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Bioactivity of *Terminalia catappa* Linn fruit and seed

Bioatividade da polpa e amêndoae sementes da *Terminalia catappa* Linn

Bioactividad de la fruta y semilla de *Terminalia catappa* Linn

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Resumo

As propriedades bioativas de frutos não convencionais têm sido de interesse de pesquisas recentes. O objetivo deste estudo foi avaliar a bioatividade da polpa e amêndoa da Terminalia catappa Linn através de sua atividade antioxidante e antimicrobiana contra microrganismos patogênicos. A polpa e a amêndoa foram analisadas quanto aos parâmetros físico-químicos de pH, aw, umidade, cinzas, carboidratos, proteínas e lipídios. Após a secagem, as farinhas da polpa e da amêndoa foram avaliadas quanto à bioatividade (fenólicos totais, taninos, antocianinas e flavonóides e antioxidante-DPPH) e atividade antimicrobiana. A polpa apresentou maior teor de carboidratos (15,65%), enquanto a amêndoa foi caracterizada por alto teor lipídico (48,52% ± 0,31) e proteínas (26,40% ± 0,10). A farinha da polpa apresentou quantidades significativas de taninos (1662,68 mg.100-1 g ± 1,27) e atividade antimicrobiana inibindo Staphylococcus aureus, Pseudomonas putida, Pseudomonas aeruginosa, Clostridium perfringens e Listeria monocytogenes. A farinha da amêndoa apresentou atividade antioxidante (46,05 mg.100-1 g ± 0,23), mas não apresentou poder inibitório contra as cepas bacterianas testadas. A fruta Terminalia catappa Linn pode ser um novo ingrediente alternativo com ação antioxidante natural.

Palavras-chave: Amêndoa indiana; Antimicrobiano natural; Bioatividade.

Abstract

The bioactive properties of unconventional fruits have been of interest in recent research. The objective of this study was to evaluate the bioactivity of Terminalia catappa Linn fruit and seed through its antioxidant and antimicrobial activity against pathogenic microorganisms. The fruit and the seed were analyzed for the physical-chemical parameters of pH, aw, humidity, ashes, carbohydrates, proteins and lipids. After drying, pulp and almond flours were evaluated for bioactivity (total phenolics, tannins, anthocyanins and flavonoids and antioxidant-DPPH) and antimicrobial activity. The fruit had a higher carbohydrate content (15.65%), while the seed was characterized by high lipid content (48.52% ± 0.31) and protein (26.40% ± 0.10). The pulp flour showed significant amounts of tannins (1662.68 mg.100-1 g ± 1.27) and antimicrobial activity inhibiting Staphylococcus aureus, Pseudomonas putida, Pseudomonas aeruginosa, Clostridium perfringens and Listeria monocytogenes. The seed flour presented antioxidant activity (46.05 mg.100-1 g ± 0.23), but did not present inhibitory power against the tested bacterial strains. Terminalia catappa Linn fruit can be an alternative new ingredient with natural antioxidant action.

Keywords: Bioactivity; Indian almond; Natural antimicrobial.

Resumen

Las propiedades bioactivas de las frutas no convencionales han sido de interés para investigaciones recientes. El objetivo de este estudio fue evaluar la bioactividad de la pulpa y la almendra de Terminalia catappa Linn a través de su actividad antioxidante y antimicrobiana contra microorganismos patógenos. La pulpa y la almendra se analizaron para determinar los parámetros fisicoquímicos de pH, aw, humedad, cenizas, carbohidratos, proteínas y lípidos. Después del secado, se evaluó la bioactividad de las harinas de pulpa y almendras (fenólicos totales, taninos, antocianinas y flavonoides y antioxidante-DPPH) y actividad antimicrobiana. La pulpa presentó un mayor contenido de carbohidratos (15,65%), mientras que la almendra se caracterizó por un alto contenido de lípidos (48,52% ± 0,31) y proteínas (26,40% ± 0,10). La harina de pulpa mostró cantidades significativas de taninos (1662.68 mg.100-1 g ± 1.27) y actividad antimicrobiana inhibiendo Staphylococcus aureus, Pseudomonas putida, Pseudomonas aeruginosa, Clostridium perfringens y Listeria monocytogenes. La harina de almendras mostró actividad antioxidante (46.05 mg.100-1 g ± 0.23), pero no mostró poder inhibidor contra las cepas bacterianas analizadas. Terminalia catappa Linn fruit puede ser un nuevo ingrediente alternativo con acción antioxidante natural.
Palabras clave: Almendra india; Antimicrobiano natural; Bioactividad.

