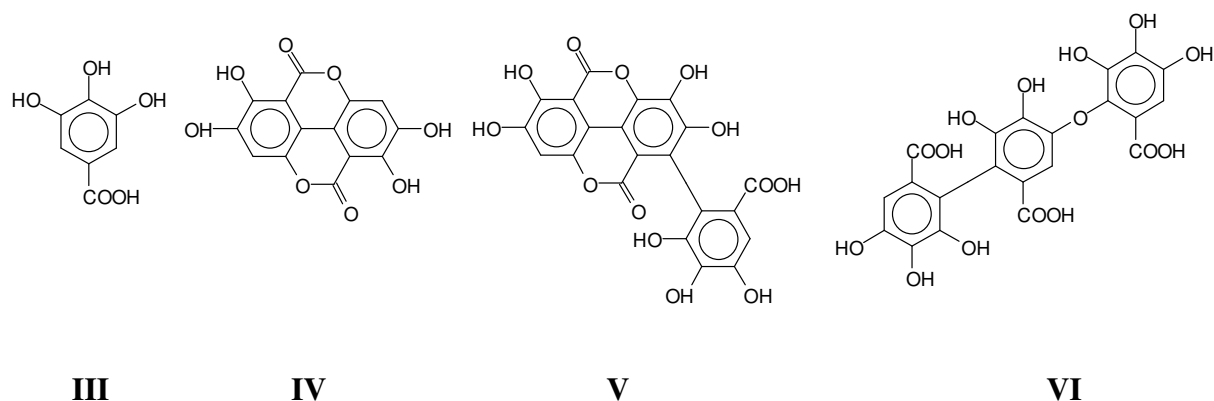
Chestnut tannin, the major exponent of the commercial hydrolysable tannins class has been proposed as an antimicrobial or for reducing mycotoxins (Bargiacchi et al 2019; Miele et al 2019). Other applications proposed for hydrolysable tannins, both gallotannins and ellagitannins, is for treating bacterial infections (Mathee et al 2009), cytokines secretion regulation (Feldmann 2000), antihistamine, antiasthma and for preventing rhinitis (Nakahara et al 1996), and for inhibiting HIV propagation in human cells (Lee et al 1989). Report of effectiveness of different tannin-derived compounds against obesity, arteriosclerosis and thrombosis as well as lowering triglycerides (Hayashi et al 2001), and for inhibiting *Staphylococcus aureus*, other Gram-positive bacteria and for leukaemia treatment (Chen et al 2012) have also been reported.

Equally patents on the use of non-commercial tannins for the treatment of cognitive, neurodegenerative and metabolic disorders, diabetes 2 and obesity (Rinsch and Dupraz 2019), hypertension and hypercholesterolemia (Lopez-Mas et al 2008) have been reported.

A great number of species can be used to extract tannins with medical/pharmacological activity. As regards all the tannins, condensed or hydrolysable, all present anti-inflammatory, bactericidal and antimicrobial activity.

Among the condensed tannins are those extracted from *Acacia mearnsii* (sold commercially as mimosa or wattle bark tannins) (Pasch et al 2001; Xiong et al 2016; dos Santos et al 2017) and *Acacia nilotica* (Rather et al 2015; Safari et al 2016) both presenting on top of the general tannins activities being also antipyretic, with the first also presenting a proven antidiarrheic activity (van Zyl 1972). Spruce bark (*Picea abies*) tannins presenting antioxidant activity on top as the same as the preceding two (Raitanen et al 2020). Quebracho wood (mixed species of *Schinopsis lorentzii* and *Schynopsis balansae*) commercial tannin extract from Argentina (Pasch et al 2001) presenting also anthelmintic activity on top of the general ones of all tannins. Sumac (*Rhus coriaria*) tannin extract, a mixed hydrolysable (mainly) and condensed tannin (Pasch and Pizzi 2002) has been proposed for the general activities (anti-inflammatory and antibacterial) but also as presenting immunomodulatory activity (Khalilpour et al 2019; Isik et al 2019). Applications for treatment of blood pressure, hypertension and in particular for calming hemorrhoidal problems (the flavonoid quercetin) have now been commercial for considerable time already.

In the same context hydrolysable tannins have been proposed for a number of pharmacological activities, the anti-inflammatory, bactericidal and antimicrobial activity being always present. The main commercial tannin of this class, Chestnut (*Castanea sativa*) wood extract is reported to present moreover antidiabetic, cardioprotective, antifungal and antidiarrheic activity (Brizi et al 2016; Chiochio et al 2020; Cardullo et al 2018; Chiarini et al 2013; Silva et al 2020; Stumpf et al 2020; Reggi et al 2020; Esposito et al 2019; Bonelli et al 2018). The predominant components of this tannin extract are structures I and II (Fig. 1) and pentagalloyl glucose oligomers (Fig. 2) and low amount of small molecular weight species as gallic acid (III), Ellagic acid (IV), flavogallonic acid (V) and valoneic acid (VI).



Walnut (*Juglans regia*) and the related Pecan (*Carya illinoensis*) (Pasch et al 2001) from the interior pellicles tannin extracts are reputed to be antiplatelet and cardioprotective (Reguero et al 2014; Papoutsis et al 2008; Sun et al 2018) and is mainly composed of delphinidin flavonoids (gallocatechin). Oak (*Quercus robur*) tannin extract is reputed to be antioxidant and antidiabetic (Muccilli et al 2017; Horvathova et al 2014; Natella et al 2014). Common

grape vine (*Vitis vinifera*) tannin extract (from grape skins and grape pips) (Ricci et al 2017) is known for being among others, a antiobesity agent (Arunkumar and Rajarajan 2018). Pomegranate (*Punica granatum*) peel tannin extract (Saad et al 2015) is reported to have antiviral (hepatitis B and herpes simplex) activity as well as being antidiabetic. There are many other sources of hydrolysable tannins, some commercial such as the Tara (*Caesalpinia spinosa*) pods tannin extract from Peru, Myrabolans (*Terminalia chebula*) tannin extract, and many other sources from species not exploited commercially but that could well be.

While many studies have been conducted on a variety of tannins derived from a wide variety of plants as an anticancer treatment, some studies on the possibility of using tannin for other medical applications have also been highlighted. Condensed tannins are traditionally used for the treatment of intestinal problems (Quideau et al 2005; Funatogawa et al 2004) or urinary infections (Noce et al 2020). This is due to their complexation ability with other molecules and their antioxidant behavior. The extract of *Stryphnodendron rotundifolium* and of other tannins has proven their effectiveness against ulcers by functioning as a protective coating of the gastrointestinal tract (Fiori et al 2013; Audi et al 1999; Lai et al 2016). Other possible mechanisms of action of phenolic plant extracts as herbal medicines against ulcers and gastritis have also been described (Saito et al 1998; Audi et al 1999; Lai et al 2016). Tannins from myrabolans (*Terminalia chebula*) fruits have been reported to attenuate induced anxiety-like behaviour via modulation of neurotransmitters (Chandrasekhar et al 2018) and in traditional Asian medicine have been used as a good astringent dentifrice in loose gums, and for bleeding and ulceration in gums.

