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Original Research Article

Evaluation of the preliminary phytochemical profile and antioxidant activity of an aqueous extract processed from *Myrciaracemosa* leaves

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Abstract

This work describes the preliminary phytochemical profile of the aqueous extract of *Myrciaracemosa* leaves, the evaluation of the antioxidant activity through the determination of the total phenolic content by the conventional method of Folin-Ciocalteu and evaluation of the antioxidant activity, measured through the ABTS. The results suggest that the aqueous extract of *Myrciaracemosa* presents in its composition phenolic compounds (tannins and flavonoids), antioxidant action around the average concentration 33668.82 mg/100g, standard deviation equal to 398.0 and coefficient of variance equal to 1.2% and the total phenolic compounds concentration in the extracts was 205.98 mg / 100g, standard deviation 24.89 and coefficient of variance 0.01%. From the evaluation of the obtained results it can be suggested that the studied plant extract presents redoxi property in relation to the contents of saponins, phenols, tannins and denoted favonoids.

Keywords: *Myrciaracemosa*, antioxidant activity, favoids, tannins, phenols, saponins.

INTRODUCTION

Medicinal plants have always enjoyed prestige since ancient times. Primitive peoples inserted into their culture the use of plants in an attempt to lessen their ills. The plants contain active principles (secondary metabolites) capable of curing various diseases, and it was from the recognition of these therapeutic properties that the emergence of modern allopathic medicine emerged (LORENZI and MATOS, 2002; BRASIL, 2009A).

Phytotherapy or plant therapy is one of the oldest therapeutic practices of mankind. It dates back to about 8,500 BC and has origins in both popular (ethnobotanical) knowledge and scientific (ethnopharmacology) experience. The growing consumption of herbal medicines by the Brazilian population is notorious. The factors contributing to this consumption could be related to advances in the scientific area, which allowed the development of recognized and safe herbal medicines and the search for less aggressive therapies by the population (YUNES et al, 2001). The use of medicinal plants is an important practice for both folk medicine and health. The availability of herbal and phytotherapeutic plants by Brazilian Unified Health System (SUS) is enabling the use of scientifically based phytotherapy extracted from the set of plants used by successive generations of a population that had as only option to treat their evils, the empirical use of medicinal plants easy access in each region of the country (LORENZI and MATOS, 2002; BRASIL, 2009A).

The discovery of the components present in medicinal plants as well as their mechanisms of biological action

has been one of the major challenges for chemistry, biochemistry and pharmacology. However, despite the increase in studies in this area, it is observed that only about 20% of the existing plant species were studied for their medicinal potential (MARQUES, 2000).

Among the pharmacological properties studied in natural products is the antioxidant action. The evaluation of this activity has been an important issue considering its importance on human health, since the natural antioxidant agents present low health risk, when compared to the synthetic ones that present toxic effect (SOUZA et al., 2007; REID et al., 2005; SOBRAL-SOUZA et al, 2013). As a result of the possible problems caused by the high consumption of synthetic antioxidants, the research has been focused on finding natural products with antioxidant activity, which will allow to replace the synthetic ones or to make an association between them (SOUZA et al., 2007; REID et al. al., 2005). The literature also mentions that free radicals and other oxidants are a major cause of aging and degenerative diseases associated with aging (cancer, cardiovascular diseases, diabetes, cataracts, immune system decline and dysfunction cerebral). Oxidative stress, for example, may also play a key role in the acute hepatotoxicity of various drugs, such as paracetamol, analgesic and antipyretic used worldwide (REID et al., 2005).

The Myrtaceae family is divided into two subfamilies known as *Myrtoideae* and *Leptospermoideae* (VIEIRA et al., 2004; CARDOSO and SAJO, 2006). Comprising about 5500 species, distributed in approximately 142 genera (THORNHILL et al., 2012), this family is widely found in tropical and subtropical regions and some of its specimens are part of the Brazilian flora (DE OLIVEIRA et al., 2006). According to Romagnoloand Souza (2006), in Brazil, they can be found around 23 genera with about 1000 species (ROMAGNOLO and SOUZA, 2006). Several species of this family present economic value, such as eucalyptus (Eucalyptus spp.), Used in the production of wood and flavorings, and the guava (*Psidiumguajava*), fruitful whose fruits are appreciated *in natura* and industrialized.