1. Introduction

Terminalia catappa Linn is a tree belonging to the *Combretaceae* family, characteristic of tropical and subtropical regions, being easily found in warmer areas of India and Malaysia, Australia, Sri Lanka, and Pakistan, among other places in South Asia (Mylangam et al., 2016).

Terminalia catappa Linn was introduced in Brazil as an ornamental tree, adapting easily to the soil and climate conditions of the country, resisting variations of heat and cold, wind, water scarcity and salinity (Silva et al., 2010). Studies report that the species has anticancer potential, healing, antidiabetic, anti-inflammatory, analgesic, immunomodulatory, hepatoprotective and aphrodisiac properties (Nagappa et al., 2003; Zhou et al., 2011; Khan et al., 2013; Azrul et al., 2014).

Terminalia catappa Linn fruit is a drupe, being constituted by a fibrous fruit that covers a hard kernel which contains a single seed inside. This fruit shows coloration that passes from green and then becomes yellow or totally red when mature (Anand et al., 2015). Several fruits are holders of bioactive compounds such as phenols, flavonoids and terpenoids with compounds that have antioxidant activity and act by eliminating free radicals, becoming an interesting target for use as natural preservatives in foods instead of synthetic additives (Kalem et al., 2017; Venkatesan et al., 2017).

In this sense, studying the bioactivity of unconventional fruits such as *Terminalia catappa* Linn makes it possible to obtain an alternative for application in food products. Therefore, the objective of this study is to characterize the physicochemical characteristics of *Terminalia catappa* Linn fruit *in natura*, and to evaluate the bioactive compounds, antioxidant and antimicrobial activity of the fruit and seed flour from *Terminalia catappa* Linn fruit.

2. Material and methods

Fruit collection

Terminalia catappa Linn fruits were collected in the city of Santa Luzia, in the interior of Paraíba, during the morning and selected in mature maturation state (as observed by purple red coloration). The fruits were stored in polyethylene bags under freezing temperature (-18 °C) until analysis.

Sample preparation

The *Terminalia catappa* Linn fruits were submitted to hygienization process, washed in running water and left for 15 minutes in chlorine solution (100 ppm), then rinsed for the removal of chlorine residues. The pulp was separated from the kernel with a stainless steel knife, where the fruit and kernel were dried in an air circulation oven (Solab - Solab brand, model SL 102/630, Piracicaba, Brazil) for 6 hours at 60 °C. *Terminalia catappa* Linn fruit was ground in an industrial blender (Urano - Urano brand, model UCB 950F, Manaus, Brazil) and sieved in 70-mesh sieve. The seed were removed from the dry kernels with the help of a stainless knife, crushed in an industrial blender (Urano - Urano brand, model UCB 950F, Manaus, Brazil) and sieved in a 70-mesh sieve. The flour was defatted (DAF) in Soxhlet for 6 hours using hexane as the solvent, and dried in an oven at 105 °C for 1 hour to remove the solvent.

Characterization of the fruit and seed

The fruit and seeds were evaluated *in natura* for pH, moisture content, ashes, lipids and proteins (Association of Official Analytical Chemists, 2005). The carbohydrate content was obtained by the difference. The water activity (A_w) was measured directly in an AquaLab apparatus (Decagon, Pulman-AquaLab, model 3TE, Washington, USA) at a temperature of 25 °C.

Bioactivity of fruit flour (FF) and defatted seed flour (DSF)

Determination of the total phenolics and tannins contents of the FF and DSF were performed according to the method described by Waterhouse (2006), using a Folin-Ciocalteu reagent with reading in a digital spectrophotometer (Spectrophotometer-Biospectro®, Spectrophotometer model SP 220, Curitiba, Brazil) at a wavelength of 765 nm. Gallic acid was used as standard for determining total phenolics and tannic acid as the standard for tannin determination. The results were expressed as mg of acid x 100 g^{-1} sample.