There are several fields of medical and pharmaceutical application of tannins that merit a deeper insight.

3.1. Cancer, Anticancers and Virucides: a critical review

While there are a considerable number of studies on the anticancer and virucide properties of tannins these have concentrated on (i) their positive effects, without considering the downside of their toxicity to the patient treated, and (ii) frequently, but fortunately not always, on non-commercial vegetal species the tannins of which are found with difficulty, and (iii) on the mechanisms of action of these materials on various viruses and cancers.

All this is very laudable but what is of importance in the use of these materials for medical applications against viruses are the Minimum Inhibitory Concentration (MIC) required to reduce virus-induced cytopathogenicity by 50 per cent, and even more important, to simultaneously determine the Minimum Cytotoxic Concentration (MCC) required to cause a microscopically detectable alteration in the normal cell morphology. The lower the MIC value, the better is the antiviral capacity of the tannin tested, while simultaneously the higher the MCC, the less toxic is the tannin to the patient cells and safer it is as an antiviral compounds. Consequently, what is sought for any compound, here of a tannin, to be a valid antiviral is the lowest possible MIC and the highest possible MCC. Only with these two conditions are satisfied one can talk of a valid antiviral remedy. This fundamental balance is ignored in the numerous studies on the virucidal capacity of tannins, and very few studies are published where MIC and MCC are measured against a variety of virus types.

The second neglected critical point is that the use of scarce species to be extracted for unusual tannins does not allow the amount of material needed for a worldwide therapeutical

application if one of these tannins is found with the due balance of MIC and MCC. Thus, a study of the tannins antiviral effectiveness should first be focused on tannins that are easily obtainable in quantity, thus on tannins that are extracted already commercially/industrially for other purposes, or that can be extracted with ease of availability of plant materials by existing tannin extraction factories.

There are very scarce data combining the two requirements mentioned, namely MIC and MCC measurement and commercial or potentially commercial availability. In one of these rare studies (Pizzi 2008), different commercial tannins appeared to be rather effective to counteract several viruses. The most logical explanation of their effectiveness is the capacity of the polyphenolic tannins to complex proteins and to associate with the carbohydrates of the virus membrane. Such a complexation is analogous to their tanning hide proteins to give leather. While this interaction is mainly due to a sum of secondary forces between tannin and a protein, any protein, more recently modern techniques have shown in a different field that both covalent and ionic bonds, thus even stronger linkages, do form between tannins and proteins by the amino groups of the non-skeletal side chains of aminoacids such as arginine, lysine, hydroxylysine and possibly others aminating the phenolic hydroxyl groups of the tannin, and by esterification by the carboxylic acid side chain groups of aminoacids such as glutamic and aspartic acid esterifying the aliphatic alcoholic hydroxyl on the C3 site of the heterocycle ring of some flavonoid tannin units heterocycle (Pizzi 2021; Ghahri et al 2018ab, 2021).

In this *in vitro* study (Pizzi 2008), MIC and MCC were determined for a number of commercially available hydrolysable and condensed tannins which have been tested, namely mimosa (*Acacia mearnsii*) bark tannin extract and its derivatives, chestnut (*Castanea sativa*) wood tannin extract, tara (*Caesalpinia spinosa*) pods tannin extract, quebracho (*Schinopsis lorentzii* and *Schinopsis balansae*) wood tannin extract both sulphited and natural, pecan (*Carya illinoensis*) nut membranes tannin extract, pine (*Pinus radiata*) bark tannin extract, sumac (*Rhus coriaria*) tannin extract, and spruce (*Picea abies*) bark tannin extract (Pizzi 2008). The viruses against which all these have been tested are highly varied, such as HIV-1 and HIV2, Herpes simplex 1 and 2, Vaccinia virus, vesicular stomatitis virus, Coxsackie virus B4, respiratory syncytial virus, Influenza A H1-N1, Influenza A H3-N2, Influenza B, Human Corona virus, Reovirus-1, Feline Corona virus, Sindbis virus, para-influenza 3 virus, and Punta Toro virus (Pizzi 2008). Examples of the results obtained are shown in Tables 1, 2 and 3. For more results the reader should refer to the original work

The results with these tannins were also compared with the MIC and MCC of known antiviral drugs, to assess at what kind level was their medicinal effectiveness. Extensive correlation tables are reported in this study, but the best effectiveness tannin type against type of virus were as follows: mimosa bark tannin extract against Herpes simplex Virus and Vaccinia virus (for both MIC 16 µg/ml; MCC 200 µg/ml). Tara+Chestnut tannins mix against Vesicular stomatitis virus (MIC 43 µg/ml; MCC 400 µg/ml). Pecan nut tannin against Respiratory syncytial virus (MIC 9 µg/ml; MCC 400 µg/ml) and mimosa bark tannin for the same virus (MIC 12 µg/ml; MCC 400 µg/ml). In this respect a relevant example is the one of Sumach tannin extract which presents good MCCs of only 16 µg/ml against Vesicular stomatitis virus, Coxsackie B4 virus and Respiratory syncytial virus, but that it has a MIC of only 80 µg/ml, being an example that not only the positive effects should be reported but also the cytotoxicity to the patient, before considering a tannin suitable for medical application.

Table 1. Examples of cytotoxicity and antiviral activity of compounds in cell cultures, Herpes and vesicular stomatitis viruses. Tannins added before virus administration.

Cytotoxicity and antiviral activity of compounds in E ₆ SM cell cultures						
Compound	Minimum cytotoxic concentration ^a (µg/ml)	Minimum inhibitory concentration ^b (µg/ml)				
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 TK ⁻ KOS ACV ^r
1 Mimosa tannin	200	40	16	16	>80	40
2 Chestnut tannin	≥40	40	16	16	>80	47
3 Tara+Chestnut mix	≥40	40	48	16	>80	47
4 Quebracho standard	40	47	36	16	>80	47
5 Quebracho purified	40	80	36	16	>80	47
6 Quebracho sulphited	≥40	40	40	16	>80	36
7 Pecan nut tannin	40	16	16	16	>80	36
8 Cube Gambier tannin	8	>80	>80	>80	>80	>80
9 RadiataPine tannin	40	40	>80	16	>80	40
10 Maritime pine tannin	40	>80	>80	80	>80	47
11 Sumach tannin	40	36	36	16	>80	36
23 Spruce tannin	40	>16	>16	>16	>16	>16
Brivudin	>400	0.128	400	16	>400	>400
Ribavirin	>400	>400	>400	400	>400	>400
Acyclovir	>400	0.384	0.128	>400	>400	48
Ganciclovir	>100	0.0064	0.0064	100	>100	2.4
^a Required to cause a microscopically detectable alteration of normal cell morphology.						
^b Required to reduce virus-induced cytopathogenicity by 50 %.						

