REVIEW

Biological activities and chemical constituents of *Araucaria angustifolia*: an effort to recover a species threatened by extinction

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Abstract

Background: *Araucaria angustifolia* (Bert.) O. Kuntze (*A. brasiliensis*), known as Paraná pine, is the sole native gymnosperm of the Atlantic forest in Brazil and has great economic, cultural and social importance. Its seed, known as *pinhão*, has been consumed since prehistoric times. Besides the nutritional aspects, different parts of *A. angustifolia* are also used in the Brazilian folk medicine for the treatment of rheumatism, respiratory infections, fatigue, anemia, among other disorders. Timber exploration has dramatically reduced the species population, and currently, *A. angustifolia* is classified as vulnerable regarding the risk of extinction.

Scope and Approach: This review presents the most recently uncovered details about the chemical composition of the various parts of the plant. Emphasis is given to the main isolated and identified compounds or fractions and their corresponding bioactivities.

Key Findings and Conclusions:.Apart from the nutritional properties of the *pinhão*, particularly as a starch source, this review reveals that a number of biological activities have been found in different parts of *A. angustifolia* (leaves, bark and *pinhão* coat), such as protection against DNA UV-induced damage, antioxidant, antiinflammatory, antiviral and digestive enzyme inhibiting activities. Further investigations should include parts of *A. angustifolia* that are currently discarded, such as the bark, bracts and the *pinhão* coat, with potential for use in pharmaceutical and cosmetic industries. Studies on *A. angustifolia* must combine two important elements: the need for preservation of a typical ecosystem and the implementation of the *A. angustifolia* forests as a true economic alternative for local residents.

Key words: *Araucaria angustifolia*; bioactive compounds; chemical composition; nutritional composition; phenolics; starch.

1. Introduction

Araucaria is a genus of evergreen coniferous trees in the family Araucariaceae. The genus *Araucaria* includes approximately nineteen species, all confined to the Southern Hemisphere (Kershaw and Wagstaff, 2001). Two species grow in South America, *Araucaria angustifolia* and *Araucaria araucana. A. angustifolia* covers areas of the South and South East of Brazil and North East of Argentine (Kock and Corrêa, 2010) whereas *A. araucana* is restricted to the high mountains in the South of Argentine and Chile (Cardemil and Riquelme, 1991).

Araucaria angustifolia (Bert.) O. Kuntze (*Araucaria brasiliense*), popularly known as Paraná pine, Brazilian pine or simply "Araucaria", is the sole native gymnosperm of the Atlantic forest in Brazil and has great economic, cultural and social importance (Auler et al., 2002). Originally, the natural forests of Araucaria covered 185,000 km² in Brazil (Carvalho,1994; Astarita et al., 2003). Timber exploration has dramatically reduced the species population and nowadays only 2-4% of the original population still exists (Mantovani et al., 2004; Santos et al., 2015). Most of the populations of *A. angustifolia* are concentrated in the Southern Brazilian States (Paraná, Santa Catarina and Rio Grande do Sul), with some representative forests in the Southeastern Brazilian States (São Paulo, Minas Gerais and Rio de Janeiro) (**Figure 1**).

Currently, *A. angustifolia* is classified as vulnerable regarding the risk of extinction (Brazilian Decree 42,099, 2002). Due to this situation the cultivation of the species has received strong encouragement from governmental agencies related to environment and agriculture, and many efforts have been carried out in order to propagate and conserve the species (Balbuena et al., 2011; Vieira et al., 2012; Kuhn and Mariath, 2014).

The edible part of the seed, known as *pinhão*, is consumed by different species of animals, especially rodents, as well as by humans and has a high nutritional value. Indians of Southern Brazil (Caingang and Guarani) are used to eat *pinhão* since prehistoric times. Especially during winter, cooked or in the form of flour, the *A. angustifolia* seed often becomes the most important food for survival (Cordenunsi et al., 2004). Besides the nutritional aspects, different parts of *A. angustifolia* are also used in the Brazilian folk medicine (Aslam et al., 2013). Tinctures extracted from the nodes are traditionally used orally or topically for the treatment of rheumatism and infusions of the nodes are used orally for the treatment of kidney diseases and sexually transmitted diseases. Infusions of the bark are used topically to treat muscular tensions and varicose veins, while the syrup produced from the resin is used for the treatment of respiratory infections. Infusions of leaves are used as emollient, antiseptic, against respiratory infections, rheumatism, fatigue and anemia, and dyes are used for the treatment of wounds and herpes eruptions (Auler et al., 2002; Andrighetti-Frohner et al., 2005; Freitas et al., 2009). In view of the above, this review aims to summarize and evaluate the main findings related to the nutritional and therapeutic uses of *A. angustifolia*, with special attention to the chemical composition of the preparations. This includes discussions on the main isolated and identified compounds and their corresponding bioactivities.

2. Plant morphology

With respect to morphology, *A. angustifolia* is characterized as a tall tree of 20-50 meters height, with an upright trunk 90-180 cm in diameter (**Figure 1A**). When young, the plant canopy is cone-shaped and, as it reaches adulthood, the canopy achieves the goblet shape due to the natural loss of the lower branches. Its acicula (leaves) are leathery, glabrous, acute-pungent and 3 to 6 cm in length (Carvalho, 1994). *A. angustifolia* is a dioecious plant that has separate male and female trees, with pollination required for seed production. Pollination occurs from October to December, mainly by wind and the ripening of the pinecones takes place two years later. In natural populations, seed production usually occurs after 15 to 20 years of age, each tree producing annually from 40 to 200 cones (BRDE, 2005).

