

An in vitro analysis of the total phenolic content, antioxidant power, physical, physicochemical, and chemical composition of *Terminalia Catappa* Linn fruits

Composição física, físico-química, química, análise do teor de fenólicos totais e poder antioxidante in vitro de frutos de Castanhola (Terminalia Catappa Linn)

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Abstract

This study assessed the antioxidant, total phenolic, and physicochemical properties of in vitro *Terminalia Catappa* Linn (locally called *castanhola*) using the DPPH assay. The *castanhola* fruits had an average weight of 19.60 ± 0.00 g, combining shell, pulp, and seed weight, and a soluble solids content of 8 °Brix. The chemical composition was determined with predominance of carbohydrates ($76.88 \pm 0.58\%$). The titration method was used to determine Vitamin C content using 2,6-dichlorophenolindophenol (DCFI), known as reactive Tillmans resulting in no significant levels. Aqueous extracts of castanhola pulp showed a higher concentration of phenolics, 244.33 ± 18.86 GAE.g⁻¹ of fruit, and alcoholic extracts, 142.84 ± 2.09 GAE.g⁻¹ of fruit. EC₅₀ values of the aqueous extract showed a greater ability to scavenge free radicals than the alcoholic extracts. The fruit had a significant content of phenolic compounds and high antioxidant capacity.

Keywords: tropical fruit; functional properties; bioactive compounds.

Resumo

Este estudo teve como objetivo determinar as características físicas, físico-químicas e químicas, teor de fenólicos totais e poder antioxidante da castanhola (*Terminalia Catappa* Linn) in vitro, por meio de descolorimento de radical DPPH (2,2-difenil-1-picrihidrazil). O fruto da castanhola apresentou peso médio de $19,60 \pm 0,00$ g, quando somados os pesos da polpa, casca e semente e um teor de sólidos solúveis de 8 °Brix. Determinou-se a composição química, obtendo-se uma predominância de carboidratos ($76,88 \pm 0,58\%$). A quantificação da Vitamina C foi feita segundo o método titulométrico, usando-se o reagente 2,6-diclorofenolindofenol (DCFI), conhecido como reagente de Tillmans, obtendo-se teores não significativos. Os extratos aquosos da polpa da castanhola apresentaram uma concentração maior de fenólicos totais, com resultados de $244,33 \pm 18,86$ GAE.g⁻¹ do fruto e os extratos alcoólicos um teor de $142,84 \pm 2,09$ GAE.g⁻¹ do fruto. Os valores de EC₅₀ dos extratos aquosos apontaram uma maior capacidade de sequestrar radicais livres do que dos extratos alcoólicos. O fruto tem um conteúdo significativo de compostos fenólicos e elevada capacidade antioxidante.

Palavras-chave: fruto tropical; características funcionais; compostos bioativos.

1 Introduction

The *Terminalia Catappa* Linn tree is known as *castanheira*, *castanhola*, *castanholeira*, *chapéu-de-sol* e *sete-copas*, belongs to the Combretaceae family. Its trunk can be straight or tortuous and ranges from 25 to 45 m in height and 50 to 150 cm in diameter. Originally from South Asia (specifically India, Malaysia, Philippines, and Indonesia), it was introduced to Brazil for ornamental purposes in urban and rural afforestation and reforestation (CAVALCANTE et al., 1986; GILMAN; WATSON, 1994; OLIVEIRA et al., 2000; ANGEL et al., 2003; THOMSON; EVANS, 2006).

The fruits are popularly called almonds or Indian almonds. They are edible and are used in food, especially for children, birds, and other animals. In addition to the pulp, the nut inside the seed is also edible – a source of proteins and lipids

(CAVALCANTE et al., 1986; MATOS et al., 1992; IVANI et al., 2008).

Many species of the genus *Terminalia* have been used for medicinal purposes in Asian countries and to treat infectious diseases in western Africa (OLIVEIRA et al., 2000; SOUSA et al., 2007).

Currently, several studies show that free radicals and other oxidants play a key role in the development of diseases that are related to premature aging and chronic non-communicable diseases (ATOUI et al., 2005; SOUSA et al., 2007).