Mimosa, Chestnut, Quebracho, Radiata pine, Maritime pine tannins had better performance than most known antiviral drugs against Influenza A H1N1 and Influenza B viruses (MIC between 1.4 and 5.5 µg/ml; MCC 100 µg/ml) while even if their performance was

respectable against Influenza H3N2 virus it was less effective than known antiviral drugs. The same 5 tannins performed better than known antiviral drugs against Para influenza-3, Reovirus-1, Sindbis, Coxsackie-B4, and Punta Toro viruses (for all MIC 80 µg/ml; MCC 400 µg/ml).

Table 2. Examples of cytotoxicity and antiviral activity of compounds in HeLa cell cultures, for vesicular stomatitis, Coxsackie and respiratory syncytial viruses

Cytotoxicity and antiviral activity of compounds in HeLa cell cultures				
Compound	Minimum cytotoxic concentration ^a (µg/ml)	Minimum inhibitory concentration ^b (µg/ml)		
		Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus
1 Mimosa tannin	400	>80	>80	12 +/-5
2 Chestnut tannin	400	>80	>80	43 +/-4
3 Tara+chestnut tannin	400	43.4 +/-4	>80	35 +/-7
4 Quebracho standard	400	>80	>80	40 +/-0.2
5 Quebracho purified	400	>80	>80	40 +/-0.2
6 Quebracho sulphited	400	>80	>80	43 +/-5
7 Pecan nut tannin	400	>80	>80	9 +/-1
8 Cube Gambier	400	>80	>80	>80
9 Radiata pine tannin	400	>80	>80	40 +/-0.2
10 Maritime pine tannin	400	>80	>80	40 +/-0.2
11 Sumach tannin	80	>16	>16	>16
12 Spruce tannin	≥80	>80	>80	>80
Brivudin	>400	>400	>400	>400
(S)-DHPA	>400	400	>400	>400
Ribavirin	>400	48	240	9.4

^aRequired to cause a microscopically detectable alteration of normal cell morphology.

^bRequired to reduce virus-induced cytopathogenicity by 50 %.

The same approach, namely effectiveness vs. cytotoxicity must be taken into account for the action of tannins on cancers. The same report (Pizzi 2008) studied the effect of the same tannins on their inhibitory effect on the proliferation of murine leukemia cells, murine mammary carcinoma cells and human T-lymphocyte cells, as their IC₅₀ (the concentration of an inhibitor where the response (or binding) is reduced by half). In brief the IC₅₀ is a quantitative measure that indicates how much of a particular inhibitory substance is needed to inhibit, in vitro, a given biological process or component by 50%, the lower the better.

For murine leukemia cells the best tannin inhibitor was quebracho tannin extract ($IC_{50} = 12 \mu\text{g/ml}$), while Sumach tannin was the best inhibitor for murine mammary carcinoma cells ($IC_{50} = 18 \mu\text{g/ml}$), and Cube Gambier and Sumach being the best inhibitors for human T-lymphocyte cells ($IC_{50} = 17\text{-}18 \mu\text{g/ml}$).

The effectiveness of the same series of commercial tannins was tested for cytotoxicity and antiviral activity human SARS Corona virus, Quebracho tannin at EC_{50} of $44 \mu\text{g/ml}$ and CC_{50} at $>100 \mu\text{g/ml}$ resulting the best in term of their EC_{50} (which is the concentration of a compound that gives half-maximal response) and their CC_{50} , this being The 50% cytotoxic concentration defined as the extract concentration that reduces the cell viability by 50% when compared to an untreated control. Chestnut wood tannin extract performed better than the other tannins against HIV-1, its concentration required to protect cells against the cytopathogenicity of HIV-1 by 50% yield a EC_{50} of $1.4 \mu\text{g/ml}$, but showed the same value of the EC_{50} of all the other tannins at $>20 \mu\text{g/ml}$.

Table 3. Examples of cytotoxicity and antiviral activity of compounds in HeLa cell cultures, for Corona viruses

	<i>Feline CORONA virus (FIPV)</i>		<i>Human Corona (SARS) virus</i>	
	EC_{50} ($\mu\text{g/ml}$)	CC_{50} ($\mu\text{g/ml}$)	EC_{50} ($\mu\text{g/ml}$)	CC_{50} ($\mu\text{g/ml}$)
1 Mimosa tannin	52 ± 19	> 100	> 100	> 100
2 Chestnut tannin	49 ± 17	> 100	> 100	> 100
3 Tara + chestnut mix	43 ± 2	> 100	> 100	> 100
4 Quebracho standard	49 ± 10	≥ 100	44 ± 10	> 100
5 Quebracho purified	55 ± 19	> 100	49 ± 21	> 100
6 Quebracho sulphited	32 ± 1	> 100	40 ± 1	> 100
7 Pecan Nut tannin	72 ± 40	> 100	> 100	> 100
8 Cube Gambier tannin	≥ 100	> 100	> 100	> 100
9 Radiata pine tannin	20 ± 21	> 100	> 100	> 100
10 Maritime pine tnnin	44 ± 5	> 100	56 ± 13	> 100
11 Sumach tannin	7.8 ± 8.0	81 ± 13	> 100	> 100
12 Spruce tannin	63 ± 32	> 100	> 100	> 100

Several experimental studies on the pharmaceutical use of tannins have been published with antitumor and anti-oncogenic activities particularly well documented (Krifa 2014a,b, 2015; Yang et al 1999; Nakamura et al 1999; Myamoto et al 1999; Almalki et al 2021; Kashiwada et al 1992; Pizzi 2008; Liu et al 2016; Quideau et al 2003, 2005; Khalifa et al 2020) but most again with some of the shortcoming indicated above.

Tannins extracted from unusual species have also shown antiviral capability. For instance, an extract of pomegranate (*Punica granatum*) containing mainly punicalagin, gallic and ellagic acids has been tested against herpes simplex virus 2. Punicalagin alone presented significant antiviral activity but when the tannin extract itself was tried a higher virus inhibition concentration was needed (Arunkumar and Rajarajan 2018). Punicalin and geraniin were

found to act against hepatitis B virus (HBV) by interfering with the synthesis, stability or transcription of the viral DNA (Liu et al 2016). Dengue virus has been found to be inhibited in vitro by common nettle (*Urtica dioica*) extracts.