Determination of anthocyanins and flavonoids was performed according to the method described by Francis (1982), where 0.5 g of the sample was weighed and 25 mL of solution was added with 95% ethanol + 1.5 mol/L HCl (85:15, v/v), and maintained in the absence of light under refrigeration for a period of 24 hours. The material was filtered and then read for anthocyanins at the wavelength of 535 nm in a digital spectrophotometer and at 374 nm for flavonoids. The results were expressed in mg x 100 g^{-1}.

The antioxidant activity was determined according to the methodology described by Fernandes et al. (2015), with inhibition of the free radical DPPH (2-diphenyl-1-picrihydrazyl) being analyzed in a digital spectrophotometer (Spectrophotometer-Biospectro®, Spectrophotometer model SP 220, Curitiba, Brazil) at 515 nm. The calibration curve was performed with solutions of DPPH at 600 μM, Trolox at 500 μM and distilled water, with final concentrations of 17.5, 35, 70, 105, 140 and 175 mg mL^{-1}. The control was prepared from a solution with 3150 μL of DPPH and 350 μL of the solvent and readings were performed at time 0 and after 30 minutes. Next, 2 g of sample diluted in 10 mL of methanol was used for the *Terminalia catappa* Linn flour preparations (FF and DSF). The aliquot used for the flours was 100 μL. The analysis was carried out in a dark place and the sample readings were carried out 30 minutes after the reaction.

The antioxidant activity was expressed as the inhibition percentage of free radical oxidation and calculated by the following formula:

$$\text{Inhibition\%} = ((A_{DPPH} - A_{Am\,/\,Trolox})/(A_{DPPH})) * 100$$

The results were generated in a graph of Trolox concentration in μM versus inhibition% and calculated in linear regression obtaining an equation of the line of: $y = 2.88x + 7.73$ and $R^2 = 0.99$. The antioxidant activity values were expressed in Trolox equivalents per milligram of the sample (μg TE mg^{-1}).

Antimicrobial activity

The antimicrobial activity was performed following the methodology of Silveira et al. (2009), with modifications. The disc diffusion method was used, and the bacterial strains were previously inoculated into Brain Heart Infusion (BHI) broth at a temperature of 37 °C for 24 hours. The inoculum standard was adjusted to 10^8 CFU.mL^{-1}, according to the turbidity of the 0.5 McFarland scale. Bacterial inocula were evenly distributed using a sterile swab on petri dishes containing Mueller-Hinton Agar. The 6 mm diameter discs were subsequently placed on the surface of the plates using sterile forceps and soaked with 10 μL of the flour in the water dilutions of 100%; 75%; 50%; 25% and 10%, 10 μL of the positive control (antibiotic Norfloxacin 20 mg.ml^{-1}) and 10 μL of the negative (sterilized water). The plates were incubated in an oven at 35 °C for 24 hours. After the incubation period, the inhibition halos formed around the discs were measured with a digital caliper. Dilutions with inhibition halo ≥ 7 mm (seven millimeters) were considered as having antimicrobial potential against the tested strains. (Araújo et al., 2011).

Nine (9) strains were tested: *Staphylococcus aureus* (ATCC 23235, INCQS 00102), *Pseudomonas putida* (ATCC 15175, INCQS 00113), *Pseudomonas aeruginosa* (ATCC 15442, INCQS 00025), *Clostridium perfringens* (ATCC 13124, INCQS 00130), *Salmonella enterica subsp. Enterica serovar Typhimurium* (ATCC 14028, INCQS 00150), *Salmonella enterica subsp. Enterica serovar Enteritidis* (ATCC 13076, INCQS 00258), *Bacillus cereus* (ATCC 11778, INCQS 00445), *Listeria monocytogenes* (ATCC 19117, INCQS 00327) and *Escherichia coli* (ATCC 35218, INCQS 00325), which were obtained from the Reference Collection of Microorganisms in Sanitary Surveillance (*Coleção de Microrganismos de Referência em Vigilância Sanitária-CMRVS, FIOCRUZ-INCQS*) (FIOCRUZ, Rio de Janeiro-RJ, Brasil).