The family Myrtaceae is one of the most species-rich families in restinga vegetation. The *Myrtaceae* family comprises about 150 genera and 5,500 species distributed predominantly in tropical and subtropical regions, including fruit and medicinal species (AGRA et al., 2007, 2008). The leaves are widely used as anti-inflammatory, healing, antiseptic and especially as antidiarrheals (DI STASI and HIRUMA-LIMA, 2002; LORENZI and ABREU MATOS, 2002). The species *Myrcia* has been used in popular medicine as astringents, diuretics, anti-hemorrhagic, in the treatment of hypertension, ulcers and, mainly Diabetes mellitus (RUSSO et al., 1990; CERQUEIRA et al., 2006).

The main compounds identified in the genus *Myrcia* are: heteroside flavanones (myrciacitrins I, II and III), acetylated glycosidic flavanones (myrciacitrinas IV and V) heterosídeosacetophenones (myrciafenona A and B) and heterosídeosflavonols (YOSHIKAWA et al., 1998; MATSUDA et al., 2002). It is worth noting that all effects of flavonoids are partially associated with the properties of free radical sequestration (BLOCK, PATTERSON and SUBAR, 1992; FREI, 1995; GEI, 1995; GILLMAN et al., 1995; NESS and POWLES, 1997; INGRAM et al., 1997; PETERSON and DWYER, 1998; TSAO et al., 2003; DE MORAIS RODRIGUES et al., 2016).

Therefore, it is relevant to study representatives of this genus, considering that possibly contain compounds with antioxidant properties. In this sense, the objective of this study was to verify the preliminary prospection of some phytochemical constituents through qualitative tests, the in vitro evaluation of antioxidant activity and quantitatively determine the total phenol content.

MATERIAL AND METHODS

Preparation of Vegetable Extract

Plant samples (aerial parts, including leaves and stems) were collected at 6:30 am of a plant specie (*Myrcearacemosa*) belonging to the remaining restinga vegetation located at the end of the Pedra de Itaúna street, in the CondomínioPedra de Itaúna, Avenida das Américas-Pista Central, S/ n, Barra da Tijuca, Rio de Janeiro - RJ -23.011041, -43.423824. The samples were conditioned in low density polyethylene bags (LDPE) and immediately transported to the Laboratory of Chemical and Biological Analysis (LAQB) of the Foundation State University Center of the West Zone (UEZO).

In the laboratory of the LAQB (UEZO), the leaves underwent a selection and sanitization with distilled water, crushed. Subsequently, 900 g of the vegetable material was weighed, weighed (class II Bel Mark 2500) and then infused for 60 minutes in water at 80°C.

At the end, the infused extract was filtered to remove solid waste, packed in amber glass and subjected to refrigeration at a temperature of -20°C. After freezing, the samples were lyophilized (Lyophilizer, LIOTOP 220) and the final concentration adopted for the assays was 50 mg/ mL.

Preliminary phytochemical evaluation

The lyophilized extract was submitted to preliminary phytochemical screening, according to a methodology described by Abreu Matos (1988), according to the classes of secondary metabolites included by the genus. Tests were carried out for the identification of phenols and tannins, Anthocyanins, Anthocyanidins and Flavonoids; Leucoantocyanidins, Catechins and Flavon-

Table 1. Determination of the extractive solution and mass

Sample	Mass (g)	Dilution in Acetone 70%	Concentration (g/L)	Absorbance	Concentration AG (mg/L)
Extract	0.0166	0.025	0.66	0.570	56.36
	0.0165	0.025	0.66	0.567	56.05
	0.0161	0.025	0.64	0.545	53.75

AG = Gallic acid

ones; Flavanones, Flavanones, Flavanones and Xanthones.

Preparation of the extract

For the preliminary phytochemical evaluation, 7 test tubes, numbered from 1 to 7, containing extract diluted in ethyl alcohol PA, were prepared in the proportion 1 part of the extract to 2 parts of solvent (1mg of extract to 2mL of solvent).

Tests for the identification of phenols and tannins

In the tube 1, 3 drops of $FeCl_3$ (Ferric Chloride) were added, shaken and the staining observed. For the reference standard 3 mL of distilled water was used and 3 drops of $FeCl_3$ (Ferric Chloride) were added, also agitated and the coloration was observed. It was verified the formation of an intense blue coloration for the extract with the addition of $FeCl_3$, not evidenced in the standard, which is, according to the methodology described by Abreu Matos (1988), a qualitative indication of the presence of Phenols in the extract sample.

