DOI: 10.5433/1679-0367.2017v38n1p99

Free radical scavenging activity of ethanol leaves extracts of Anacardiaceae

Atividade de sequestramento de radical livre dos extratos etanólicos das folhas de Anacardiaceae

Sérvio Quesado Junior[1], Robéria Lima de Oliveira[2], Márcia Maria Mendes Marques[3], Ana Raquel Araújo da Silva[4], Maria Izabel Florindo Guedes[5]

Abstract

Free radicals are responsible for causing many chronic and degenerative diseases. Antioxidants are substances capable of scavenging free radicals and preventing cell damage. In this context, antioxidant activity of alcoholic extracts from leaves of *Anacardium occidentale* and *Myracrodruon urundeuva* was evaluated by 2,2-diphenyl-1-picrylhydrazyl DPPH and 2,2'-azinobis-(3-ethyl-benzothiazolin-6-sulfonic acid) ABTS methods. Phenolic content and phytochemical analysis were performed for each species. Results showed that both species exhibited free radical scavenging activity. These results are directly related to high phenolic content found in the extracts. *M. urundeuva* showed antioxidant activity similar to butylhydroxytoluene (BHT) and could be considered a promising plant source of natural antioxidant.

Keyword: Natural antioxidants. *Anacardium occidentale. Myracrodruon urundeuva*, phenolic content, antioxidant activity.

Resumo

Os radicais livres são responsáveis por causar muitas doenças crônicas e degenerativas. Os antioxidantes são substâncias capazes de eliminar radicais livres e impedi-los de causar danos celulares. Neste contexto, a atividade antioxidante dos extratos alcoólicos das folhas de *Anacardium occidentale* e *Myracrodruon urundeuva* foi avaliada pelos métodos de 2,2- difenil-1-picrilhidrazil (DPPH) e 2,2'-azinobis-(3-etil-benzothiazolin-6-sulfónico ácido) (ABTS). O conteúdo fenólico e análise fitoquímica dos extratos também foram avaliados. As duas espécies exibiram atividade sequestradora de radicais livres. Estes resultados estão relacionados com alto teor de compostos fenólicos encontrados nos extratos. *M. urundeuva* mostrou atividade antioxidante semelhante ao butilhidroxitolueno (BHT), se tornando uma fonte promissora de antioxidante natural.

Palavras chave: Antioxidantes naturais. *Anacardium occidentale. Myracrodruon urundeuva.* Conteúdo fenólico. Atividade antioxidante.

[1] Docente do Instituto Federal de Educação, Ciência e Tecnologia do Ceará - IFCE; Camocim, Ceará, Brasil.
[2] Discente da Universidade Norte do Paraná - UNOPAR; Londrina, Paraná, Brasil.
[3] Docente da Universidade Federal do Piauí - UFPI; Picos, Piauí, Brasil.
[4] Docente do Instituto Federal de Educação, Ciência e Tecnologia do Ceará - IFCE; Crato, Ceará, Brasil. E-mail: anaraquelaraujosilva@yahoo.com.br
[5] Docente da Universidade Estadual do Ceará - UECE; Fortaleza, Ceará, Brasil.

Introdução

Increased production of free radicals can cause damage to cell components such as proteins, DNA, and cell membranes by stealing their electrons through oxidation. Several diseases are associated with free radicals, such as atherosclerosis, diabetes, cardiovascular diseases, cataract, etc., and free radicals are also related to the aging process. Aerobic organisms have developed several mechanisms against free radical-induced oxidative stress, consisting of endogenous (enzymes production in response to oxidative stress) and exogenous (from food) mechanisms (ROCHA et al., 2007; UTTARA et al., 2009). Cellular components are not fully protected by endogenous antioxidants, and it is well established that dietary antioxidants are indispensable for proper defense against oxidation; therefore, they play an important role in maintaining health (CERQUEIRA; MEIROS; AUGUSTO, 2007; HALVORSEN et al., 2002).

Synthetic antioxidants are commonly added to oils and fatty foods in order to inhibit or slow down lipid oxidation. However, toxicological studies in animals have shown the possibility that such products could carry carcinogenic effect.

Considering the evidence of problems that can be caused by the consumption of synthetic antioxidants, research has been conducted to find natural products with antioxidant activity, which will allow the replacement of the synthetic ones or to make associations between them, aiming to reduce their quantity in food (AUGUSTYNIAK et al., 2010; RAMALHO; JORGE, 2006). Special attention is focused on plant sources (cereals, fruits, vegetables, and beverages) for identifying new antioxidants, for example, the natural antioxidants found in pomegranate fruit can protect humans against the oxidative stress and, consequently, reduce the risk of chronic diseases and prevent disease progression (VIUDA-MARTOS et al., 2010).

