Tannins obtained from medicinal plants extracts against pathogens: antimicrobial potential

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The molecular diversity found in popularly used medicinal plants is much higher than the one derived from chemical synthesis processes. Thus, vegetables constitute an excellent source for searching new antimicrobial drugs. The condensed tannins stand out among several chemical substances which present important pharmacological activities and are isolated from medicinal plants. The condensed tannins are constituted of catechin-type monomers, known as flavonoids. The therapeutic potential of the tannins is assigned to the phenolic hydroxyl group situated on the surface of tannins. This group binds to protein adhesins and promotes inhibition of enzymes, rupture of the plasma membrane and deprivation of the microbial substrate. Due to its antiseptic property, the tannins are currently being tested against pathogenic organisms. In this chapter, we properly approach the use of antimicrobial substances obtained from extracts of tannin-rich plants found in the Brazilian Semi-arid.

Keywords: antimicrobial; vegetable extracts; Gram-positive bacteria; Gram-negative bacteria; popular medicine; Brazilian semi-arid

1. Introduction

From the beginnings of humankind to present days, plant species are widely used by most of the world's population for therapeutic purposes, aiming at curing and/or treating symptoms of numerous diseases [1]. Surveying this popular knowledge starting from the experiences of the community itself or sources which are external to the community is important in order to prove, perpetuate and identify it [2]. Studies of this nature are known as ethnobotany [3].

Brazil has a huge plant biodiversity, including about 22% of the known species in the world [4]. Because of that, plant species are commercialized and grown in residential backyards both in poor regions and in big cities aiming at its medicinal use [5].

The plant species used as medicinal plants by the population present higher molecular diversity than the drugs derived from chemical synthesis processes, making the vegetables an excellent source for searching new drugs, including the antimicrobial ones [6]. Several classes of pharmacologically active chemical substances have been isolated from various medicinal plants [7-9]. These substances play distinct roles in plants as phytohormones, structural compounds and secondary metabolites [10]. Compounds called tannins perform various biological functions in the plant organism such as insect, fungi and bacteria control, and stand out among the secondary metabolites [11-13].

Tannins are polyphenols with high molecular weight (500 to 3,000 kDa) which react with proteins through hydrogen bonds and/or hydrophobic interactions when not oxidized; however, they are converted into quinone when oxidized, generating covalent bonds with some functional groups of proteins such as the sulfidric groups of cysteine and the ε-amino groups of lysine [14]. They are classified into hydrolysable and condensed, the latter being currently more studied due to its therapeutic properties as an antimicrobial product [12, 15-19].

The tannins bind to the proteins and adhesins present in mucosal cells with this action being attributed to the phenolic hydroxyl group situated on the surface of this molecule [20, 21]. They form a protective cover, inhibiting the action of microbial enzymes [21], which promotes the disruption of the plasma membrane and deprivation of substrates required for the microbial growth by forming a tannin-protein and/or polysaccharide complex, thus preventing the growth of microorganisms [12, 20-22].

2. Origin, concentrations, classification and extraction methods

2.1 Origin of tannins

The tannins may present mineral, synthetic or vegetable origins. Mineral tannins are obtained from inorganic salts based on chromium or zirconium [23]. Synthetic Tannins are derived from the condensation of phenol, cresol and naphthalene with an aldehyde, such as furfuraldehyde [23]. The natural tannins, in turn, can be found in various parts of a plant, such as the bark, leaves, heartwood, fruits, seeds and sap. They are known, therefore, as vegetable tannins, and are extracted...
mainly from the bark, stem or heartwood of the plant. Their properties may vary between different species or within the same species depending on the plant tissue, age, time or even the place where it was collected [24, 25]. Tannins are produced by plants in adverse environmental conditions, being responsible for their protection against herbivores and pathogenic diseases and are essential for the growth and reproduction of the plants [25].