Driven by concerns about their future health and the daily benefits that a correct diet can offer, Brazilians have been seeking healthy foods, especially foods that are

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inexpensive and can be supplied locally (SANTOS, 2007). Phenolic compounds are aromatic compounds that result from the secondary metabolism of plants (KIM; JEONG; LEE, 2003). They have an adaptive-protective role, and their importance has been shown in numerous studies, particularly the importance of flavonoids, anthocyanins, and anthoxanthins, which proves the ability to capture free radicals and thus promote the prevention of cardiovascular and circulatory diseases, cancer, diabetes, and Alzheimer's disease (ISHIGE; SCHUBERT; SAGARA, 2001; WANG; MAZZA, 2002; STOCLET et al., 2004; ABDILLE et al., 2005; KHATOON et al., 2008).

The bark of plants of the genus *Terminalia* is a source of terpenoids, flavones, and phenolic compounds, and the presence of tannins in the peel of the fruits is a characteristic of this genus (KHATOON et al., 2008).

The aim of this study was to determine the physicochemical composition of *castanhola* fruits (*Terminalia Catappa* Linn) and also to quantify their total phenolics and antioxidant capacity.

2 Material and methods

2.1 Raw material

Twenty mature fruits were randomly selected from trees at the Ininga campus of the Federal University of Piauí-UFPI.

Once selected, the fruits were rinsed in water and measured. They were then peeled manually with the aid of stainless steel knives to separate the pulp, which was homogenized for subsequent analysis of chemical, total phenolic, and antioxidant properties.

2.2 Physical parameters

The following measurements of physical variables were performed in the 20 randomly selected fruits: the average dimensions of the fruits (using Mitutoyo callipers) and the weight of the whole fruit, peel, core, and the pulp to obtain a final average weight using a digital weighing scale (MARTE model AY220). These data were used to calculate the ratio among shell, core, and pulp. The pH was measured using a pH meter (WTW model pH 330i/SET) and calibrated with pH 4.0 buffer solution. The soluble solids were determined using a refractometer (QUIMIS model Q767A1).

2.3 Chemical measurements of the pulp

The measurement of moisture and ash content were calculated using the method described by Association of Official Analytical Chemists (2000). The ether extract was measured using the method recommended by the Institute Adolfo Lutz (2008). Fatty matter was extracted from the sample with hexane using a Soxhlet extractor. The protein content was determined according to the Association of Official Analytical Chemists (2000) method and included evaluation of total nitrogen following the method of Kjeldahl. The quantity of protein was calculated by multiplying the total nitrogen content of the sample by 6.25.

The carbohydrate content was determined by subtracting values of protein, fat, ash, and moisture from 100 according to the analytical standards of the Institute Adolfo Lutz (2008). The titration method was used to determine Vitamin C content using 2,6-dichlorophenolindophenol (DCFI), known as reactive Tillmans.

2.4 Obtaining extracts and chemicals and standards

The sample was previously dried in a ventilated oven at 50 °C. After grinding, 5 g of the sample was used in the sequential extraction using ethyl ether (PROQUIMIOS®), ethanol (VETEC®), and distilled water. The residues were filtered using Whatman N° 4 filter paper according to the method described by Larrauri, Rupérez and Saura-Calixto (1997), adapted by Lima (2008).

The study used SIGMA® brand reagents and standards (such as the DPPH). Folin Denis Reagent was purchased from DINAMICA®, and the sodium carbonate, Gallic acid, and methanol were supplied by VETEC®.

2.5 Determination of total phenolic compounds in the extracts

The quantification of total phenolics was measured using Folin's reagent (SWAIN; HILLIS, 1959, adapted by LIMA, 2008). A calibration curve was created with different solutions of gallic acid, and the results were expressed as mg GAE (gallic acid equivalent). The reading at 720 nm in a CELM spectrophotometer was achieved by transferring 0.5 mL of the solution of the extracts of the fruit to a 10 mL container and by adding 0.5 mL of Folin Denis reagent. The solution was homogenized, allowed to stand for 3 minutes, and then 1 mL of saturated solution of sodium carbonate (NaCO₃) was added. The final volume was completed with distilled water.