Anacardiaceae family includes more than 700 species of 82 genera with pantropical distribution. Some genera, however, extend into the temperate zone. Some species of this family are cultivated throughout the world for their edible fruits and seeds, medicinal compounds, valuable timber, and landscape appeal (PELL, 2004).

In folk medicine, *Anacardium occidentale* L. (cashew) is used as antidiabetic, astringent, anti-diarrheal, depurative, tonic and anti-asthmatic. It is used topically as antiseptic and anti-inflammatory (SOUSA et al., 2004). Razali et al. (2008) showed potential antioxidant of extract from shoots of *A. occidentale*. The cashew nutshell liquid has an important therapeutic activity, for the treatment of leprosy and other skin diseases, and antimicrobial activity against microorganisms responsible for dental caries and acne (LORENZI; MATOS, 2008). Cashew nutshell liquid and the contained anacardic acids demonstrated to contain antioxidant, lipoxygenase inhibitory, anti-*Helicobacter pylori* and antitumor properties (MORAIS et al., 2010).

Additionally, *Myracrodruon urundeuva* Fr. All. (aroeira) is used to treat inflammation, respiratory diseases, ulcers and wounds, among other diseases (MAIA, 2004). The inner bark of this medicinal plant is used for gynecological purposes in post-partum housing treatments as well as in skin injuries (revised for (BANDEIRA; MATOS; BRAZ-FILHO, 1994). The methanol extract of the aroeira bark reduced DNA oxidation, suggesting that it is a potent antioxidant (DESMARCHELIER et al., 1999).

In this study, it was evaluated the antioxidant properties of ethanol leaf extracts from two species of Anarcadiaceae (*A. occidentale* and *M. urundeuva*) native to Northeastern Brazil.

Material and Method

Collection of plant material

Fresh leaves from *Anacardium occidentale* L. and *Myracrodruon urundeuva* Fr. All. were collected in June 2013 from cashew plantation areas of Fortaleza and Crateús, Ceará, Brazil, respectively. The identification of plant material was confirmed by plant taxonomist Prof. Dr. Edson de Paula Nunes and a voucher specimens has been deposited at the Herbarium of the Department of Biology, Federal University of Ceará (UFC), Brazil, with numbers 45,744 for *A. occidentale* and 45,743 for *M. urundeuva*.

Chemicals

DPPH (1,1-diphenyl-2-picrylhydrazyl), $ABTS^{+\bullet}$ [2,2-azino-bis-(3-ethylbenzothiazolin-6-sulfonic acid)], gallic acid and BHT (2,6-di-tert-butyl-4-methylphenol) were purchased from Sigma Chemical Co. All solvents used for the extraction were of analytical grade.

Preparation of extracts

The leaves of *A. occidentale* L. and *M. urundeuva* were dried and cut into small pieces. Then, their ethanol extracts were obtained by soaking powdered leaf samples in 95% ethanol at room temperature (25-28°C) for 7 days. The solution was filtered through a paper filter and evaporated in a rotary evaporator at 60°C. The extracts were concentrated in a water bath with temperature held below 70°C. *A. occidentale* L. and *M. urundeuva* extract yields were 300 g and 260 g, respectively. The extracts were stored at -20°C until further use.

Phytochemical analysis

The stock solution was prepared from ethanol extracts (0.3 g) and was dissolved in 70% ethanol (100 mL). The obtained stock solutions were used for phytochemical analysis. Tests were performed for the detection of phenols, flavanonols, hydrolysable tannins, condensed tannins, flavanones, flavonols, xanthones and saponins according to the methodology proposed by Matos (2009), through observation of color changes or precipitate formation, characteristic for each class of substances. All tests were performed in triplicate.