Tests for the identification of flavonoid phenols, leucoanthocyanidinscatechins and flavanones

For these tests, we acidified tube 2 to pH 3 with drops of 1N hydrochloric acid and alkalized tube 2 at pH 8.5 and pH 3 at 11, both with drops of 1M caustic soda. After the pH adjustments, we observed that in tubes 2 and 3 there was no change in staining, while in tube 3 there was a red coloration at pH 11. According to the methodology described by Abreu Matos (1988), the results indicated us qualitatively that the extract sample has the presence of Flavonoids in its composition. The tubes were not discarded, being used for comparison in the test identification of Leucoanthocyanidins, Catechins and Flavonones.

Tests for the identification of Leucoanthocyanidins, Catechins and Flavonones

For this test, we acidified tube 5 to pH 1 with 1M hydrochloric acid and tube 6 basified to pH 11 with 1M caustic soda. The tubes were heated in Bunsen spout for 2 to 3 minutes. At the end of the warm-up period, we observed that in tube 5 there was no change in staining and in tube 6 there was a more intense red coloration, both compared to tubes 2,3 and 4. These results showed qualitatively, according to the methodology described by Abreu Matos (1988), that the extract presents flavonoids in its composition. Tube 5 was not discarded and used as a comparison for the identification of Flavonols, Flavonones, Flavononols and Xanthonones.

Tests for identification Flavonols, Flavonones, Flavononols and Xanthonones

To the tube 7 were added centigrams of magnesium and 0.5 mL of concentrated HCl. At the end of the reaction indicated by the end of the effervescence, we observed the formation of an intense red coloration, not formed in the tube 5, which shows qualitatively, according to the methodology of Abreu Matos (1988), the presence of constituents Flavonols, Flavonones , Flavononols and Xanthonones in the extract.

Folin-Ciocalteu Reagent Assay

The determination of the total phenol content present in the samples of aqueous extract of *M. racemosa* was done by ultraviolet - visible spectroscopy using the Folin - Ciocalteu method (GEORGÉ et al., 2005). Approximately 0.0166 g of the aqueous extract of the leaves of *M. racemosa* was diluted in 25 mL of 70% acetone in a 50 mL round-bottomed flask, which was left in magnetic stirring for 30 minutes. Shortly thereafter the extract was filtered on Waters HBL ([polyvinyl-co-N-vinylpyrrolidone) SPE cartridge]. The cartridge was preconditioned with 4 mL of methanol and then effluent twice with 4 mL of distilled water. The extract was then filtered. An aliquot of 500 µL of the filtrate was diluted in distilled water in a 5 mL flask.

For the reaction, a 500 µL aliquot of the diluent was used. In test tubes, 2.5 mL of the Folin-Ciocalteu reagent was added and 2 minutes later, 2 mL of 7.5% Sodium Carbonate was added, the tubes were incubated at 50 °C for 15 minutes. Extractions were done in triplicates (Table 1). They were placed in an ice bath and read on a

Table 2. Aliquot concentrations for curve construction

Volume of mother liquor (mL)	Final volume (mL)	Gallic acid concentration (mg / L)	Abs1	Abs2	Abs3	Average
0.5	10	5.45	0.070	0.072	0.066	0.052
0.5	5	10.90	0.111	0.115	0.130	0.102
1	5	21.80	0.218	0.226	0.237	0.210
2	5	43.60	0.472	0.414	0.431	0.422
3	5	65.40	0.603	0.611	0.616	0.593
4	5	87.20	0.807	0.801	0.824	0.794
5	5	109.00	1.083	1.126	1.096	1.085
0	5	0.00	0.021	0.020	0.010	0.017

Abs = Absorbance

Table 3. Data for obtaining the Trolox standard curve in ethanol.The readingswereperformed in triplicates

Volume of mother liquor (mL)	Final volume (mL)	TroloxConcentration (mM)	Control	Abs1	Abs2	Abs3	Average Abs	Average Abs – Abs Control	Average
0.05	5	20.18	0.703	0.698	0.689	0.703	0.696	0.007	0.696
0.1	5	40.35	0.701	0.692	0.685	0.699	0.692	0.009	0.692
0.2	5	80.71	0.701	0.673	0.682	0.676	0.678	0.024	0.678
1	5	403.53	0.701	0.589	0.587	0.584	0.588	0.113	0.588
2	5	807.06	0.698	0.500	0.462	0.461	0.481	0.217	0.481
3	5	1210.60	0.700	0.334	0.318	0.328	0.326	0.374	0.326
4	5	1614.13	0.698	0.225	0.185	0.182	0.205	0.493	0.205

Abs = Absorbance

760nm Ultraviolet spectrophotometer shortly thereafter.