2.6 Evaluation of antioxidant activity of the extracts DPPH radical method

In this study, the antioxidant activity was assessed in terms of hydrogen-donating or radical scavenging ability of extracts. A methanolic solution (1 mL) of the extract at four different concentrations was added to 3 mL of DPPH solution (6 · 10⁵ M in methanol). The decrease in the absorbance at 517 nm was measured using a CELM spectrophotometer until the reaction reached the steady state in the dark (BRAND-WILLIAMS; CUVELIER; BERSET, 1995). Radical scavenging activity was expressed as the inhibition percentage and was calculated as follows (Equation 1):

$$\% \text{ Protection} = (\text{Abs control} - \text{Abs sample}) \times 100 / \text{Abs control} \quad (1)$$

The present study also assessed the kinetic parameters of the capture reaction of the DPPH radical at four different times: 5, 10, 15, and 20 minutes of reaction (SÁNCHEZ-MORENO; LARRAURI; SAURA-CALIXTO, 1998).

3 Results and discussion

3.1 Physical and physicochemical characteristics

Since this is not a commercial fruit, the data about the physical and physicochemical properties of *castanholas* are not

easily found in the literature. Table 1 shows the comparative data of physical parameters found in this study and the study of Cavalcante (1986).

The analysis of the data shows that there was a difference in the average weight of whole fruit (19.60 ± 0.00 g) and the average weight of the seed (7.28 ± 0.59 g) when compared with Cavalcante's data (1986), who obtained an average weight of whole fruit of 38.37 ± 0.00 g and average weight of seed of 20.23 ± 0.00 g (Table 1). However, higher pulp yield (57.34%) was obtained even with lower average weights. The dimensions of the fruit showed similar results, which shows that the differences may be caused by differences in location, planting techniques, and soil type. Nevertheless, the fruits maintain a certain regularity of physical characteristics.

The °Brix and vitamin C values could not be compared because there are no studies in the literature that address these aspects of *castanhola* fruits.

Analysis of the nutritional composition of the fruit shows that there is a high quantity of carbohydrates and water in its composition (Table 2). The consumption of 100 g of castanhola supplies 17.09% of the caloric needs of an adult based on a diet of 2,000 kcal.

The reduced levels of vitamin C in the *castanhola* fruits is mainly due to the high rainfall in the region during the harvest period, which probably diluted both the soluble solids and the ascorbic acid present in the cellular juice of the fruit, as revealed by the level of humidity found in the composition of the fruit.

3.2 Quantification of in vitro total phenolics and antioxidant capacity

This study assessed the total phenolic content of aqueous and alcoholic extracts of *castanhola*, and the results are presented in Table 3. According to the data, the two extracts that were examined had significant quantities of phenolic compounds.

A comparison of the efficiency of solvent extraction, in Table 3, shows that pure water had a higher power to extract phenolic compounds present in the *castanhola* when compared with the alcoholic solution. This demonstrates that the majority of the phenolic compounds of these *castanholas* have a higher polarity and are therefore more hydrosoluble.

Recent research has shown that phenolic compounds, in addition to playing a role in the capture of free radicals, may also be involved in other physiological mechanisms that stimulate the activity of antioxidant enzymes or as cellular signaling substances that activate and/or inhibit the expression of some enzymes related to the cancer process (KATSUBE et al., 2003, SHAHIDI; ALASALVAR; LIYANA-PATHIRANA, 2007). Therefore, studies that quantify these compounds in foods are of vital importance.

According to Nagappa et al. (2003), the presence of phenolic compounds indicates that the fruit has the potential to prevent and possibly treat certain diseases. In this regard, extracts from the castanhola fruits (*Terminallia* C. Linn) were described as being anti-hypoglycemic and capable of regenerating β -cells in the pancreas.

Table 1. Physical and physicochemical parameters of castanholas in different studies. Teresina-PI, 2010.

Parameters	Values obtained	Values obtained by Cavalcante (1986)
Number of fruits	20	86
Average weight of fruits (g)	19.60 ± 0.00	38.37 ± 0.00
Pulp (g)	11.24 ± 2.35	13.72 ± 0.00
Shell (g)	1.08 ± 0.00	2.32 ± 0.00
Seed (g)	7.28 ± 0.59	20.23 ± 0.00
Pulp yield (%)	57.34	35.80
Shell percentage	5.51	6.20
Seed percentage	37.14	52.50
Length/ Height(cm)	4.32 ± 0.00	4.11 ± 0.00
Width (cm)	3.16 ± 0.00	3.42 ± 0.00
Thickness (cm)	2.57 ± 0.00	-
Soluble solids (°Brix)	8 ± 0.00	-

Table 2. Chemical composition of Castanhola fruits. Teresina-PI, 2010.