The flowering of *A. angustifolia* produces a fruit, which is called pinecone (**Figure 1B**). Each of these has a diameter of 10 to 25 cm and contains approximately 700-1200 scales with about 150 seeds with a weight ranging from 0.61 to 4.1 kg (**Figure 1C**) (Lima et al., 2007). In a pinecone of 2.3 kg (weight average) there are about 0.8 kg seeds (*pinhão*, plural *pinhões*). The edible part of the *pinhão* almond) consists of a starchy mass with tougher texture when raw and soft after cooking. At its center, there is a filament where about 4/5 of length is occupied by the embryo, called filiform. The edible part is covered by two structures, an inner membrane firmly attached to the almond and an outer coat, highly resistant.Both the inner membrane and the outer coat have colors ranging from yellow to dark red and are removed after cooking (Leite et al., 2008). The *pinhão* is about 3 to 8 cm in length, from 1 to 2.5 cm wide and weights in average 8.7 g (**Figure 1C**).

In Brazil, the *pinhões* are found in greater amounts from April to June (Amarante et al., 2007). For consumption, the seeds are usually cooked in water or roasted. Flours of raw or cooked *pinhão* are used in the preparation of regional dishes, cakes, breads and cookies. In regions where the *A. angustifolia* tree occurs, a common practice is cooking in water, followed by preservation in salt and vinegar (Leite et al., 2008) (**Figure 1D**).

The use of the *pinhão* in the Brazilian cuisine is far from being widespread and intense due to the lack of methods for preserving it fresh and for industrial processing. Thus, techniques for conservation and sustainable use have been investigated to encourage preservation and marketing during out of the production season periods (Amarante et al., 2007; Stahl et al., 2007; dos Santos et al.,2008; Balbuena et al., 2009). A more attractive market would encourage extraction and marketing by rural producers, promote cultivation and automatically discourage illegal logging.

3. The *pinhão*

3.1. Proximate composition and nutritional aspects of pinhão

The particular composition of each *pinhão* almond can result from variations in the stage of development, temperature and irrigation techniques or simply reflect genetic characteristics (Astarita et al., 2003). The accumulation of nutrients occurs during the dehydration of the seeds in the final stages of ripening, generally during the months from April to May, when the protein content increases. The edible part of the seedscontains about 50% moisture. The contents of ash, crude protein, total lipids, total fibers and other carbohydrates are 1.50%, 3.42%, 1.67%, 1.29% and 48.42%, respectively (da Silva et al., 2016). Among carbohydrates, starch is the main component,comprising around 31-36%, averaging 30% amylose in the raw seed (Bello-Pérez et al., 2006; Conforti and Lupano, 2007; 2008). The content of soluble sugars in the *A. angustifolia* seed is relatively low, especially after cooking (Cordenunsi et al., 2004). Glucose is the most common sugar, followed by fructose and sucrose. Thirteen fatty acids were recently identified in the edible seed (da Silva et al., 2016). The main fatty acids are linoleic acid (18:2n-6), oleic acid (18:1n-9) and palmitic acid (16:0).

The amino acid composition of the proteins in the *A. angustifolia* seeds has already been determined (Leite et al., 2008). After milling, the *pinhão* flour was dried at two temperatures, 50 and 80^{o}C. Both preparations were rich in glutamate and aspartate residues and contained minor amounts of histidine and cysteine. Although the *A. angustifolia* seeds are considered essentially a source of starch, the *pinhão* flour is comparable to other protein sources used in the human diet: the amino acid composition resembles that observed for cereals, such as wheat and corn and is comparable to those of legumes, which are poor in lysine and histidine (Young and Pellet, 1994). Additionally, the contents of phenylalanine, tryptophan and valine found in the *pinhão* flour are similar to those of casein and it also presents higher contents of valine and methionine than soybean. For this reason, the *pinhão* flour has been considered as an adequate substitute for up to 20% casein as a complementary source of proteins in diets for growing rats (Leite et al., 2008). The presence of trypsin inhibitor activity in the flour, particularly lectin (see further), has limited the use of higher proportions of *pinhão* flour in the diet. Concerning free amino acids, aspartic acid and glutamic acid are the most abundant in the mature seed (Astarita et al., 2003). The seed also presents considerable amounts of tocopherols and phytosterols (da Silva et al., 2016), magnesium and copper (Cordenunsi et al., 2004).

3.2. Properties of the **pinhão** *starch*

Studies characterizing the *pinhão* starch concluded that its isolation is quite simple and that it is stable for a year at room temperature without changing color and flavor (Bello-Pérez et al., 2006).The yield of the *pinhão* starch isolation was the same using fresh and frozen seeds (freezing may be required, because the *pinhões* are harvested once a year), namely 70%. Even when the content of proteins was low, there was no difficulty in separating them from starch. Low contamination by proteins is an important characteristic of starch preparations, since the technology used in the food industry has to be adapted to the content and type of protein. This characteristic suggests an advantageous use of the *pinhão* starch in the production of glucose and fructose syrups.