The inhibitory effects of these tannins have also been tested on proliferation of murine leukemia cells, murine mammary carcinoma cells, and human T-cells (Kashiwada et al 1992).

Acutissimin A is a bound flavonoid with an ellagitannin. It is formed by the interaction of a wine flavonoid with the vescalagin generated by the barrel's oak tannin (Arankumar and Rajarajan 2018; Liu et al 2016). Acutissimin A has been found to present an effectiveness 250 times higher to stop tumors growth than the drug Etoposide (Quideau 2003). Khalifa et al (2020) have recently studied the inhibition of SARS-CoV-2 by an in silico approach the effect of with 19 different hydrolysable tannins of different structure.

However, there are critical reports of the clear correlation of excess consumption in foods, beverages and masticatories of high tannins content that have led to the awareness of their carcinogenicity and presenting some danger for human health. This applies to tea (*Camelia sinensis*), but the clearest cases being the one of the tannins of Yerba Maté tea (*Ilex paraguayensis*) (Fig. 7) in Argentina and Khat (*Catha edulis*) which when abundantly consumed over ten or twenty years have been found to trigger esophageal and tongue cancer in excessive consumers (Morton 1989).

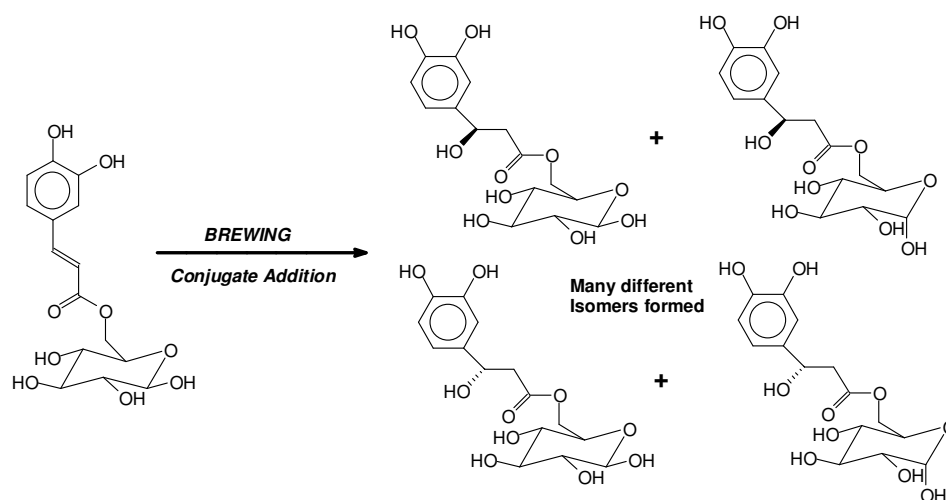


Figure 7. Yerba Maté tea (*Ilex paraguayensis*) toxic isomers formation with hot brewing

A study on bush-tea and popular medicine plants in Curacao, namely *Acacia glauca*, *Melochia tomentosa* and *Krameria ixina*, produced tumors in 100% of the tested laboratory animals. The earliest surge of tumors was caused by *Krameria*, this being the main component of bush-tea in some northern Venezuela areas where occurs the highest incidence of esophageal cancers. Esophageal cancer high risk areas correlations of this type were also found in the teas in the cancer belt of south Russia, north Iran, and Turkey, by red sorghum in northern China Honan region, In South Africa Transkei region by tea and red sorghum, in Curacao by tannin-rich red sorghum and popular remedies, in north France by high-tannin apple cider, and in southern Chile Aysen region by *Krameria cistoidea* and *Limonium guaicuru* which are traditional popular medicine plants (Morton 1989).

3.2. Antimicrobials

Tannins bactericidal, antifungal and antiviral properties are proven and have been described in many articles (Smeriglio et al 2017; Pizzi 2019). The reasons for such effect is to be found in their characteristic action of binding irreversibly to proteins, the same mechanism outlined above in the critical review of their anticancer and virucidal capability. Stronger linkages, on top of secondary forces linkages do form between tannins and proteins mainly by the amino groups of the non-skeletal side chains of some aminoacids such as arginine, lysine, hydroxylysine and possibly others aminating the phenolic hydroxyl groups of the tannin. Esterification by the carboxylic acid side chain groups of aminoacids such as glutamic and aspartic acid of the alcoholic C3 hydroxyl of the heterocycle ring of flavonoid tannin units also appear to (Pizzi 2021; Ghahri et al 2018ab, 2021) (Fig. 8).

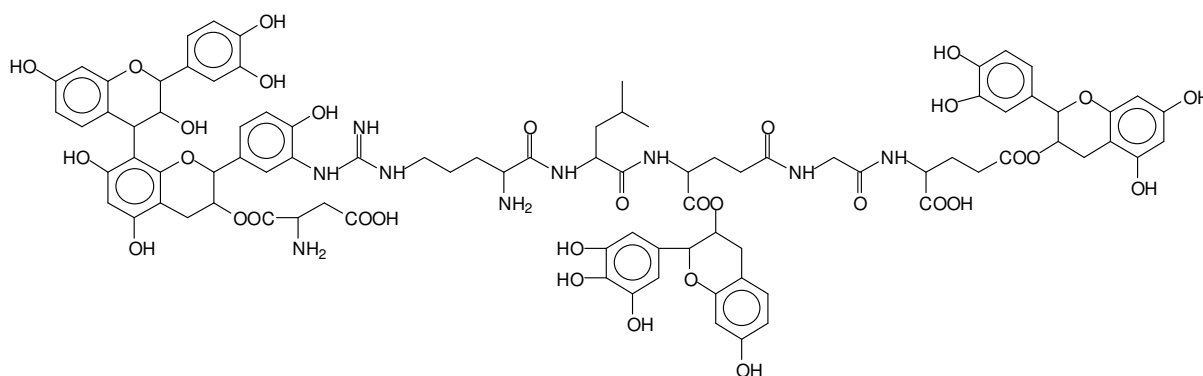


Figure 8. Example of condensed tannin dimers and monomers linked to a polypeptide sequence from collagen. The figure shows a sequence of aspartic acid-(fisetinidin-catechin dimer)-arginine-leucine-aspartic acid-(robinetinidin)-glycine-glutamic acid-catechin (Pizzi 2021).