Nutrients	Castanhola (mean \pm SD)
Humidity (%)	17.2 ± 1.13
Ash (%)	0.83 ± 0.24
Lipids (%)	2.79 ± 0.58
Proteins (%)	2.30 ± 0.00
Carbohydrates (%)	76.88 ± 0.58
Vitamin C content (mg.100 g ⁻¹)	0.22 ± 0.00
Total Energetic Value (TEV) (kcal.100 g ⁻¹)	341.83

Table 3. Content of total phenolic compounds in castanhola fruits. Teresina-PI, 2010.

Fruit	Extraction (mg GAE.g ⁻¹ of fruit) alcoholic aqueous	
	(Mean \pm SD)	(Mean \pm SD)
Castanhola (mg.100 g ⁻¹)	142.84 ± 2.09	244.33 ± 18.86

These functions of phenolic phytochemicals are due to their ability to efficiently scavenge radicals. Studies of tea have found a maximum quantity of 50 mg GAE.g⁻¹. When comparing phenolic quantifications in the cashew fruit, it was found that the castanhola has a higher composition of these compounds compared to that of the cashew pseudo-fruit with 2.8 to 10.4 mg of gallic acid/g of bagasse (ASOLINI; TEDESCO; CARPES, 2006; BROINIZI et al., 2007; SOARES et al., 2008).

The quantity measured in this study is comparable to the phenolic content found in 100 g the Niagara and Isabella varieties of grape, 183.04 ± 11.63 and 196.83 ± 16.97 mg GAE, respectively, which are widely recognised as potential sources of phenolic compounds (ASOLINI; TEDESCO; CARPES, 2006; BROINIZI et al., 2007; SOARES et al., 2008).

The DPPH method is one of the most widely used chemical methods to determine antioxidant capacity because it is considered to be practical, fast, and stable (SOARES et al., 2008).

Table 4. Percentage of inhibition of the DPPH radical by castanhola extracts, after 20 minutes. Teresina-PI, 2010.

Extracts	Concentrations ($\mu\text{g.mL}^{-1}$)				EC_{50} ($\mu\text{g.mL}^{-1}$)
	12.5	25	50	100	
Aqueous (%)	50.00 (± 0.00)	57.73 (± 2.72)	61.81 (± 3.44)	71.09 (± 4.33)	0.70
Alcoholic (%)	33.67 (± 0.00)	34.44 (± 0.61)	46.94 (± 1.11)	51.36 (± 5.31)	85.99

The antioxidant activity of the aqueous and alcoholic extracts of the *castanhola* fruits obtained using the DPPH radical scavenging method is shown in Table 4. The results were expressed as EC_{50} (the quantity of antioxidant in the extracts that is capable of reacting with 50% of the radical in the DPPH solution). Therefore, the lower the EC_{50} value, the greater the antioxidant activity of the extract analyzed.

There is a higher antioxidant activity in the aqueous extract with EC_{50} values of $0.70 \mu\text{g.mL}^{-1}$, which also had a higher concentration of phenolic compounds. In this case, the concentration of these compounds was directly related to the antioxidant activity.