Compared to corn starch the *pinhão* starch has lower temperature and enthalpy for gelatinization, lower retrogradation degree and greater water absorption capacity, solubility and viscosity at low temperatures, and shows higher susceptibility to amylolytic attack (Wosiacki and Cereda, 1989).These properties result in advantages such as soft texture, the possibility of being stored for long periods and the perspective of developing new products with thermolabile ingredients (Bello-Pérez et al., 2006, Stahl et al., 2007; Thys et al., 2013).

Stahl et al. (2007) evaluated the physical and chemical properties of phosphorylated *pinhão* starch in comparison with those of the phosphorylated corn starch. Phosphorylated starches are prepared by chemical methods in order to obtain clear pastes with greater consistency, freeze-thawing stability and high capacityof absorbing water (swelling), which can be used as stabilizers in foods such as fermented milk and ice cream. They can also be used as texture enhancers and for water retention in cheese. It has been found that the native *pinhão* starch, when compared to native corn starch, presents a higher swelling power, increased solubility, reduced syneresis and a delayed loss of clarity of the paste during storage at 5°C. Phosphorylation of the *pinhão* starch yielded a starch phosphate similar to corn starch. For both, corn and *pinhão* starch, medium and high degrees of phosphorylation increased the coldwater absorption capacity and clarity of the paste, decreased syneresis and induced a loss of birefringence to similar extents (Stahl et al., 2007).

A comparative study of the seed starch of *A. angustifolia* and *A.araucana* revealed that the amylose content was higher in the *A. angustifolia* starch and similar to that of potato and corn starch (22.4%) (Conforti and Lupano, 2008).The starch granules of both species are round or slightly oval, with a central hilum, and the gelatinization temperature of the*A. angustifolia* starch is higher than that of the *A. araucana* starch, due to the higher amylose content of the former. The average size of the granules of *A. angustifolia* starch (12.2 µm) was larger than that of *A. araucana* starch (8.4 µm), which also presents more heterogeneous granules.

Recent studies have been conducted for the determination of the properties of *pinhão* starch such as the crystallinity, granule size, granule surface, susceptibility to enzymatic attack and oil absorption capacity (Pinto et al., 2012; 2015). Such characterization will help broaden the applications of *pinhão* starch in food and nonfood industries. For example, the *pinhão* starch was recently characterized for its pharmaceutical properties and applicability as pharmaceutical excipient (Daudt et al., 2014). The native *pinhão* starch shows poor flow properties due to particle cohesion,what is commonly observed in commercial starch from different sources. It also presents very low solubility, which has equally been observed in other native starches. Native *pinhão* starch granules also exhibit a homogenous size with a narrow size distribution. This characteristic allows the powder to be easily mixed for pharmaceutical application.

The most difficult step of the starch isolation process from the *pinhão* seed is the removal of the outer coat and especially the inner one, which is adhered to the almond and contains a high amount of phenolic compounds (345 mg catechin equivalents/g fresh weight) (Cordenunsi et al., 2004; Bello-Pérez et al., 2006). The incomplete removal of the inner coat canresult in starch with undesirable color due to the action of polyphenoloxidases, which use the phenolic compounds as substrates and are responsible for enzymatic browning. The properties and characteristics of a polyphenoloxidase of *A. angustifolia* seed have been investigated at the prospect of learning how to control the enzyme's activity during the production of starch and other products that use raw seeds (Daroit et al., 2010). The enzyme presents an optimal activity at pH 5.0, and maximal activity at the 30-35°C range when using catechol as substrate.

3.3. New technologies for using the **pinhão** *flour*

The flour of the edible part of the *pinhão* can be regarded as a new technological option in terms of raw material utilization and as a nutritional source for possible formulations of food products, including gluten-free breads (Basso et al., 2015), and as a coating in β-carotene microencapsulation by freeze-drying (Spada et al., 2012). No doubt that such products would add substantial value to the seed of *A. angustifolia*. However, due to the recalcitrant characteristics of the seeds, the viability of their effective use can be compromised by the drying processes such as those used with corn, rice, beans and others (Fonseca and Freire, 2003). In this way, many studies have looked for storage alternatives aiming the *pinhão* conservation. Capella et al. (2009) analyzed the chemical composition of the *pinhão* flour with respect to the conditions of pretreatment and dehydration as a technological option for food products. They observed that the drying time at 65°C required to achieve the moisture standards recommended by the Brazilian Sanitary Surveillance Agency (ANVISA) was approximately 5 hours for both raw and cooked seeds. The flour showed distinct characteristics with regard to color, since the raw *pinhão* flour resembled the common wheat flour in terms of color and texture, and the cooked *pinhão* flour showed a yellowish color and a higher density due to the incorporation of phenolic compounds from the coat and water adsorption. Drying caused losses with significant differences in most constituents as revealed by comparisons of the contents of cooked and raw seeds with their respective flours (Capella et al. (2009). Some studies indicate that the *pinhão* starch is darker than that of corn and wheat due to the oxidation of phenolic compounds and the presence of phosphate, but even so it use has been proposed in the formulation of bakery products, pasta, biscuits and others, with promising results (Acorsi et al., 2009; Basso et al., 2015).