Total phenolic contents

The total phenolic content was determined by using the *Folin-Ciocalteu* assay with modification (SOUSA et al., 2007). Each ethanol extract was diluted in methanol in order to obtain final concentration of 150 µg/mL. An aliquot (100 µL) of the diluted extract was taken in a test tube, and, then, it was added 50 µL *Folin-Ciocalteu* reagent and 6 mL of distilled water. After one minute, 2 mL of Na_2CO_3 15% was added to the solution and shaken for 30 seconds. Finally, distilled water was added to fulfill 10 mL. The test tube was kept in the dark for 2 h. Absorbance was measured at 750 nm in UV–vis spectrophotometer (SPEKOL 1100). A standard solution of gallic acid serial dilution (50-500 µg/mL in ethanol) was used to prepare a calibration curve. These data allowed to generate the lineal regression; the direct line equation was obtained and it was used for samples concentration calculus. Total phenolic content was expressed as mg Gallic Acid Equivalents (GAE) per g of extract. Each sample was measured in triplicate.

Determination of anti-free radical activity of A. occidentale and M. urundeuva extracts

Free radical scavenging activity of the ethanol extracts was determined by DPPH and ABTS assays according to Silva et al. (2012). All tests were performed in triplicate. BHT was used as positive reference compound.

DPPH assay: Briefly, a solution containing DPPH radicals (6.5×10^{-5} mol/L) in methanol was prepared. An aliquot of 3.9 mL of this solution was mixed with 100 µL of different concentrations (5-5000 mg/L in methanol) of extracts or reference compound. After 1 h, the reduction of the DPPH radical was measured at 515 nm in UV-Vis spectrophotometer (SPEKOL 1100). The scavenging percentage of DPPH radical was calculated according to the formula: % scavenging effect = $100 \times [(A_{DPPH} - As)/A_{DPPH}]$, where A_{DPPH} is the DPPH solution absorbance and As is the mixture absorbance of extract with DPPH solution. The results are expressed as IC_{50} values.

$ABTS^{+\bullet}$ assay: A standard solution of $ABTS^{+\bullet}$ (7 mM, 5 mL) was mixed with 88 µL of potassium persulfate (140 mM). The mixture was shaken and kept in the dark at room temperature for 16 h. Then, 1 mL of this solution was added to 99 mL ethanol and absorbance was measured at 734 nm, it should be approximately 0.715. Solutions of decreasing concentrations (5-5000 mg/L in ethanol) of extracts or reference compound were prepared and 3.0 ml of $ABTS^{+\bullet}$ solution was added up to 30 µl of these solutions. After 6 minutes, readings were taken at 734 nm in UV-Vis spectrophotometer (SPEKOL 1100). The percentage scavenging of $ABTS^{+\bullet}$ radical was calculated according to the formula: % scavenging effect = $100 \times [(A_{ABTS} - As)/A_{ABTS}]$, where A_{ABTS} is the $ABTS^{+\bullet}$ solution absorbance and As is the mixture absorbance of extract with $ABTS^{+\bullet}$ solution. The results are expressed as IC_{50} values.

Statistical analysis

All the experiments on the antioxidant effect were calculated as means ± standard deviation (SD). We performed the one way analysis of variance test (ANOVA) to determine the statistical differences among Anacardiaceae extracts and standard, that was followed by Tukey's Multiple Comparison. The significance level was set at $p < 0.05$.

Results and Discussion

Phytochemical composition analysis of leaf extracts of *A. occidentale* and *M. urundeuva* revealed that both species presented phenols and flavononols, while flavanones, flavonols, xanthones and saponins were not detected. However, these species showed distinction in tannin content: condensed tannins in *M. urundeuva* and hydrolysable tannins in *A. occidentale* (Table 1). Chemical prospection of the inner bark of *M. urundeuva* showed compounds such as catechins, condensed tannin, dimeric chalcones (A, B and C) and flavonoids (SOUSA et al., 2007), and steroids, flavonoids, tannins, catechins and other phenols in *A. occidentale* (LORENZI; MATOS, 2008). Current results demonstrate that *A. occidentale* and *M. urundeuva* leaves have chemical composition similar to their inner bark, plant tissue that is most frequently used in folk medicine; however, their continuous use destroys the plant.

Table 1 – Chemical compounds of the ethanol extracts of *Anacardium occidentale* L. and *Myracrodruon urundeuva* Fr. All. leaves.