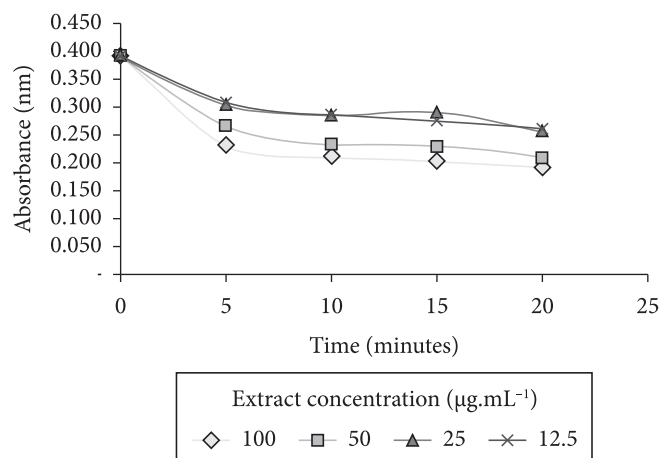
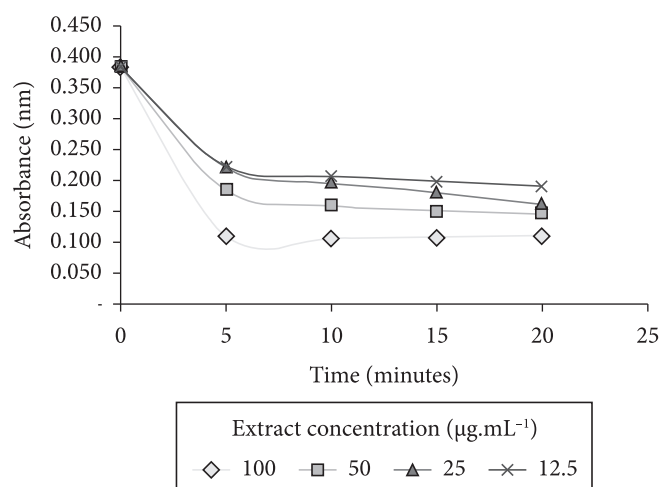
Compared with Brazilian cerrado fruits, extracts of the castanhola are more effective antioxidants. Roesler et al. (2007), in their study on *pequi*, *araticum*, *cagaita*, and *lobeira* pulps obtained EC_{50} values in alcoholic extracts of $298.75 \pm 3.80 \mu\text{g.mL}^{-1}$, $148.82 \pm 0.98 \text{mg.mL}^{-1}$, $387.47 \pm 8.70 \text{mg.mL}^{-1}$ and $162.97 \pm 2.05 \mu\text{g.mL}^{-1}$, respectively. In aqueous extracts, the results were respectively $534.43 \pm 7.32 \mu\text{g.mL}^{-1}$, $198.28 \pm 8.24 \mu\text{g.mL}^{-1}$, $879.33 \pm 11.70 \mu\text{g.mL}^{-1}$, and $1328.98 \pm 9.42 \mu\text{g.mL}^{-1}$.

Figures 1 and 2 show the kinetic curves of degradation of the DPPH radical by the different extracts at different concentrations of the *castanholas*. Each extract had a distinct behavior according to the concentration. The aqueous extract had a strong antioxidant capacity in the first five minutes of reaction with a significant reduction of DPPH radical. It was found that the higher the concentration of the extract, the higher the antioxidant activity in both the aqueous and alcoholic extract.

The efficiency of extraction of phenolic compounds with water was demonstrated once more by the fact that the aqueous extract, under the same conditions of concentration and reaction time of the alcoholic extract, changed the color of the DPPH to a greater extent, which shows that the antioxidant power of the aqueous extract is greater than the other extract and directly proportional to the quantity of extracted phenols.

When the same extraction conditions were used with fruits from the Brazilian Cerrado, such as *murici* and *jenipapo*, there was also a greater carrying efficiency of large amounts of hydrophylic phenols. As with the castanhola fruits, the ability to change the color of the DPPH radical is directly linked to the amount of total extracted phenols (MOREIRA-ARAÚJO et al., 2010).

In the sample analyzed, the vitamin C content was not a determining factor in the high antioxidant potential of the fruit because of the low level of the vitamin ($0.22 \text{mg.}100 \text{g}^{-1}$).

**Figure 1.** Kinetic curve of the antioxidant potential of the alcoholic extract of castanhola fruits using the DPPH assay. Teresina-PI, 2010.**Figure 2.** Kinetic curve of the antioxidant potential of the aqueous extract of castanhola fruits using the DPPH assay. Teresina-PI, 2010.

4 Conclusion

It was concluded that although the castanhola fruit is not commercially exploited, it has a high calorific content and can be used as a source of carbohydrates, has a high pulp yield, and is a source of phenolic compounds with antioxidant properties that are comparable to the best known sources to date. This study showed that the majority of these compounds are hydrophilic and that water is the best solvent for extraction of castanhola. High levels of phenolic compounds extracted in aqueous media suggest high extraction efficiency in the human body during normal digestion since human body is composed mostly of water.

Further studies including other methodologies should be carried out to address the in vivo and in vitro antioxidant potential of this fruit and demonstrate its health benefits for humans.

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