2.2 Classification of vegetable tannins

The vegetable tannins are classified into hydrolysable and condensed. The hydrolyzable tannins are mixtures of simple phenols and esterified sugars, especially glucose, suffering acidic, alkaline or enzymatic hydrolysis [26]. The basic structural unit of this type of tannin is a polyol, usually D-glucose, with its hydroxyl groups esterified by gallic acid (gallotannins) or by hexahidroxifênico (ellagitanins) [27]. Figure 1 shows the chemical structure of tannic acid (hydrolyzable tannin).

![Fig. 1 Chemical structure of hydrolysable tannin. Source Battestin et al. (2004) [27].](image)

Condensed tannins (or proanthocyanidins), on the other hand, are composed by flavanol units: flavan-3-ols (catechin), or flavan 3,4-diols (leucoantocianidine). They are structurally complex, resistant to hydrolysis, can be soluble in aqueous organic solvents, have a rich structural diversity, contain phenolic-hydroxyl groups in sufficient amount to allow crosslinking with proteins [4, 27-31] and precipitate in formaldehyde and hydrochloric acid through the Stiasny reaction [32].

The condensed tannins form the most important group, accounting for over 95% of commercial tannins production and exceeding the mark of 350,000 t/year [33]. They are responsible for the astringency in fruits, juices and wines and are, in many cases, bioactive compounds in medicinal plants [27]. Figure 2 shows the chemical structure of the condensed tannins.

![Fig. 2 Chemical structure of Condensed tannin. Source: Battestin et al. (2004) [27].](image)

2.3 Concentrations in plant species


2.4 Vegetable tannins extraction methods

There are different extraction methods of tannins from parts of plants. The extraction is usually simple and done with hot water in autoclaves [24, 34]. The use of water as solvent in the extraction of tannin is the most common process, due to the economy and good efficiency of the extraction. In addition, some companies have been choosing extraction with water because of the consumers’ requirement for environmentally friendly products. However, to maximize the extraction and the quality of the tannins, substances such as sodium sulfite (Na₂SO₃), sodium metabisulphite (Na₂S₂O₅) or sodium carbonate (Na₂CO₃) are added to the water in some cases [35, 36]. The works cited verified that the addition of salts assisted the extraction of tannins from barks of Pine and Oak from the Southern United States.

When assessing the tannin content extracted from the bark of “angico-vermelho” - *Anadenanthera colubrina* (Vell.) Brenan var. cebil (Gris.) Alts., Diniz (2008) [37] concluded that the extraction with distilled water extracted less total solids when compared to other extractors (sodium hydroxide and/or sodium sulfate). However, the extract with total solids, which is represented by the content of tannin and non-tannin substances, presented a greater concentration of tannic substances.

The extraction temperature may vary from 70 to 105°C, according to the species [24, 34]. For acacias, temperatures between 94 and 100°C are the ones that best correspond to maximum extraction. In species of *Pinus sp.*, temperatures...
below 70°C are used, and for the genus *Eucalyptus*, the extraction temperatures usually used are 70 and 100°C, depending on the species.

Carneiro and Vital (1999) [38] evaluated the yield of aqueous extraction from the bark of *Eucalyptus grandis* (Maiden) and observed that the amount of non-tannin material increased as the extraction temperature was raised. Temperatures above 100°C, for most of the already studied species, do not improve the yield of phenolic material and can facilitate the extraction of non-phenolic material [36, 38], hindering the extract purification.

In the Brazilian Semi-arid, specifically in the Municipality of Cabaceiras, State of Paraíba, a cooperative called Arteza - Cooperative of Leather Tanners and Artisans from Ribeira, in Portuguese, uses the tannins from the bark of “Ángico Vermelho” - *Anadenanthera colubrina* (Vell.) Brenan Var. Cebil (Gris.), to turn goat skins into leather. The homemade process consists in the management of the species, with the bark of the most ancient species being cropped. The bark is grounded and soaked; the sap (tannin) is removed and put on animal skin which passes from the decomposition state to the noble condition (leather), fortified and soft.