Calibration curve

The calculation of total phenolic content was performed using a calibration curve having gallic acid (GA) as the standard (GEORGÉ et al., 2005). The GA mass used was 0.0109g diluted in 100 mL (concentration of the stock solution = 0.109 g/ L) and the result expressed in mg equivalent of Gallic acid (EAG).$100g^{-1}$ extract (Table 2; GEORGÉ et al. al., 2005). The UV reading was done in triplicate. The absorbance mean obtained at each reading was then calculated.

Reduction of the ABTS radical in TEAC

This assay was performed based on the method described by Re et al. (1999). The preparation of stock solution of the ABTS + radical. (6-hydroxy-2-5-7-8-tetramethylchromo-2-carboxylic acid) was done the day before analysis. The ABTS + working solution was prepared by diluting the stock solution in ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. In a 96-well microplate, 20 µL of the extract diluted in ethanol, standard and white (ethanol). The plate was placed in the plate reader at 37 °C, where 180 µL of the ABTS + solution was added, absorbance was read after 6 minutes at 734 nm and the results were expressed in µmolTrolox/ g extract (Table 3).

RESULTS AND DISCUSSION

Preliminary phytochemical evaluation

The aqueous extract of the *Myrciaracemosa* leaf was submitted to a series of phytochemical characterization reactions: phenols and tannins, flavonoids, Leucoantocyanidinscatechins and flavanones; flavan-ones, flavanones and xanthones, and finally, saponins, according to a methodology described by Abreu-Matos (1988). The presence or absence of the secondary metabolite groups was verified from the observation of the expected or non-expected characteristic reaction, indicating the presence of positive or negative results for each group and constituent analyzed (ABREU-MATTOS, 1988; Table 4).

Table 4 shows the potential of this species for antimicrobial activity due to the phytochemical constituents present as saponins, tannins, flavonoids and phenolics (OKEKE, 2001), which may be associated with antioxidant and antimicrobial activity (SOUZA et al., 2013). The literature indicates that the presence of these metabolites in extracts of medicinal plants may respond

Table 4. Preliminary phytochemical screening of *Myrciaracemosa* aqueous extract (MATOS, 1988)

Constituents	Results
Saponins	+
PhenolsandTannins	+
Flavonoids	+

RM = Mayer's reagent; sign + means that the result was positive for identification of the constituent in question

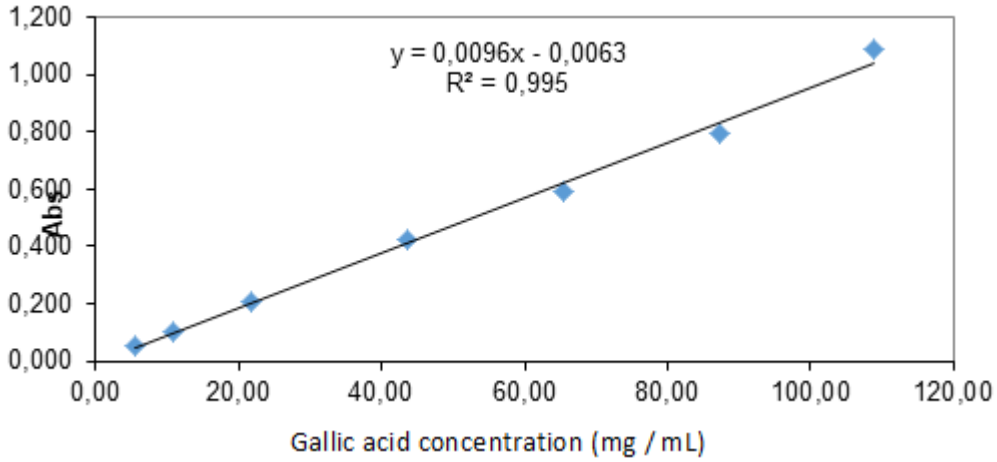

Figure 1. Analytical curve of gallic acid for quantification of total phenols

mainly to biological activity, although their form of action is usually conjugated to determined bioactivity. Saponins have, for example, hemolytic, molluscicidal, anti-inflammatory, antifungal, antibacterial, antimicrobial, antiparasitic, cytotoxic and antitumor activity, among others (SPARG et al., 2004). In addition, phenolic compounds have the antioxidant capacity to neutralize the activity of free radicals generated in the body, with associations to various chronic-degenerative diseases such as diabetes, cancer and inflammatory processes, also inhibiting the risk of cardiovascular diseases. The tannins present antioxidant and anti-infective activity, antibacterial, antifungal and antiprotozoal action, in tissue repair, enzymatic and protein regulation, stimulation of phagocytic cells and tumor action (ROBBERS et al., 1997) in wound healing processes such as small ulcerations, antiulcerogenic and antimicrobial activity, as well as the antioxidant, antiproliferative and anti-inflammatory activities, antiallergic, hepatoprotective, antitrust, antiviral and anticarcinogenic (MIDDLETON JUNIOR et al., 2000). In addition, the results were very similar to those identified in the family *Myrtaceae* (DE MORAIS RODRIGUES et al, 2016) and in the genus *Myrcia*spp (DE MORAIS RODRIGUES et al, 2016; WUBSHET et al., 2015).