Again, the relevant studies in this area are divided between tannins from unusual and rather scarce species and commercial tannins. Thus, walnut leaves have recognized antimicrobial effect and are an approved treatment to relieve mild skin inflammation. Oak fruits ethanol extract at high doses inhibits the growth of methicillin-resistant *Staphylococcus aureus* while at low dosage reduces bacterial adherence. Its content is of gallic acid, ellagic acid, and their derivatives, flavonoids such as quercetin, catechin, naringin and higher molecular weight tannins. The use of this same extract has been consequently advised as a treatment for skin and soft tissue infections (Quave et al 2008). In the case of the Kaki Persimmon (*Diospyros kaki*) tannins, mainly composed of catechin, catechin-3-gallate, gallic acid, and gallic acid-3-gallate units bound into condensed tannins oligomers (Fig. 9), the mechanism of action appeared to be due to the physical damage of the bacterial proteic cell walls (Liu et al 2020).

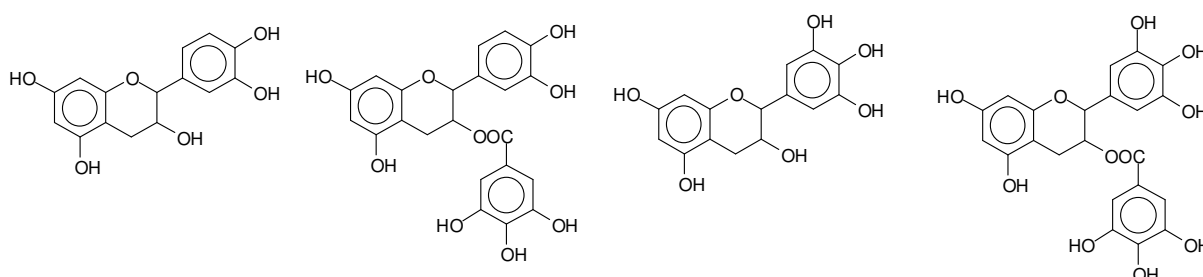


Figure 9. From left to right: catechin, catechin-3-gallate, gallicocatechin, and gallicocatechin-3-gallate

This is logical if one thinks that tannins do “tan” by interacting heavily with proteins by secondary forces and even react covalently with hide proteins in leather making disrupting their spatial structure (Pizzi 2021; Ghahri et al 2018a,b, 2021). The most common commercial mimosa (*Acacia mearnsii*) tannin extract showed bactericidal character against the growth of *Staphylococcus aureus*, against *Aspergillus niger*, against *Escherichia coli*, and against *Candida* sp.(dos Santos et al 2017) as the equivalent quebracho (*Schinopsis* spp) wood extract (Saraiva et al 2013).

Tannins bactericidal mechanism also appears to inhibit the bacterial ion pumps in their effect against *Helicobacter pylori* (Martini et al 2009) as shown by the tannins of thornless wild blackberry (*Rubus ulmifolius*). The same tannin showed antiameobal and bactericidal activity. It also showed excellent activity against the infected wounds by *Escherichia coli*, *Staphylococcus agalactiae* and *Candida albicans* but less effective against others (Hajaji et al 2017). Its methanol extract showed to possess notable antifungal capability against filamentous fungi (Sisti et al 2008). Chestnut (*Castanea sativa*) tannin components from different plant parts such as shells, shells inner membranes and leaves have shown bactericidal capability. They were also capable of inhibiting the growth of some Gram-positive and Gram-negative bacteria (Silva et al 2020). Two of the main purified components of commercial Chestnut wood tannin extract, namely vescalagin and castalagin, have shown bactericidal capacity on *Escherichia coli*. Moreover, the raw tannin extract of Chestnut wood presented an even stronger bactericidal action than the purified vescalagin and castalagin derived from it, due possibly to the concurrent sum of effects of the other molecules present in it (Elizondo et al 2010; Reggi et al 2020).

3.3.Antioxidants

The antioxidant effect of tannin is now starting to be exploited for a number of different applications, such as in food conservation for example. The antioxidant effect of tannins is due to the characteristic of any phenol to stabilize free radicals and to inhibit further damage these may otherwise cause. In the medical field the antioxidant effect of tannins is well known and there are numerous articles on the subject. A critical review of these articles shows that they again appear to concentrate on the tannins positive antioxidant effects without really defining what are the parameters that need to be considered to define their antioxidant activity and to correlate these with the level of antioxidant activity found.

It is critical to understand that a number of parameters affect the radicals control of tannins, these being (Noferi et al 1997) in no particular order of importance: (1) the ease or difficulty of tannin solvation (2) the proportions of hydroxyl groups on their A- and B-rings, (3) the heterocyclic pyran C-ring ease of cleavage opening, (4) their colloidal state in water, (5) their interflavonoid bond conformation. These are the parameters determining the capacity of a tannin to act as an antioxidant. The reasons for the influence of these parameters are because the tannin's antioxidant ability is measured by two different variables (Noferi et al 1997):

(1) The rate of decay of a tannin phenoxyl radical. This indicates the level of stability as a function of time of such a radical. To slower decay rates correspond a lower tannin-added material degradation. This indicates that the tannin is a better antioxidant.

(2) The rate of uptake of a radical by a tannin from another material's radical. This reflects how easily a tannin is able to uptake free radicals from other materials. The faster and easier is the uptake rate the more antioxidant is the tannin.

To define tannins antioxidant activity in function of these variables is of fundamental importance in the medical/pharmaceutical field both their anti-inflammatory and wound repair ability depend and is related with their antioxidant capability.

There are many studies on the effect of tannins as antioxidants in the pharmaceutical/medical field (Rodriguez-Perez et al 2019; Sienawska 2015), and on a variety of different tannins (Sung 2012, Cardullo et al 2020). For example, purified tannic acid, a generic term for hydrolysable tannins, prevents photo-damage by partially inhibiting oxidation levels and photoageing (Daré et al 2020). Condensed tannins are also known to have this characteristic as particularly apt at protecting human skin from solar UV radiation and radioactive surges. Further studies have shown that tannins structures such as galloyl moieties, catechin and catechin gallate inhibit lipid peroxidation (Okuda et al 1983; Iwata et al 1987; Beninger and Hosfield 2003; Tian et al 2012; Horvathova et al 2014). The antioxidant characteristics of a purified extract from commercial mimosa tannin (*Acacia mearnsii*, formerly *mollissima*, de Wildt) appeared to reverse the negative effects of acroleine (propenal, an unsaturated aldehyde, related to neural degenerative illnesses) induced cytotoxicity in human neuroblastoma cells (Huang et al 2010) and cells apoptosis inhibition.