3.4. Phenolic compounds and antioxidant activity of **pinhão**

During cooking colored compounds present in the internal and external coats migrate not only to the water but also to the surface of the edible part of seed (almond) (Cordenunsi et al., 2004).This is revealed by both the astringent flavor and the brown color of both water and almond, the latter showing the brown color basically on its surface. Compared to other commonly consumed foods, oil seeds, for example, which have a much higher lipid content, the *pinhão* almond presents a lower phenolic content. However, the phenolic content of the *pinhão* almond is similar to that of several carbohydrate-rich foods, such as baked beans and potatoes. Both the raw and cooked *pinhão* almonds revealed to contain significant amounts of catechin, more specifically 17.5 mg/100 g seed (edible part) and 21.1 mg/100 g seed (edible part), respectively. The amounts of catechin in the *pinhão* seed are comparable to those found in other catechin rich foods such as raw apples (9.0 mg/100 g), apricots (11.0 mg/100 g), grapes (17.6 mg/100 g) and blackberries (18.7 mg/100 g) (Han et al., 2007; USDA, 2003). Gallic acid and quercetin have also been identified. The raw almond contains 0.36 mg/100 g seed of gallic acid whereas in the cooked almond the content increases to 0.82 mg/100 g seed. The quercetin content also increases after cooking, from 0.07 mg/100 g seed to 0.7 mg/100 g seed. The contents of protoanthocyanidins in the seed increased from 22.50 mg/100 g of seed to 2,035.00 mg/100 g seed after cooking. It is clear that cooking promotes migration of phenolics from the coat to the almond (Koehnlein et al., 2012). The analysis of antioxidant activity of hydroalcoholic extracts from raw and cooked for four different methods (1,1-diphenyl-2-picrylhydrazyl free radical scavenging, 2,2’-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid scavenging, ferrous ions chelating activity and lipid peroxidation inhibition), revealed significant improvement in the antioxidant activity after cooking. This improvement is certainly due to migration of phenolics from coat to seeds after cooking.

3.5. Lectins from **pinhão**

Lectins are a special group of proteins of non-immune origin able to agglutinate cells and precipitate glycoconjugates because of their property of binding reversibly to carbohydrates (Santi-Gadelha et al., 2006). They exhibit a wide range of biological properties not yet fully elucidated. Two lectins with high binding capacity were purified from the *pinhão* seed (Datta et al., 1991, 1993). One of them, a N-acetyl-D-glucosamine lectin, presents agglutinating activity against rabbit erythrocytes (Santi-Gadelha et al.,2006). It also presents significant antimicrobial activity against mainly Gram-negative bacteria due to its ability to form complexes with microbial glycoconjugates. It also acts againstacute cellular inflammation when administered intravenously to rats with peritonitis and paw edema. Administration of 0.01, 0.1 and 1 mg/kg of the *pinhão* lectin significantly reduces the paw edema in a dose-dependent mode. This effect was suggested to involve a lectin domain, since incubation of the lectin with its ligand N-acetyl-D-glucosamine prevented its antiedematogenic activity, an action that was not observed upon incubation with mannose. In the carrageenan-induced peritonitis model, the *pinhão* lectin injected intravenously at the doses of 0.1 and 1 mg/kg inhibited neutrophil migration by 69% and 92%.

Mota et al. (2006) studied the effects of the lectin from *A. angustifolia* seeds in a rat paw edema model and observed that intravenous injection of lectin (0.1-1 mg/kg) dose-dependently inhibited the increase in vascular permeability and edema induced by dextran and serotonine. This action was due to the association of the lectin with the N-acetyl-glucosamine (Glyc-NAc) binding domain. The treatment of animals with the *pinhão* lectin as the sole anti-inflammatory agent (1 mg/kg IV) for 7 days did not affect the body weight of rats, liver, kidney, spleen and stomach, blood leukocyte count, urea, creatinine, or serum transaminase activity. Systemic toxicity was evident only with the administration of doses much higher (88.3 mg/Kg) than those required for an anti-inflammatory action. Thus, the *pinhão* lectin exerts anti- and pro-edematogenic actions by interactions with its specific domain. These actions may share a common pathway involving both the activation and inhibition of inflammatory mediators of resident mastocytes (Mota et al., 2006).

The action of the *pinhão* lectin on the central nervous system of rats was also evaluated (Vasconcelos et al., 2009). The authors administered intraperitoneally lectin (at 0.1, 1 and 10 mg/kg) or saline (control) to male mice 30 minutes before the administration of pentylenetetrazol (85 mg/kg, intraperitoneal injection), pilocarpine

(400 mg/kg, subcutaneous injection) or strychnine (75 mg/kg, intraperitoneal injection). The following parameters were evaluated: latency of the first seizure or death, the percentage of convulsing animals and the percentage of surviving animals.Additionally the authors performed the open field test by administering intraperitoneally lectin (at concentrations of 0.1, 1 and 10 mg/kg), saline (control), diazepam (1 mg/kg) or flumazenil (1 mg/kg). The authors concluded that the lectin of*A. angustifolia* exerts a depressant activity on the central nervous system, acting via a GABAergic mechanism. This conclusion is supported by the observations that the *pinhão* lectin was able to increase the latency for convulsions and the death rate in models induced by pentylenetetrazol and strychnine, and to reduce dose-dependently thelocomotor activity in the field test, using diazepam as a positive control. The latter was reversed by pretreatment with flumazenil. These findings are interesting because they provide opportunities for the development of drugs with central depressant activity.

3.6. The **pinhão** *coat*

The coat of the cooked or raw *pinhão* is usually discarded into the environment. It is estimated that approximately 10 tons of *pinhão* coats are discarded annually (Brasil et al., 2006). As this coat takes a long time to decompose, several investigations have searched for possible uses.