2.5 Condensed tannins quantification methods

The aqueous extraction of tannins from bark or wood of forest essences involves the concomitant removal of other groups of compounds (waxes and greases, flavonols, sugar, pectin, xylan, ashes, lignins and polyphenolic acidic structures). Therefore, when a certain extract is obtained, it is necessary to know the percentage of the extracted solids that corresponds to the condensed tannins [39]. For this evaluation, one of the methods used nowadays is based on the Stiasny reaction. Through this reaction, it is possible to gravimetrically quantify the percentage of the solids of tannic extract that effectively corresponds to the condensed tannins [40].

The tannin extracts may be quantified on the content of total solids, on the Stiasny index and on the condensed tannin content. The total solids content expresses the sum of tannic and non-tannic substances contained in extracts obtained from the barks. The Stiasny index is a measurement of the extract percentage that reacted with formaldehyde: the higher the Stiasny number, the greater the amount of condensed tannins present in the extract. The condensed tannin content is the product of the total solids content by the Stiasny index.

3. Therapeutic potential and popular use

Tannins are found in a wide variety of plants with significant amounts when compared to other metabolites; they can be found in the roots, bark, leaves, seeds, sap and in the fruits, where they are responsible for the astringency of many of them as unripe, when the decrease in astringency results from the production of molecules with affinity to tannins [41]. They protect plants against pests and insects, since they bind to insects digestive proteins and form an insoluble compound, inactivating digestive enzymes [4, 41]. The concentrations of tannins may vary according to climatic and geographical conditions and also according to their chemical composition, about which there is little knowledge [4].

Among the species traditionally used by the population for therapeutic purposes, the following stand out:

3.1 *Mimosa tenuiflora*

*Mimosa tenuiflora* (Willd.) Poiret is a Leguminosae, subfamily Mimosoideae, easily found in the Brazilian Caatinga and is a typical species of semi-arid areas of Brazil [42]. It is drought-resistant, with great ability to regrowth throughout the whole year. Popularly known in Northeastern Brazil as “jurema preta”, it is shrub sized, 5–7 m tall, forming erect, very sharp and taller than 1.5 m rods [43]. The stem is erect or slightly inclined, with abundant branching, which falls into thin scale-form portions. The branches are reddish-brown and sparsely aculeate. The plant presents rough, longitudinally slotted and slightly fibrous bark [44]. It is preferentially found in secondary formations of floodplain with proper levels of humidity and in deep, alkaline and fertile soils [43].

*M. tenuiflora* stands out among medicinal plants for presenting a high percentage of tannins when compared to other species [45]. The decoction of the stem bark of *M. tenuiflora* is popularly used in the treatment of topical wounds, gastric ulcers, inflammations, burns or wounds and symptoms related to menorrhrea, dysmenorrhea and endometritis [43,46]. On the other hand, *M. tenuiflora* is part of an important group of toxic plants reported by Da Silva et al. (2006) [47] who observed that the ingestion of large quantities of the plant cause abortion and, in the first 60 days of pregnancy, fetal malformations. Although a small amount of tannins is found in the leaves, Heldt (1997) [30] reported that trees predated by antelopes released ethylene, which is the only plant hormone, inducing an increment of tannins synthesis in those trees and in the ones nearby in approximately 30 minutes; thus, eating these leaves can lead animals to death. The interaction between tannins and digestive proteins of herbivores was described by Heldt (1997) [30], who believes that this interaction possibly occurs through hydrogen bonds between the phenolic groups of tannins and certain sites of the proteins, with an enduring stability of these substances. The anti-nutritional potential of the tannins is well known, however, some plants and animals use strategies to reduce the astringency and the effects caused by these compounds by inducing a large release of salivary proteins [48]. Nevertheless, there is no specific treatment to reverse the symptoms in cases of poisoning by ingestion of the plant; thus, preventing access of sheep and goats to areas with *M. tenuiflora* is the only way to avoid poisoning [47].
The antimicrobial activity of the alcoholic extract obtained from the stem bark of *M. tenuiflora* was tested both in vitro and in culture of Gram-positive and Gram-negative bacteria such as: *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Aeromonas caviae*, *Proteus mirabilis*, *Shigella sonnei*, *Klebsiella pneumoniae*, *Thrichophyton mentagrophytes*, *T. rubus*, *Staphylococcus epidermidis*, *Acinetobacter calcoaceticus*, *Salmonella typhimurium*, and *Chaetomium indicum*. It was also tested in culture of fungus such as *Candida albicans* [43, 49, 50]. Pereira *et al.* (2015a) [51a] demonstrated antimicrobial activity of tannins isolated from the stem bark of *M. tenuiflora* on *S. aureus* of animal origin with minimum inhibitory concentration (MIC) of 31.2 μg/ml. The authors emphasize that the strains of *S. aureus* were not able to grow under these experimental conditions.