Folin-Ciocalteu reagent assay

The phenol content for the aqueous extract of the leaves of *Myrciaracemosa* was evaluated by the Folin-Ciocalteu reagent, which has been widely used in the determination of several extracts. The reagent consists of phosphomolybdic and phosphotunguistic acids. They suffer reduction in the presence of phenolic compounds of extracts forming bluish colorations of the solution due to the reduction of the oxidation state of molybdenum and tungsten metals to +5. Through the reaction between gallic acid and Folin-Ciocalteu reagent the linear regression analysis was determined, with the equation of the line y = 0.0096x-0.0063 with correlation coefficient (R2) equal to 0.995. Figure 1 shows the line determined in the concentration range of 0 to 120 $mg.L^{-1}$. The extract analyzed with the Folin-Ciocalteu reagent and obtained a total phenol concentration of 33668.82 ± 398.20 $EAG.g^{-1}$ (equivalent of gallic acid per gram of extract) with a relative standard deviation of 1.2% between measures.

Reduction of the ABTS radical in TEAC

The results of the antioxidant activity by the ABTS method were expressed as total antioxidant capacity equivalent to Trolox (TEAC values). From the results, the aqueous extracts of *Myrciaracemosa* acerola pulp showed high antioxidant capacity, with a TEAC value of 2045.98 ∓ 24.89 mMTrolox/ g, determined by the linear regression analysis with a coefficient of (R2) equals 0.9965, equation of the line y = 0.0003x-0.0057 (Figure 2), with a relative standard deviation of 1.2% between

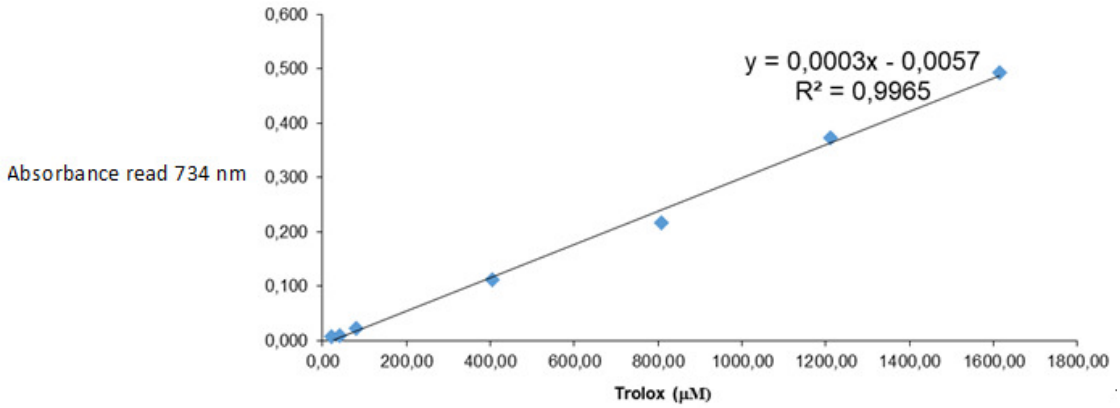

Figure 2. Analytical curve of trolox to determine the antioxidant activity by the ABTS method.

measurements.

The results found in this scientific investigation corroborate with those described by Silva et al. (2017), where it was speculated that the aqueous extract of *Myrciaracemosa* expresses substances with redox properties, exhibiting a bactericidal effect observed in the induction of lethality in the studied bacteria, besides theantioxidant effect depending on the level of oxidative stress, according to the minimizing effect of the $SnCl_2$ effect on bacterial strains mutant Escherichia coli type BW. It is interesting and motivating that other studies are carried out to elucidate the mechanisms of action involved in the effects of the extract of *Myrciaracemosa* in its different levels both potentially therapeutic as well as toxicity.

CONCLUSION

According to the experimental data obtained and analyzed we can speculate that the aqueous extract of *Myrciaracemosa* possesses substances with redoxi properties like saponins, phenols, tannins and favonoids.

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