Several authors (Lima et al., 2007; 2008; Royer et al., 2009; Calvete et al., 2009; 2010) studied the use of the *pinhão* coat as an alternative for promoting the adsorption of metal ions and dyes, potentially carcinogenic, from aqueous solutions in the treatment of industrial effluents. All studies argue that the *pinhão* coat can be a powerful and inexpensive tool for removing heavy metals and dyes in the treatment of effluents from tannery and metallurgical industries. The intense brown color of the *pinhão* coat is due to the presence of tannins that are primarily responsible for the adsorption of metal ions, such as copper, for example (Lima et al., 2007).

Besides this alternative of environmental protection, it is important to mention the content of phenolic compounds in the *pinhão* coat. The amounts of phenolics extracted from raw and cooked coat were 77.56 and 31.63 mg catechin equivalents/g dry weight, respectively (Koehnlein et al., 2012). Fourier transform-infrared spectroscopy analysis of the*A. angustifolia* seed coat tannins revealed a higher proportion of procyanidins to prodelphinidins when compared to the tannins of black wattle (*Acacia mearnsii*) (Silva et al., 2014). As other tannin rich extracts (Kusano et al., 2011; Manaharan et al., 2012; de Sales et al., 2012), those of the *A. angustifolia* seed coat strongly inhibited both human salivary and porcine pancreatic α-amylase (Silva et al., 2014). Inhibition of α-amylase resulted in delayed carbohydrate digestion and glucose absorption with attenuation of postprandial hyperglycemic excursions. The observation that the rich in tannins *pinhão* extract is an effective inhibitor of salivary and pancreatic α-amylases, suggests that it could be used to suppress postprandial hyperglycemia in diabetic patients. Furthermore, it was also suggested that the *pinhão* coat tannins could, in principle at least, be used to promote weight loss and to combat obesity, perhaps even as a kind of functional food. The possibility that the tannin rich extract could be active on other enzymes in addition to the α-amylases, as α-glucosidases, for example, cannot be excluded.

Based on the previously mentioned observation that the *pinhão* coat extract inhibits amylases, Oliveira et al. (2015) investigated a possible action on the pancreatic lipase. Inhibition was indeed found, thus confirming the previous hypothesis. The *pinhão* coat extract inhibits the pancreatic lipase by a non-competitive parabolic mechanism. Consistently, the *pinhão* coat extract was also effective in reducing plasma triglyceride levels in rats after an olive oil load. This observation is probably the consequence of an indirect inhibition of triglyceride absorption via inhibition of pancreatic lipase. Taken together with the similar inhibition of glucose absorption after a starch load (Silva et al., 2014) these effects represent a potential anti-obesity activity, as suggested for other polyphenols or preparations rich in tannins.

Pinhão coat extracts rich in condensed tannins were also explored for possible uses in cosmetic formulations thanks to their high antioxidant activity (Mota et al. 2014). The *in vitro* and *in vivo* antioxidant activity as well as the antigenotoxic activity of bracts (undeveloped seeds) of *A. angustifolia* were confirmed and attributed to their high contents in phenolics, especially catechin, epicatechin, quercetin, apigenin, and rutin (Michelon et al., 2012, Souza et al., 2014). The aqueous extract of the bracts was able to scavenge DPPH radicals, and exhibited potent superoxide dismutase and catalase-like activities.The extract significantly protected MRC5 cells against H_2O_2-induced mortality and oxidative damage to lipids, proteins, and DNA.

4. Leaves of *A. angustifolia*

Studies have demonstrated that a fraction of the leaves of *A. angustifolia* containing biflavonoids was effective in protecting DNA from damage induced by ultraviolet radiation and in inhibiting lipid peroxidation (Yamaguchi et al., 2009; Souza et al., 2014). In leaves of *A. angustifolia*, six main biflavones were identified: amentoflavone, mono-*O*-methylamentoflavone, di-*O*-methylamentoflavone, ginkgetin, tri-*O*-methyl amentoflavone and tetra-*O* methyl-amentoflavone (**Figure 2**) (Yamaguchi et al., 2005; Souza et al., 2014). The fraction rich in biflavonoids obtained from the leaves of *A. angustifolia* showed a greater ability to eliminate the singlet oxygen radical when compared with quercetin and ginkgetin. The same fraction also showed the greatest inhibition of single-stranded plasmid DNA breaks formation. On the other hand, the fraction rich in biflavonoids was not able to protect plasmid DNA against single strand breaks generated by the Fenton reaction, contrary to quercetin and rutin. Moreover, at least in two papers (Yamaguchi et al., 2005; Souza et al., 2014) it was observed that, similar to quercetin and rutin, the fraction rich in biflavonoids proved to be able of protecting liposomes against lipid peroxidation induced by ultraviolet (UV) radiation, which is responsible for damage caused to the skin (Souza et al., 2014). Although the biological activity of the biflavone fraction obtained from the leaves of *A. angustifolia* is not as effective as quercetin, rutin, alpha-tocopherol and troloxin protecting against single DNA strand breaks,it can notwithstanding be regarded as an excellent candidate for successful employment as antioxidant and sunscreen. A recent study has demonstrated that the biflavonoids of the leaves from *A. angustifolia* were more efficient in preventing the formation of thymine cyclobutane dimers induced by UV-B radiation than the compounds commonly used in cosmetics, but were not as efficient against UV-A radiation (Yamaguchi et al., 2014).