Research work on walnut (*Juglans regia*) tannins have shown that its hydrolysable ellagitannins, such as digallic acid glucoside can release ellagic acid a known antioxidant (Papoutsi et al 2008). Its main constituents are 3-hexahydroxydiphenylglucose, pedunculagin and 2,3,4,6-tetragalloylglucose. Pedunculagin (Fig. 10) was the predominant constituent presenting strong antioxidant activity of the extract of walnut pellicles.

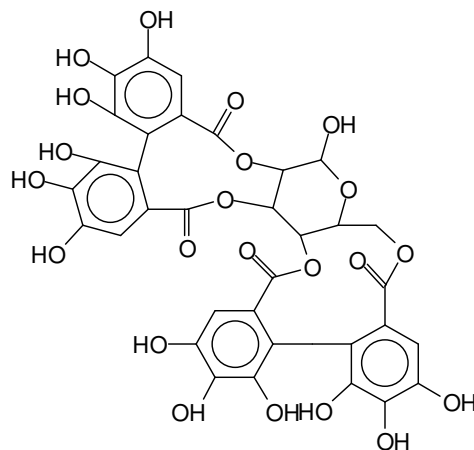


Figure 10. Structure of pedunculagin

Examples of the tannins use as food antioxidants are also on record and this application is growing. For example, purified tannin from quebracho wood has been used as meat patties antioxidant (Fruet et al 2020).

3.4. Antinflammatory

The antinflammatory action of tannins is one that is intimately related to their antioxidant characteristics (see above). There are a number of recent works on the anti-inflammatory action of tannins and some even addressing their mechanism of action or identifying the substances which allow such an effect. Slight antipyretic effects are reported with *Acacia* spp. tannins (Safari et al 2016). Again, purified mimosa (*Acacia mearnsii*) tannin extract inhibited cytokines expression and inflammatory enzymes, the action being ascribed to the richness of this extract in its mixed composition of profisetinidin (30%) and prorobinetinidin (65%) oligomers, with some catechin (5%) present too (Roux 1965; Pizzi 1983). Chestnut (*Castanea sativa*) spiny burs tannin extract, a hydrolysable gallotannin, showed also anti-inflammatory effects showing cytoprotection in tumor necrosis factors (Chiocchio et al 2020). Among the hydrolysable tannins Sumach (*Rhus coriaria*) extract has showed treatment, prevention and reversion capacity of necrotizing enterocolitis, as well as showing anti-inflammatory, immunomodulation and anti-apoptosis capacity (Isik et al 2019). Among the condensed tannins procyanidins tannins (formed exclusively of linked catechin and epicatechin) from grape pips reduced hyperlipidic diets inflammation (Terra et al 2009; Park et al 2014). It is to note that procyanidins tannins of equal type are also available from a variety of other sources, such as spent grape skins, pine bark and others. As tannins anti-inflammatory and antioxidant effects are effective against many illnesses or infection due to, or based on, oxidation and inflammation such as heart infections, diabetes and wound repair.

3.5. Antidiabetic

Tannins, both hydrolysable and condensed, from a number of sources such as Chestnut (*Castanea sativa*), Oak (*Quercus robur*), Quebracho (*Schinopsis lorentzii*) and Myrabolans (*Terminalia chebula*) have been reported to present antidiabetic effect as such disease is related to cellular inflammation and oxidation processes. Well-known are the works of Senthilkumar and Subramanian (2008) on effect of myrabolans on the levels of glycoproteins in induced experimental diabetes in rats, and of Matsumoto et al (2012) on the effect and its mechanism of persimmon kaki (*Diospyros kaki*) tannin on type 2 diabetes in mice. In particular hydrolysable tannins appear to inhibit alpha-glycosidase which regulates carbohydrates absorption presenting so effectiveness against type 2 diabetes mellitus or low glucose tolerance (Cardullo et al 2018). Main alpha-glucosidase inhibitors compounds in hydrolysable tannins which have been reported are valoneic acid dilactone (Cardullo et al 2018), monogalloyl glucose, hexahydroxy diphenyl glucose (digallic acid glucoside, monoellagic acid-glucoside), castaline, vescalagine and gallic acid with castalagin having the major effect (Muccilli et al 2017). For condensed tannins the main compounds from Quebracho tannin reported for alpha-glucosidase and alpha amylase inhibition were flavonoid oligomers such as mixed dimers to pentamers of fisetinidin, catechin and catechine gallate, and from the hydrolysable fraction of the same tannin from digalloyl quinic acid esters topentagalloyl quinic acid esters (Cardullo et al 2018, 2020).

3.6. Cardiovascular protection

Tannins have been found to have antiplatelet and anticoagulant properties. Research work on walnut (*Juglans regia*) tannins have shown that its hydrolysable ellagitannins, such as digallic

acid glucoside can release ellagic acid a known antioxidant (Papoutsi et al 2008). This extract and ellagic acid itself has been shown to limit the severity of myocardial infarctus (Sun et al 2018). Red raspberry (*Rubus idaeus*) extract was found to decrease by 20 percent or more the expression of the glycoprotein involved in the reception of fibrinogen and platelet activation with a decrease of almost 50% of platelet aggregation and when tested in blood platelet aggregation inhibition reduced to less than 20% (Dudzinka et al 2016). Antiatherosclerosis and cardioprotective action was shown to occur in vivo by the seeds extract of *Acacia senegal*, with a reduction of low density lipoprotein (LDL) and total cholesterol and triglycerides (Ram et al 2014). Aortic noradrenaline-induced contraction was decreased by Chestnut (*Castanea sativa*) wood extract simultaneously diminishing heart pulse rate. Such results justify the use as dietary supplement of Chestnut wood extract as it affords both beneficial cardioprotection and antioxidant functions (Chiarini et al 2013).

3.7.Wound repair

This tannins effect is also somewhat related their antioxidant activity. *Rhus imperialis* and *Rhus coriaria*, which are two different kinds of Sumac tannin extract have shown interesting results as cytoprotective, wound healing and anti-inflammatory materials without cytotoxicity of the extracts (Tonin et al 2016). This work is of importance as the cytotoxicity or its absence was well taken into consideration and measured. *Rhus coriaria* tannin extract was shown to reduce wound area and shortening epithelization and scar formation. Due to its antimicrobial activity animals with wounds infected by *Staphylococcus aureus* healed (Gabr, and Alghadir 2019). Thus tannins haven't just a cicatrization effect but also an antimicrobial, bactericidal role, this being determinant in wound healing as wounds are very often also infected.

3.8.Antidiarrhoic

Tannins are effective and safe antidiarrhoic. Their use in this situation has been demonstrated to reduce the duration of diarrhea caused by rotavirus in infants.

More recently it has been reported that children acute diarrhea duration was significantly shorter than standard rehydration treatments when tannins were instead administered (Subbotina et al 2003; Russo et al 2018).