3. Results and discussion

Considering the results for the *in natura* physical-chemical characterization of the *Terminalia catappa* Linn fruit and seed (Table 1), the fruit presented moisture (76.30% ± 0.02) and carbohydrates (15.65%) as predominant characteristics, respectively, and a low level of lipids (4.95% ± 0.07), proteins (1.70% ± 0.44) and ashes (1.40% ± 0.03). The fruit presented pH of 4.20 (± 0.02) and A_w of 0.997 (± 0.003), which makes it susceptible to rapid deterioration due to the chemical and enzymatic reactions that normally occur when water is available. In evaluating the physico-chemical composition of the *Terminalia catappa* Linn fruit flour, Santos et al. (2017) obtained values of 2.15 (± 0.15) for moisture, 16.70 (± 0.02) of proteins and 66.80% of carbohydrates, and obtained a pH of 3.83 (± 0.02) and A_w of 0.24 (±

0.01) for the *Terminalia catappa* Linn fruit flour. Based on these results, it is observed that better use of the fruit would be processed as flour, because in addition to concentrating constituents such as carbohydrates and proteins, the reduced water content prolongs its shelf life and increases the availability of its application in the food industry.

Terminalia catappa Linn fruit seed presented a low moisture content of 13.30% (± 0.20) and 7.58% carbohydrates. The ash content was 4.30% (± 0.01), constituting a value close to that found by Souza et al. (2016), who obtained 3.99% ± (0.06) in their study with *Terminalia catappa* Linn fruit seed. In this fruit fraction, lipids (48.52 ± 0.31) correspond to the predominant nutrient, followed by proteins (26.40% ± 0.10), which is common in several oilseeds. The pH was 6.18 (± 0.01) and water activity was 0.986 (± 0.002). It is known that pH is one of the factors that influence microbiological growth, and some microorganisms such as deteriorating bacteria can develop in this pH range, thus making the *in natura* seed susceptible to microbial deterioration.

Table 1 - Physical-chemical characterization of *Terminalia catappa* Linn fruit and seed.

Parameters	Fruit (%)	Seed (%)
Moisture	76.30 ± 0.02	13.30 ± 0.20
Ash	1.40 ± 0.03	4.20 ± 0.00
Proteins	1.70 ± 0.44	26.40 ± 0.10
Lipids	4.95 ± 0.07	48.52 ± 0.31
Carbohydrates	15.65	7.58
pH	4.20 ± 0.02	6.18 ± 0.01
A_w	0.997 ± 0.003	0.986 ± 0.002

Results of analyzes in triplicate (mean ± standard deviation).

The bioactives determined in the *Terminalia catappa* Linn fruit flour (FF) and the defatted seed flour (DSF) are presented in Table 2. The FF presented a high content of tannins, anthocyanins and total flavonoids. The high anthocyanin content in FF is associated with the natural coloration of the fruit (purple red). The tannin content observed in the FF corroborates the sensorial attribute of astringency found in the cashew fruit. The high tannin content can also be justified from the low pH of the *in natura* fruit, which is attributed to the presence of acids, including tannic acid. In DSF, the amount of tannin, anthocyanins and total flavonoids was lower than the amount found in FF, which should be associated with the different constituents of the fruit (pulp and seed).

Table 2 - Result of bioactive properties of the fruit and defatted seed flours from *Terminalia catappa* Linn.

Bioactive properties	FF	DSF
Tanins (mg of tannic acid.100^{-1}g)	1662.68 ± 1.27	658.75 ± 1.28
Total anthocyanins (mg.100^{-1} g)	21.45 ± 0.002	1.44 ± 0.03
Total Flavonoids (mg.100^{-1} g)	148.11 ± 0.19	53.06 ± 0.08
Total phenolics (mg of gallic acid.100^{-1} g)	860.34 ± 0.68	649.51 ± 1.37
Antioxidant activity (μg TE mg^{-1})	54.82 ± 0.10	46.05 ± 0.23

Results of analyzes in triplicate (mean ± standard deviation).