A study conducted to confirm the popular use of leaves of *A. angustifolia* against Herpes Simplex Virus (HSV-1) showed that the ethyl acetate and *n*-butanol fractions obtained from the crude hydroalcoholic extract have the best antiherpetic activity (Freitas et al., 2009). Chemical analysis of this subfraction revealed the presence of proanthocyanidins and biflavonoids (bilobetin, II-7-*O*-methyl-robustoflavona and cupressuflavone), but the authors reported that the proanthocyanidins are possibly the main responsible for the anti-HSV-1 activity.

5. Miscellaneous compounds found in various parts of *A. angustifolia*

A series of molecules have been identified in different parts of *A. angustifolia* (Maurer et al., 2010; Koul et al., 2015). **Table 2** lists some of these molecules. The biological activities of these molecules have not yet been fully investigated. In the dead bark, however, two natural product afzelechin derivatives, *epi*afzelechin protocatechuate and (-) epiafzelechin *p*-hydroxybenzoate were identified and presented strong antioxidant activities (Seccon et al., 2010).

7. Concluding remarks

This review reveals that apart from the nutritional properties of the *pinhão*, particularly as a starch source, a number of biological activities have been found in different parts of *A. angustifolia* (leaves, bark and *pinhão* coat), such as antioxidant, anti-inflammatory, antiviral and digestive enzyme inhibiting activities (**Figure 4**). The *pinhão* almond is no doubt amply used in the regional cuisine. More studies are needed, however, with respect to the technological feasibility of using it in industrialized products, especially as an alternative source of starchor as a substitute or alternative for the commonly used flours. Knowledgeabout the chemical composition and biological activities of extracts from leaves, dead bark, bracts and *pinhão* coat must also be improved. It seems at least clear from the preliminary results that there is still much to gain from a systematic and targeted continuation of the investigations. Such investigations should include also parts of *A. angustifolia* that are currently discarded, such as the bark and the *pinhão* coat, whose potential for being used as pharmaceutical and cosmetic products should not be underestimated.

Finally, it is important to mention that studies on *A. angustifolia*, a species threatened by extinction and with aparticular socio-cultural value, must combine two important elements: the need for preservation of a typical ecosystem and the implementation of the *A. angustifolia* forests as a true economic alternative for local residents.

Acknowledgments

The authors thank the Fundação Araucária for funding this study. R.F. Oliveira, R.C.G. Correa, L. Bertonha and V.G. Correa thank Coordenação de Aperfeiçoamento do Pessoal do Ensino Superior (CAPES) for the financial support provided for their post-graduate studies in Universidade Estadual de Maringá. R.M. Peralta and A. Bracht are research grant recipients of Conselho Nacional de Desenvolvimento Científico e Tecnologia (CNPq).

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Table 1. Miscellaneous of chemical compounds identified in several parts of *A. angustifolia*.

Part of plant	Compounds
Tissue cultures	**Undifferentiated callus**
(Fonseca et al., 2000)	Phenyl propanoid derivatives E and Z isomers of octadecyl *p*-coumarate and octadecylferulate
	Seedling stems
	Biflavonoids (7,4',7''-tri-*O*- methyl amentoflavone, 7,4',4''-tri-*O*-methyl amentoflavone, 4',4''-di-*O*-methyl amentoflavone)
	Seedling roots
	Diterpene (trans-cummunic acid)
	Adult stems
	Benzaldehydes (vanillin, *p*-hydroxybenzaldehyde and coniferaldehyde); lignans (pinoresinol, eudesmin and lariciresinol); isoflavones (cabreuvine and irisolidone)
Knot resin (Ohashi et al., 1992)	Norlignan (2,3-bis-(*p*-hydroxyphenyl)-2-cyclopentene-l-one)
Dead bark (Seccon et al., 2000)	Benzoic acid, *p*-hydroxybenzoic acid, protocatechuic acid, quercetin, (-)-epiafzelechinprotocatechuate, (-)-epiafzelechinp-hydroxybenzoate, (-)-epicatechin