Extract	Phenols	Flavanonols	Hydrolysable Tannins	Condensed Tannins	Flavanones, Flavonols, Xanthones	Saponins
M. urundeuva	+	+	–	+	–	–
A. occidentale	+	+	+	–	–	–

(+) detected; (–) not detected.
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The total phenol content was determined by *Folin-Ciocalteu* assay and the regression equation of the calibration curve ($y = 0.0007x + 0.0105$, $R^2 = 0.98790$) expressed in GAE as milligrams per gram of the extract. *A. occidentale* and *M. urundeuva* ethanol leaf extracts exhibited total phenol contents of 280.93 ± 19.06 mg GAE/g and 388.86 ± 23.96 mg GAE/g, respectively. Our results showed that these species were rich in phenols by presenting concentrations greater than 100 mg GAE/g (OLIVEIRA et al., 2012; VASCO; RUALES; KAMAL-ELDIN, 2008). *M. urundeuva* presented higher total phenolic content than *A. occidentale* (Table 2). Other studies showed that Anacardiaceae family is rich in phenols, for example, Chaves et al. (2010) obtained total phenol content of *A. occidentale* inner bark of 345.16 ± 16.24 mg GAE/g. *M. urundeuva* seedling leaves grown from seeds that were previously submitted or not to osmotic stress treatment containing higher phenols content (103.89 mg GAE/g and 106.02 mg GAE/g, respectively) (OLIVEIRA et al., 2012).

Table 2 – Antioxidant activity and total phenolic content of ethanol extracts of *Anacardium occidentale* L. and *Myracrodruon urundeuva* Fr. All. leaves.

Extract/standard	Phenolic content (mg GAE/g extract ±SD)	DPPH (IC_{50} ± SD)	ABTS (IC_{50} ± SD)
M. urundeuva	388.86 ± 23.96[a]	0.217 ± 0.00 [a]	0.279 ± 0.01 [a]
A. occidentale	280.93 ± 19.06[b]	0.260 ± 0.00 [b]	0.350 ± 0.02 [b]
BHT		0.165 ± 0.00 [c]	0.309 ± 0.00 [a]

EC_{50}: Medium effective concentration (mg/mL); GAE: gallic acid equivalent; SD: standard deviation; In the same column, different letters refer to statistically different ($P<0.05$).
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Antioxidant activity ethanol leaf extracts of *A. occidentale* and *M. urundeuva* were measured by ABTS and DPPH methods, expressing EC_{50} in mg/mL. Synthetic antioxidant BHT was used as standard (Table 2). When tested by DPPH method, all results were statistically different, with decreasing antioxidant capacity: BHT > *M. urundeuva* > *A. occidentale*. When evaluated by radical $ABTS^{+\bullet}$ method, *M. urundeuva* antioxidant activity (0.279 ± 0.0119 mg/mL) was statistically similar to BHT (0.309 ± 0.0075 mg/mL), and both were higher than *A. occidentale* (0.350 ± 0.0255 mg/mL). According to Cabral et al. (2009), differences in the antioxidant activity of sample may occur due to the method used. Rufino et al. (2010) demonstrated that $ABTS^{+\bullet}$ method is generally indicated for hydrophilic compounds and DPPH method may be employed routinely with aqueous-organic extracts containing hydrophilic and lipophilic compounds. Both methods are very widely used for determining antioxidant capacity *in vitro*.

Studies have demonstrated the antioxidant potential of *A. occidentale* and *M. urundeuva* (DOSS; THANGAVEL, 2011; IFESAN et al., 2013; KÄHKÖNEN et al., 1999; SÁ et al., 2009), indicating that these species represent a source of bioactive compounds.

In this study, *M. urundeuva* leaf extract presented higher content of phenolic compounds than *A. occidentale*, as well as a better antioxidant activity in both tests DPPH and ABTS. Thus, antioxidant activity is related to phenolic contents of these species extracts (Table 2). Results of other studies corroborate that antioxidant activity is due to the presence of phenols (CHAVES et al., 2010; OLIVEIRA et al., 2012; SOUSA et al., 2007).

Our results may suggest the potential use of ethanol leaf extract of *M. urundeuva* in the fat food industry as a substitute for BHT, due to its antioxidant activity similar to BHT. Previously, it is interesting to evaluate lipid oxidation by β-carotene/linoleic acid methods from *M. urundeuva.*

Conclusion

Myracrodruon urundeuva and *Anacardium occidentale* ethanol leaf extracts exhibited free radical scavenging activity directly related to their high phenolic content. *M. urundeuva* extract showed antioxidant activity statistically similar to that of synthetic antioxidant BHT and could be considered a promising natural antioxidant from plant source. Further studies are needed for isolation, purification, identification and toxicity evaluation of these species bioactive compounds, as well as to evaluate the antioxidant capacity of extracts as antioxidant supplement or their pharmacological use in clinical trials.

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Recebido em: 04 abr. 2016
Aceito em: 13 abr. 2017