### 3.2 *Piptadenia stipulacea*

*Piptadenia stipulacea* is a plant of the family *Leguminosae*, subfamily *Mimosoideae*, popularly known in Northeastern Brazil as “carcará”, “cassaco”, “jurema branca” e “rasga-beiço”. This species occurs in the semi-arid region of Northeastern Brazil and is typical of the caatinga, from Piauí to Bahia. It is arboreal or shrubby, about 2-4 m tall, with light brown bark heavily armed by strong aculeus [43] and is a pioneer plant, easily occupying roadsides [52]. It can adapt to low fertility soils and has the ability to fix nitrogen in the soil through symbiosis with bacteria in their roots, which facilitates their development [43]. It is a deciduous plant, containing 2-12 small, oval and brown seeds per pod. Its wood is light in color and its flowering occurs in rainy seasons, but it can also be found in dry seasons, followed by the fructification which extends until the dry season [53].

The population makes little use of *Piptadenia stipulacea* as a medicinal plant; however, the extract of the stem bark has anti-inflammatory and healing properties [54]. A weak antimicrobial activity of the alcoholic extract obtained from the stem bark of *Piptadenia stipulacea* on Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Aeromonas caviae* has been observed in several studies, probably due to the small amount of tannin found in its bark [43, 55]. The antimicrobial activity of tannins isolated from stem bark of *P. stipulacea* was demonstrated by Pereira *et al.* (2015a) [51a] on *Staphylococcus aureus* strains of animal origin, with MIC of 62.5 μg/mL.

### 3.3 *Mimosa arenosa*

*Mimosa arenosa* (Willd.) Poir. is a plant of the family *Fabaceae*, subfamily *Mimosoideae*, native in South America. It is popularly known in Northeastern Brazil as “jurema vermelha” or “unha de gato”. It has a shrub and thorny size and is drought resistant, with great ability to regrow throughout the year, usually reaching 3-5 m. It can be recognized for the tomentose branches, membranous pinnules, shortly pedicellate flowers and glabrous fruit [42, 56]. It has significant importance as forage for small ruminants in Northeastern Brazil. It is used for the production of firewood, charcoal and wood for building fences [56].

In the interior of Brazil, it is common to use the bark stem and the root of *M. arenosa* in preparations in which honey or sugar and water are added to produce a "home remedy" with therapeutic uses on the treatment of colds, flues and lung diseases [50]. *M. arenosa* is used in a similar way as *Mimosa tenuiflora*, since both species have the same tannin content [45].

The antimicrobial activity of tannins obtained from the stem bark of *M. arenosa* was observed on both Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* with MIC ranging between 250 and 1000 μg/ml among these microorganisms [57]. These authors observed that the antibacterial activity performed by the tannins of *M. arenosa* against all tested strains was bacteriostatic.