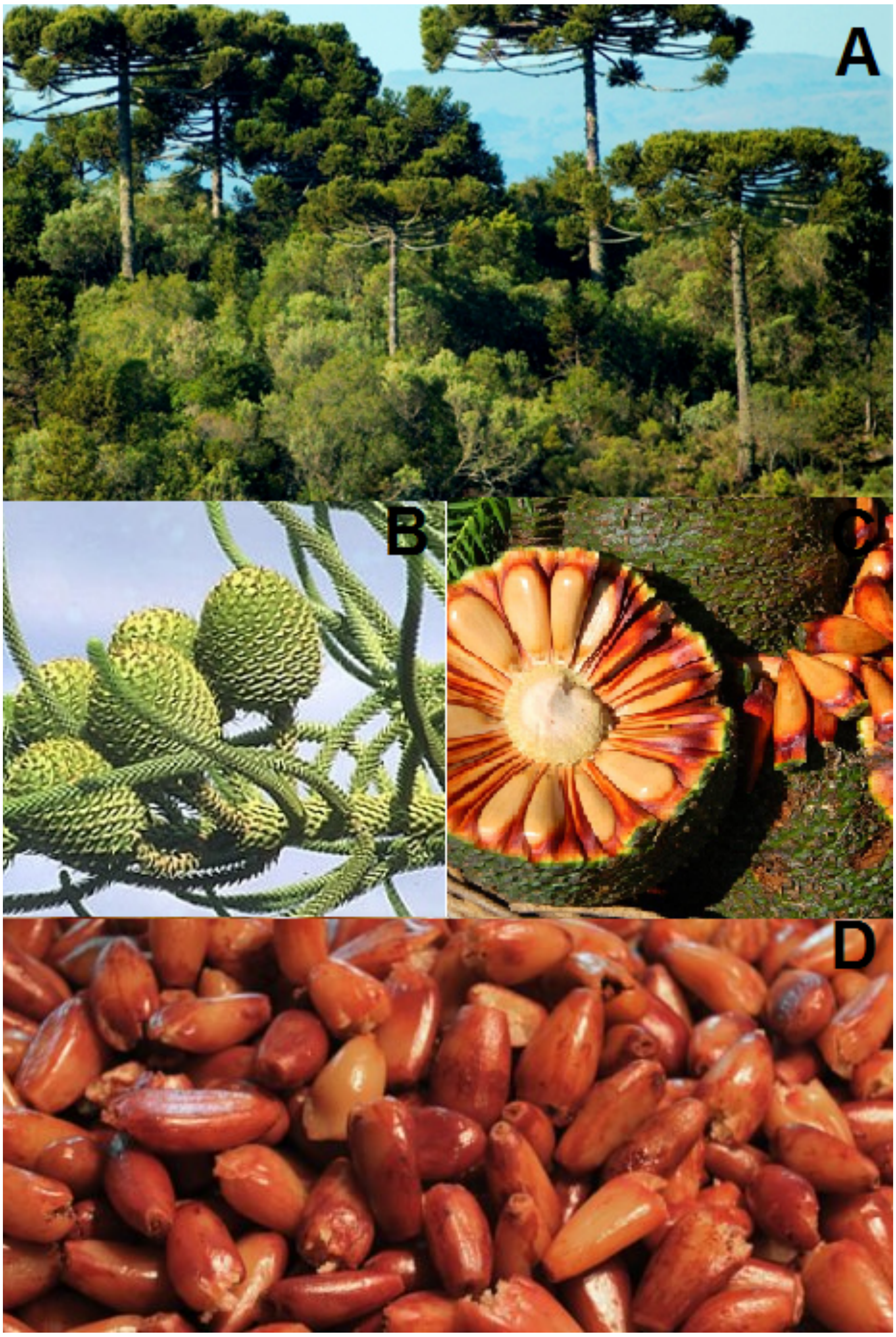


Figure 1. *Araucaria angustifolia*. (A): mature tree; (B): female cones or pine cones; (C): mature seeds; (D): cooked seed (edible part).

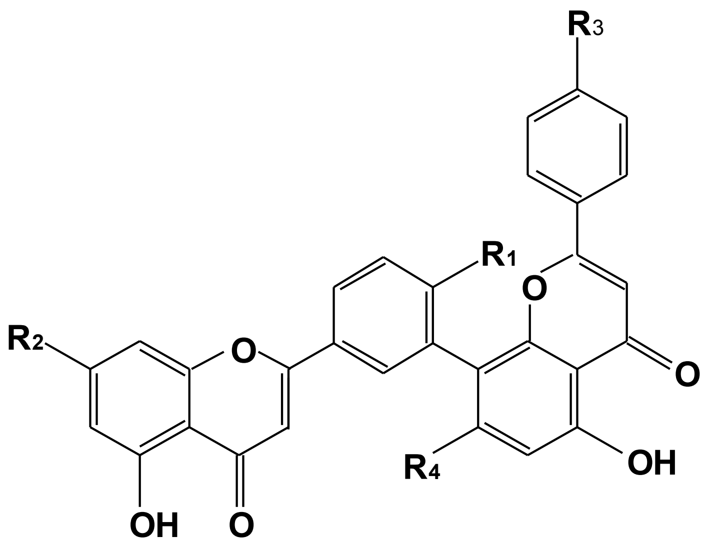


Figure 2. Six major biflavonoids present in *A.angustifolia* needles (Yamaguchi et al., 2005; 2009).

R_1=R_2=R_3=R_4=OH: amentoflavone;

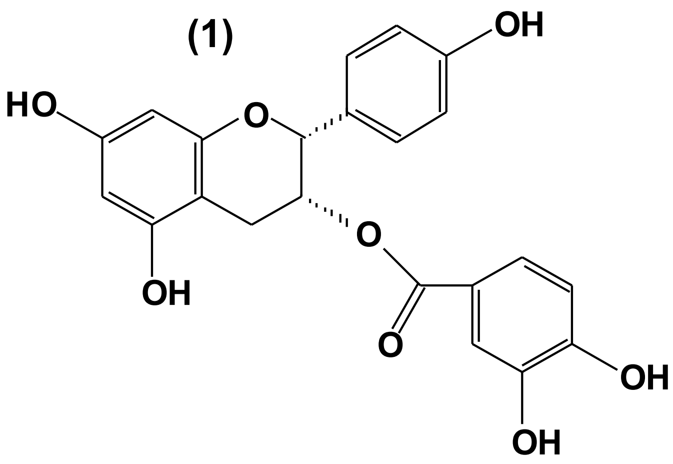
mono-*O*-methylamentoflavone;

di-*O*-methylamentoflavone;