The total phenolic content found in FF and DSF was 860.34 (± 0.68) and 649.51 (± 1.37) (mg.100^{-1}), respectively. These results show that both fruit and seed flour contain significant amounts of phenolic compounds. The antioxidant activity was 54.82 (± 0.10) (μg TE mg^{-1}) for FF, and 46.05 (± 0.23) (μg TE mg^{-1}) for DSF. The results of this work justify the values obtained for the phenolic compounds, therefore evidencing that the high amount of phenols found in the fruit and seed flours can influence its antioxidant power. In evaluating the residues of commonly known tropical fruits, Infante et al. (2013) obtained values close to those found in this work, being 68.60 ± 0.23 (μmol TE mg^{-1}) for caju bagasse and 33.03 ± 2.40 (μmol TE mg^{-1}) for mango bagasse pulp.

The results for the determination analysis of the FF and DSF antimicrobial activity are arranged in Tables 3 and 4, respectively.

Table 3 - Antimicrobial activity results of the *Terminalia catappa* Linn fruit flour (FF).

Dilutions	Inhibition Halo (mm)								
(%)	SA	PP	PA	LM	CP	EC	SE	ST	BC

100	0	11	0	12	12	0	0	0	0
75	0	14	0	11	12	0	0	0	0
50	0	12	0	11	12	0	0	0	0
25	10	12	10	8	0	0	0	0	0
PC	45	43	44	42	44	55	50	49	52
NC	0	0	0	0	0	0	0	0	0

SA=*Staphylococcus aureus*; PP=*Pseudomonas putida*; PA=*Pseudomonas aeruginosa*; LM=*Listeria monocytogenes*; CP=*Clostridium perfringens*; EC=*Escherichia coli*; SE=*Salmonella enteritidis*; ST=*Salmonella typhimurium*; BC=*Bacillus cereus*; PC = Positive Control: Norfloxacin. NC = Negative Control: Sterile distilled water.

When antimicrobial activity was determined, FF showed inhibition (Table 3) in the different tested concentrations (100, 75, 50, 25 and 10%). FF showed efficiency in the inhibition of *Staphylococcus aureus*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Clostridium perfringens* and *Listeria monocytogenes* strains, with inhibition halos of 10, 12, 10, 12 and 11 mm, respectively. This antimicrobial power may be associated with the amount of biologically active compounds of the *Terminalia catappa* Linn FF, such as polyphenols (tannins, flavonoids and anthocyanins).

DSF showed no antimicrobial activity against the tested strains (Table 4). This result may be associated with a lower amount of bioactive properties (tannins, anthocyanins and flavonoids) present in DSF compared to FF.

The positive control demonstrated efficient antimicrobial activity, inhibiting all analyzed bacteria.

Table 4 - Antimicrobial activity results of the *Terminalia catappa* Linn defatted seed flour (DSF).

Dilutions	Inhibition Halo (mm)								
(%)	SA	PP	PA	LM	CP	EC	SE	ST	BC
100	0	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0
PC	50	53	46	55	52	49	50	47	51
NC	0	0	0	0	0	0	0	0	0

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SA=*Staphylococcus aureus*; PP=*Pseudomonas putida*; PA=*Pseudomonas aeruginosa*; LM=*Listeria monocytogenes*; CP=*Clostridium perfringens*; EC=*Escherichia coli*; SE= *Salmonella enteritidis*; ST=*Salmonella typhimurium*; BC=*Bacillus cereus*; PC = Positive Control: Norfloxacin. NC = Negative Control: Sterile distilled water.

4. CONCLUSION

Terminalia catappa Linn fruit presents the highest levels of bioactive compounds and antimicrobial activity with inhibitory potential for *Staphylococcus aureus*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Clostridium perfringens* and *Listeria monocytogenes* bacterial strains. The fruit and the defatted seed flours present nutritional and bioactive substances which would allow their insertion in the food industry, with the seed flour being able to act as a natural antioxidant, and the fruit flour, besides being an antioxidant, is able to act as a non-synthetic antimicrobial agent.

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