### 3.4 *Anacardium occidentale*

*Anacardium occidentale* Linn belongs to the family *Anacardiaceae*, popularly known in Northeastern Brazil as “cajuíro” (cashew tree, in Portuguese). It is an evergreen, medium sized and low branched tree. The various cashew plants have been grouped into common type and early dwarf type. Plants of the common type are 5-15 m tall with 12-20 m of crown diameter [58]. The foliage is permanent. The leaves are simple, whole, measuring up to 20 cm long by up to 12 cm wide. The amount of fruits formed is very low in the cashew tree when compared with the amount of hermaphroditic flowers produced and it varies among plants according to the region and the time of year. Fungi and insect attacks, nutritional effects and mechanical factors such as strong winds stand out among the causes of the fall of immature fruit [58]. Ethnobotanical studies have disclosed that the cashew tree is one of the most used plants by the users of public health service [59].

Popularly, the stem bark decoction and infusion of the leaves of *A. occidentale* is used as tonic, anti-diabetic, anti-inflammatory, antibiotic, wound healing, depurative and for the treatment of ulcers in the oral cavity. It is also used to reduce the effects of hypertension, in addition to presenting astringent action [60-63]. This astringent action presented by *A. occidentale* is attributed to the presence of tannins, which are found in abundance in the stem bark.

The antimicrobial activity of the alcoholic extract obtained from the stem bark of *A. occidentale* was observed on both Gram-positive and Gram-negative bacteria and fungi, with the following standing out: *Staphylococcus aureus,*
Studies that investigated the antimicrobial and non-stick activity of tannins isolated from stem bark of \textit{A. occidentale} on microorganisms which form the dental biofilm, including \textit{S. aureus}, \textit{S. mutans}, \textit{S. mitis}, \textit{S. sanguis}, \textit{Streptococcus oralis}, \textit{Streptococcus salivarius} and \textit{Lactobacillus casei}, presented promising results [66, 67].

\subsection{3.5 \textit{Anadenanthera colubrina}}

\textit{Anadenanthera colubrina} (Vell.) Brenan var. Cebil (Griseb.) Von Reis Alstchul. belongs to the division \textit{Mimosoideae} of the family \textit{Fabaceae}, order \textit{Fabales}. It is a Brazilian native plant, occurring from the state of Maranhão to the state of Paraná. It is popularly known as “angico branco”, “angico cambui”, “angico escuro”, “angico vermelho”, “jurema preta”, among other popular names depending on the region of Brazil [68].

It is a deciduous tree with open and irregular crown, 5-15 m tall. In the semi-arid region of Brazil, however, it usually reaches up to 7 m tall. It has a cylindrical trunk with 30 to 50 cm in diameter, coated with rough skin with sparse aecules. The leaves are compound, presenting opposite leaflets [69]. The plant has white flowers arranged in inflorescences of panicles type with globose spikes. Its fruits are flat, rigid, glabrous, bright, dehiscent, brown legumes (beans), with up to 20 cm long and 5 to 10 smooth and dark seeds [70].

The bark is rich in tannins which give the plant medicinal properties such as antimicrobial, anti-inflammatory, antioxidant and astringent actions [71]. It is used for leucorrhoea, gonorrhea [72], sore throat, rheumatic pains, skin infections, chronic cough, bronchitis, inflammation and as expectorant [68]. The plant is orally administered, prepared by decoction, infusion, maceration or as juice obtained after maceration of the leaves or other plant parts in water. Some preparations, consisting of roots and stem barks, are called “garrafada” [73].

The bark stem of \textit{A. colubrina} contains from 12% to 15.38% of tannins [45]. The content is lower in the seeds, about 3% of tannins and about 1.8% of this secondary metabolite in the heartwood [68]. Other authors demonstrated that the amount of tannins in the leaves may vary from 3.21% to 11.07% [71]. The same study also demonstrated no significant differences between the levels of tannins and flavonoids in the stem bark and its leaves.