R_1=R_2=OMe, R_3=R_4=OH: ginkgetin; tri-*O*-methylamentoflavone;

R_1=R_2=R_3=R_4=OMe: tetra-*O*-methylamentoflavone

(1)



(2)

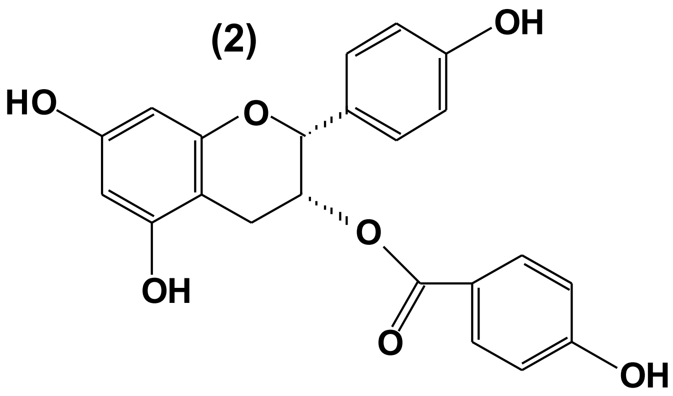


Figure 3. Two natural product afzelechin derivatives present in dead bark of *A. angustifolia*. (1) epiafzelechin protocatechuate; (2) (-)-epiafzelechin *p*-hydroxybenzoate (Seccon et al, 2010)

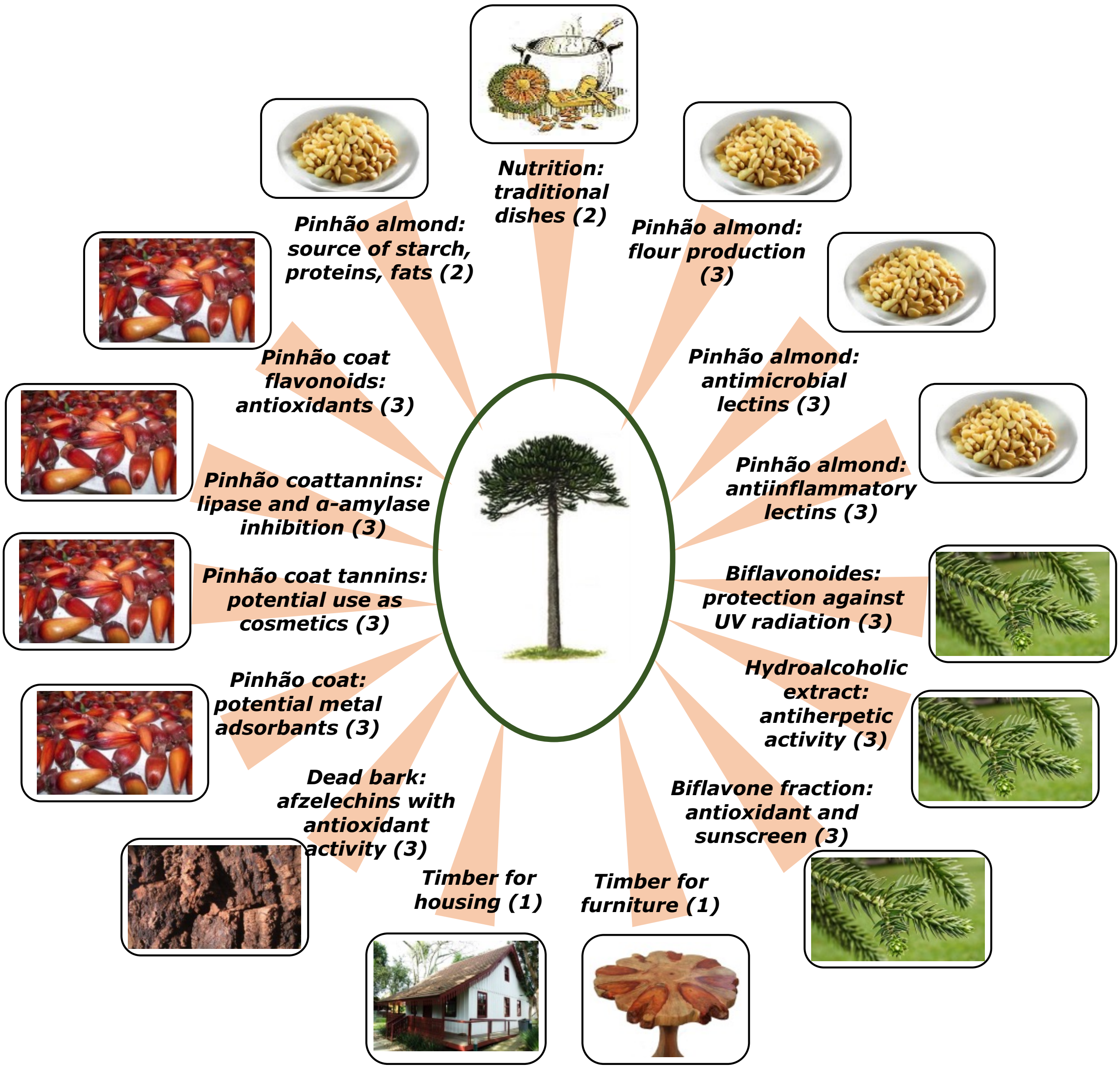


Figure 4. Uses of products derived from *Araucaria angustifolia*: (1) past; (2) present and (3) future.