Equally commercial quebracho and chestnut wood tannin extracts were examined for their antioxidant activity and metabolization in vitro digestion–fermentation tests. Degradation by intestinal microbiota was shown with the release of quercetin or sinapinic acid metabolites, higher antioxidant capacity on residual solids after fermentation and increased production of short-chain fatty acids, these latter being considered as prebiotics in the gut (Molino et al 2018). This infers the prebiotic effect of tannins on digestive microbiota.

3.9 Structural bone reparation.

Skeletal defects resulting from trauma and disease represent a major clinical problem worldwide, exacerbated further by global population growth and increasing number of elderly people. As treatment options are limited, bone tissue engineering opens the possibility to grow unlimited amount of tissue products with enhanced therapeutic potential for the management of clinical cases characterized by severe bone loss. Bone engineering relies on the use of compliant biomaterial scaffolds, osteocompetent cells, and biologically active agents.

Condensed tannins have achieved a new structural medical application for natural materials (Abdalla et al 2018, 2020). Among others materials, porous tannins as a spray-dried powder (PTSDs) is approved for human use. PTSDs has been used as bone reconstructive materials, of low cost, biocompatibility and potential ability to replace bone in vivo. In this study macro PTSDs scaffolds with defined geometry, porosity and mechanical properties have been manufactured using a combination of casting technology and porogen leaching, by mixing PTSDs and hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) with polyethylene glycol macro particles. Thus, a biocompatible bone graft for implanting into a bone wound site or screening a bone disease drug, comprising a porous scaffold structure made from a tannin-hydroxyapatite resin, a population of osteocompetent stem cells, and a growth medium was prepared (Abdalla et al 2018). Methods of synthesis and physical characterization of the porous scaffold structure are described, as well as biological testing of the osteocompetent stem cells of the bone graft. the expected results showed that the scaffolds developed could support attachment, long-term viability and osteogenic differentiation of human induced pluripotent stem cell derived mesenchymal progenitors (Abdalla et al 2018). The combination of select macro porous PTSDs scaffolds with patient-specific osteocompetent cells offers new opportunities to grow autologous bone grafts with enhanced clinical potential for complex skeletal reconstructions.

3.10. Veterinary

Tannins can have a double function in herbivores. They can decrease the excessive fermentation of crops such as alfa-alfa/lucern (*Medicago sativa*) in bovines rumen leading to dangerous and excessive gas forming, and if not relieved even to the death of the animal. Tannins-rich legumes have been used in this respect as regulators of cattle feed (Fagundes et al 2020). Conversely, tannins by their complexing action with vegetal proteins inhibits nutrition absorption from herbivores. The effect can be extreme, and it was first observed for the wild kudu antilopes in Africa dying of starvation when eating excessive amounts of wild acacia leaves, particularly rich in condensed tannins (van Hoven 1984).

Chestnut tannin extract has been shown to shorten diarrhea periods in calves and this without having affected their weight (Bonelli et al 2018). Tannins have also been used as antihelmintics and antiprotozoan parasites, in particular extracts of sumac (*Rhus coriaria*) and of European common barberry (*Berberis vulgaris*) (El-Saber Batiha et al 2020).

Poultry and swine nutrition are the most important sectors in which tannins have been used, firstly adopting tannin-rich feedstuffs and more recently, using tannin extracts from different plants. Several commercial products are available containing tannins extracted from the European chestnut tree (*Castanea sativa* Mill.) and the South American quebracho (*Schinopsis* spp.). Tannins extracted from these plants have been applied on intensive swine farms due to their ability to improve animal performance and health. These positive and prominent effects are frequently associated with the antinutritional effects in reducing feed palatability, digestibility and protein utilization of feed. Some criticisms and contrasting results regarding pig performance and intestinal health have been reported. However, proper dosages have been determined to eliminate this controversy (Caprarulo et al 2021).

Tannins when consumed by herbivores with high gut pH, undergo self-oxidation producing semiquinone radicals and quinones. These oxidation products can bind to the nutrients in the gut lumen and cause damage (Barbehenn et al 2006). Tannins action finds in mammalian herbivores gut an ideal environment for tannin–protein interactions (Verma et al 2021). When

given in moderate amounts, the protein binding ability of tannins can improve nutrient utilisation in ruminants (Mueller-Harvey 2006; Barbehenn and Constabel 2011; Verma et al 2021). Moreover, their effectiveness depends on the structure of the tannins involved. Ellagitannin-rich plants appear to have a more marked oxidative behaviour compared to plants in which galloyl glucoses or condensed tannins predominate (Barbehenn et al 2006). Condensed tannins appear to precipitate more actively the proteins than ellagitannins (Salminen and Karonen 2011; Verma et al 2021). The anthelmintic and antimethanogenic bioactivity of condensed tannins in ruminants is due to their proteins precipitation ability (Zhang and Lin 2008; Desrues et al 2017; Verma et al 2021) and their antioxidant behaviour (Naumann et al 2017; Aboagye and Beauchemin 2019; Verma et al 2021). Condensed tannins form insoluble complexes with proteins by binding to the protein surface coating it, this leading to its precipitation (Adamczyk et al 2011; Zeller 2019; Verma et al 2021). These complexes are generally based on non-covalent, secondary forces interactions between tannin and proteins such as hydrophobic interactions, van der Waals forces and hydrogen bonds (Prigent et al 2009; Verma et al 2021). There have been reports of ionic interactions and covalent bonds with amino acids or sulphur on proteins (McMahon et al 2000; Verma et al 2021) these being confirmed by the action of hydrolysable and condensed tannins on soy proteins (Ghahri et al.2018ab, 2021) and collagen (Pizzi 2021) in other field of application. Additionally, under low pH and oxidative conditions, tannins can form covalent bonds with proteins (Prigent et al 2009; Hagerman 2012; Engström et al 2016; Adamczyk et al 2017; Verma et al 2021). Very recently the formation of ionic bonds between condensed and hydrolysable tannins and soy proteins and collagen have been clearly demonstrated, with ionic bonds predominating at ambient temperature and covalent bonds starting to predominate as the temperature increase (Pizzi 2021; Gharhi et al 2018a,b, 2019).

3.11. Food additives

Wine, beer, and fruit juices contain tannins (Ricci et al 2017). The tannins presence determine the taste characteristic of these beverages. If the tannin content of these beverages is not within a determinate range or concentration their taste is organoleptically unpleasant, in brief they are either insipid (too low a tannin proportion) or too astringent (too much tannin), namely “tanning” the mouth epithelium. Hydrolysable tannins purified for human consumption are even added to some beers to maintain the required taste when this is necessary. The same applied to fruit juices.