The antimicrobial activity of tannins obtained from the stem bark of \textit{A. colubrina} was observed on both Gram-positive and Gram-negative bacteria and also demonstrates antifungal activity with MIC in hydro-alcoholic fraction of 62.5 μg/ml for \textit{Staphylococcus aureus}; 143.587 μg/ml for \textit{Shigella sonnei}, 250 μg/ml for \textit{Pseudomonas aeruginosa}; 62.5 μg/ml for \textit{Escherichia coli}; 125 μg/ml for \textit{Salmonella enterica} and 192.77 μg/ml for the fungus \textit{Candida albicans}.

Clinical studies concluded that this plant has hallucinogenic and hypnotic activities. Phytochemical analysis of the bark isolated the oxide indole alkaloid of \textit{N,N-dimethyltryptamine}, steroids (palmitate of \textit{B-sitosterol}, \textit{B-sitosterol}, glucoside), flavonoids, triterpenoids (\textit{luperona}, \textit{lupeol}) and phenolic compounds (dalbergina, 3,4,5-dimethoxidalbegiona, khulmannina). 2.1% bufotenin (psychoactive alkaloids) was found in the seeds. Some tribes of Mexico and South America (Yanomami) use the seed of \textit{Anadenanthera colubrina} to create a hallucinogen called yopo, which can be inhaled and used in religious rituals [68, 74].

\subsection{3.6 \textit{Solanum paniculatum}}

\textit{Solanum paniculatum} L. belongs to the family \textit{Solanaceae} and order \textit{Solanales}. Popularly known as “jurubeba”, “jurubeba leão do norte” and “jurubeba verdadeira”. It is native throughout Brazil. It is shrubby, 1.5 to 2.5 m tall, grows wild and is often considered by farmers as a weed. It reproduces by seed, flowering and fruiting throughout the whole year [5].

In Brazil, many species of the genus \textit{Solanum} are used in popular medicine for various purposes such as treatment of gastrointestinal verminosis in humans, treatment of ictericia and chronic hepatitis and treatment of chronic arthritis. It also presents antipyretic, anti-allergic and hallucinogenic action [5, 75, 76]. In addition, the popular medicine recommends the tea as a cardiovascular tonic and as a stimulant for appetite, liver, spleen and digestion. It presents diuretic, hypoglycemic, antianemic, febrifuge and healing action. The infusion of its stem and root in sugarcane alcohol (“cachaça”) is popularly used as an appetizer.

Regarding its antimicrobial effect, \textit{Solanum paniculatum} demonstrated activity compatible with growth inhibition of strains of Gram-positive (\textit{Staphylococcus aureus}) and gram-negative bacteria (\textit{Pseudomonas aeruginosa}, \textit{Escherichia coli}, \textit{Proteus vulgaris}, \textit{Shigella flexneri} and \textit{Klebsiella pneumoniae}). The six tested bacterial cultures showed MIC results for \textit{Solanum paniculatum} of 256 μg/ml for Gram-positive (\textit{S. aureus}) and 512 μg/ml for Gram-negative (\textit{Pseudomonas aeruginosa}, \textit{E. coli}, \textit{P. vulgaris}, \textit{S. flexneri} and \textit{K. pneumoniae}), indicating that the extract of \textit{Solanum paniculatum} has moderate antimicrobial activity [77].

Although the literature shows an increasing number of publications about the use of tannins in experimental models \textit{in vitro} with antimicrobial potential, more systematic information about their action is necessary, particularly clinical trials involving blind, double-blind, controlled and randomized experiments.
Increasing advances have been occurring in regard to the production of herbal medications from purified active principles, such as FITOSCAR, an herbal medication from the tannins of *Stryphnodendron adstringens* (Mart.) Coville, which has healing effects. Nevertheless, its action mechanism is not yet completely known.

Thus, it is relevant to adequate the treatment protocols, within previously established methodology in further studies, which allow to understand the usefulness and possible risks of the herbal medication. This is, thus, relevant to adequate treatment protocols within previously established methodology in further studies, which allow to understand the usefulness and possible risks derived from the use of medications based on tannins, reinforcing the scientific evidence of their effects and effectiveness.

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