Many applications of using tannins as food natural antioxidants have been reported. Tannic acid can be used to decrease lipid oxidation by ferrous ions by binding the metal (Hagerman 2012). The metal binding properties of tannins, both hydrolysable and condensed, is well known with the binding occurring by mean of the ortho-diphenolic –OH groups of the aromatic rings of the tannins (Pizzi 2019; Slabbert 1992). The reason for the coupling of tannins with metals is well known and is the formation of Fe³⁺ coordination complexes due to the *ortho*-diphenol hydroxyl groups on the B rings of a flavonoid tannin as shown in Figure 11.

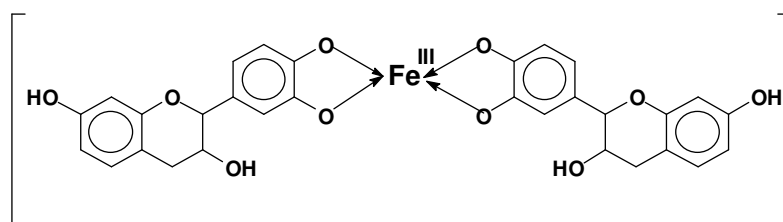


Figure 11. Example of a Fe^{+3} complex where the metal is complexed by the vicinal *ortho*-hydroxyl groups of two fisetinidin flavonoid units.

The same approach can be used for tannins from many different sources to block lipids of any nature to become rancid (Al-Hijazeen et al 2016; Raitanen et al 2020; Li et al 2020), such as meat patties. The same effect was observed with condensed tannins from quebracho, spruce and pine.

3.12. Biopesticides

Chestnut tannin has been recently commercialized in Italy as an enhancer of the defenses of plants against bioorganisms attack and as a plant corroborant by improving roots development and to reduce roots damage by parasites (Bargiacchi 2019; Pagliaro et al 2021; SKL 2021). This is a raw chestnut extract with a 75% content of tannin. This preparation is used as a biopesticide as well as for its broad antimicrobial and antifungal actions based on the inhibition of hydrolytic enzymes such as cellulases, pectinases and xylanases used by pathogens to penetrate plant tissues (Pagliaro et al 2021). It also repels insects and parasites due to the altered astringent taste of the plant tissue and its increased hardness. Very recent studies have shown that this material promoted vigorous plant growth and simultaneously acted as a pesticide against phytopathogenic bacteria on kiwi and against nematodes attack on tomato and tobacco plants (Bargiacchi et al 2017; Pagliaro et al 2021). Ecotoxicological tests have shown that all the tannin extracts used, such as chestnut, did not present any toxic effect at reasonably high concentrations (EU Evergreen Programme 2018).

4. Conclusions

The many uses of tannins for medical and pharmacological applications described above need to be put into perspective with regards to possible further advances, existing drawbacks, and future potential. Of all the applications described above, leather tanning still remains the main industrial use of vegetable tannins, but their use for this application has been progressively decreasing and limited to heavy duty leathers, as displaced for finer applications by chrome tanning and by totally synthetic materials. The commercial tannins drawback at present is that their present supply is limited. The potential world supply of tannins is really huge given the many sources from which they can be extracted, some of these sources being really extensive. What is lacking, however, is a marked increase in the factories extracting them. A few new tannin extraction factories have been created in the last decade, mainly for application in biomaterials.

Medical and pharmaceutical applications are one of the more interesting and active fields of research at present for the evaluation of vegetable tannins. While some pharmaceutical applications already exist, for further progress some of the results being developed in this

field need to be proven *in vivo*, this being an important phase of development. It is difficult to say for what new and specific pharmaceutical uses tannin might be successfully adopted in future. The main drawback here is that the balance of properties favorable and unfavorable to each application have to be evaluated. It is nonetheless an application that is likely to further flourish in the future.

More established and fully functional is the use of tannins in the beverage industry, be it wine, beer, or fruit juices. Their use will expand with the expansion of these markets due to the expansion of the population, but not for different applications in this field.

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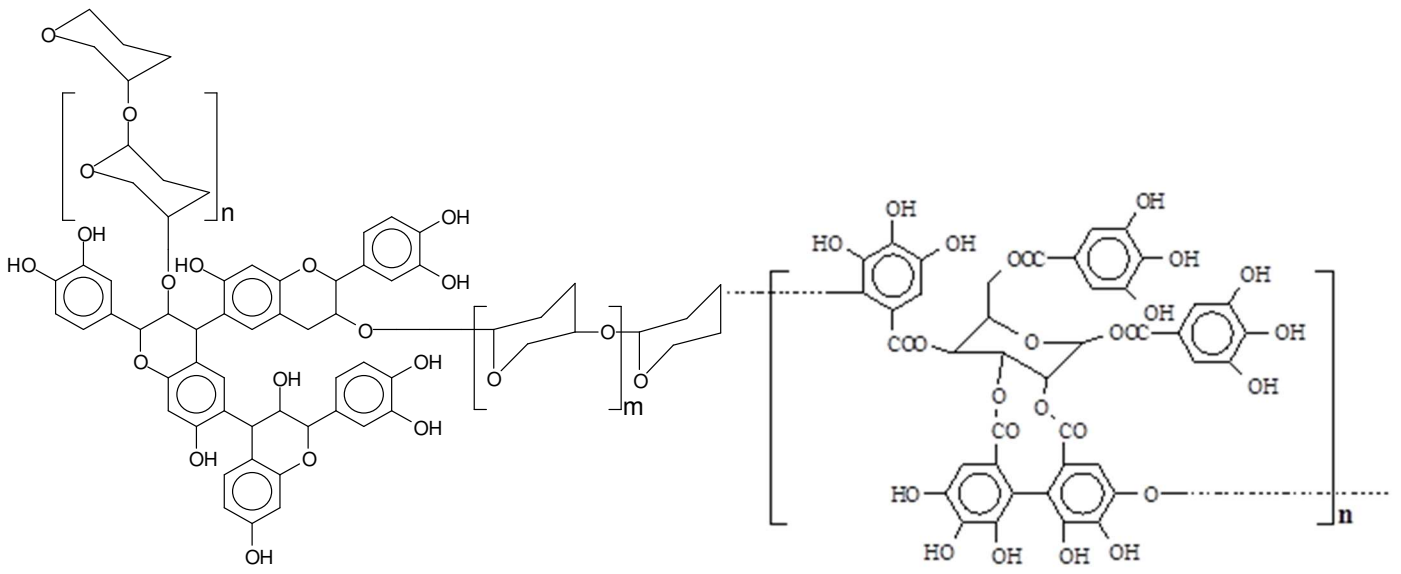
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Condensed Tannin

Hydrolysable Tannin