Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(2):309-313

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Chemical profile, total phenols, total flavonoids and antioxidant activity of five species of the Caatinga biome

Fabíola F. G. Rodrigues[1,2], Cicera J. Camilo[1], Fábio F. Galvão-Rodrigues[1], Cristiane M. U. Lopes[2], Sheyla C. X. de Almeida[2] and José Galberto M. da Costa*[1,2]

[1]Department of Chemistry Biological, Laboratory of Research in Natural Products, Regional University of Cariri, Av. Cel. Antônio Luiz, 1161. Pimenta, CEP: 63105-000 Crato-CE, Brazil
[2]Northeast Network on Biotechnology, State University of Ceará, Fortaleza 60811-905, CE,Brazil

---

ABSTRACT

Many traditional culture of communities use medicinal plants as the sole therapeutic option available. This is a natural alternative that can be exploited in all Brazilian biomes, among others, the Caatinga is the one with the greatest diversity in flora, however, the amount of ethnobotanical and pharmacological studies of these species are still few. The species studied in this work were Turneraulmifolia, Lantanamontevidensis, Alpiniazerumbete, Momordicacharantia andXimeniaamericana. The plant material was collected in Chapada do Araripe-CE, Brazil. Chemical assays to detect the presence of secondary metabolites classes have been performed. The content of total phenols was determined by the Folin-Ciocalteu method, the content of total flavonoids was evaluated by the complex formed between the flavonoids and aluminum, the antioxidant activity was determined using the free radical DPPH sequestration method, and compared with the BHT reference compound. The following classes of metabolites were identified: flavones,flavonols, xanthones, flavononols, steroids and alkaloids. Extracts of X. americanaand A. zerumbet showed good antioxidant activities, and A. zerumbet was the most effective antioxidant with IC50 36,3µg / mL. The phenolic content and total flavonoids were expressed in mg / mL, especiallyT. ulmifolia with 19.5 and 3.3 L. montevidensis with 12.5 and 3.2 for phenols and flavonoids, respectively. The results show that some extracts studied here can be good sources of antioxidant phenolic compounds. Further studies will be conducted to get to the isolation and identification of the main phenolic and flavonoids constituents of the extracts.

Keywords: Antioxidant, chemical characterization, flavonoids, phenols, Caatinga.

INTRODUCTION

Many traditional culture communities use medicinal plants as the only therapeutic resource that is available. This conventional wisdom is transmitted empirically by their ancestors. In recent years the number of surveys of plant species in the Caatinga has been expanded. Contrary to postulate this biome is the one with the greatest diversity in flora, containing a still preserved vegetation. Regarding the amount of ethnobotanical and pharmacological studies related to these species, are still few explored by the scientific society. [1]

In this biome are commonly found plants containing substances that act in defense of organisms. These substances are produced by the secondary metabolism of plants, and can act as antimicrobial, antifungal, antiviral and as antioxidants. Thus, in recent years these plants have become the subject of researches that can identify the presence of natural antioxidants. Antioxidants are responsible for the capture of free radicals, aging delay, for actions against associated degenerative diseases and the capture of reactive oxygen species (ROS) that under normal conditions must be balanced in the body, however, when excessive production occurs they are responsible for oxidative stress which can cause deleterious effects to the organism. Some classes of secondary metabolites contribute significantly

to the ROS capture, such as flavonoids and phenols. This antioxidant capacity is mainly due to its reducing properties [2,3].

Popularly some species of the Caatinga are known to have pharmacological properties which can be highlighted: *Turneraulmifolia* (Chanana) used in folk medicine as an expectorant, anti-inflammatory and anti-ulcer [4,5]. The species *Lantana montevidensis* (Chumbinho) native to Brazil, very used to treat itching, stomach ache, rheumatism and problems in the airways [6,7]. *Alpiniazerumbete*, (Colonia), a medicinal plant used in the treatment of cardiovascular problems such as high blood pressure, as a stimulant to the intestinal motility and hypotensive [8]. *Momordicacharantia* (Melão de São Caetano) species widely used in the treatment for diabetes [9] and *Ximeniaamericana* (Ameixabrava) mainly used in folk medicine for stomach ache, treatment of hemorrhoids, inflammation and mouth infections [10].

This study aimed to evaluate the antioxidant activity, identify the presence of secondary metabolites classes and quantify flavonoids and total phenols present in the extracts of five leaf species found in Araripe National Forest's Caatinga biome, Crato-CE, Brazil.

EXPERIMENTAL SECTION

Plant Material

The studied plant materials were the fresh leaves of five native species of the Caatinga biome, being collected in the Araripe National Forest, Crato-CE, in March 2013. They were selected to perform the tests in the Natural Products Research Laboratory at the Regional University of Cariri - URCA.

Obtaining extracts

The plant materials were triturated (500g of fresh leaves) individually and submitted to exhaustive soaking in 95% ethanol for 72h. The extraction solution was subjected to distillation of the solvent in a rotary evaporator under reduced pressure at an average temperature of 50 ° C. After distilling off the solvent to dryness, crude ethanolic extracts were obtained with the following percentage yields: 1.1; 2.3; 2.5; 1.9; 3.1% for the respective plants: *T. ulmifolia* ,*L. montevidensis*, *A. zerumbete M. charantia* and *X. americana.*

Phytochemical screening

Phytochemicals tests to detect the presence of secondary metabolites classes were carried out following the method described by Matos (1997). These assays are based on visual observation of color change or precipitate formation after addition of specific reagents. [11]

Antioxidant assay

The antioxidant activity of the ethanolic extracts was assessed using the in vitro photocolorimetric method of the free radical DPPH (1,1-diphenyl-2-picrylhydrazyl) according to the methodology of Mensor (2001), with some adjustments. In test tubes 2.5 mL of the samples with varying concentrations were added to 1 mL of ethanolic solution of DPPH (50 mcg / mL) for 30 minutes under light and at room temperature. The absorbance measurements were performed at a wavelength of 518nm. The blank test was prepared by replacing the DPPH ethanol in the reaction mixture. Negative controls were prepared with addition of 1 mL of DPPH with 2.5 mL of ethanol. All readings were performed in triplicate. With the means of the data obtained the differences in absorbance between samples and negative controls were calculated, being the percentage antioxidant activity obtained by linear regression. The tests were followed by a BHT positive control [12].

Determination of total flavonoids

The quantifications of flavonoids were performed according to the methodology described by Kosalec*et al* (2004), with adaptations. The technique is based on measurements of the complex's absorbances formed between the flavonoid and the color reagent's aluminum to form yellowish compounds. With varying concentrations between 250 and 1000µg /mL. The samples were added to 600µL of ethanol, aluminum chloride 40µL of 10%, 40µL of potassium acetate and 2 mL distilled water. A blank test was conducted by adding the same volume of sample and ethanol. The solutions remained incubated in the dark for 30 minutes, after this period the absorbances of the reactions were measured with a spectrophotometer, with a wavelength set to 415 nm. [13].

Determination of total phenols

The spectrophotometric determinations of the samples' phenolic compounds were carried out according to the method described by Singleton *et al* (1999) using the Folin-Ciocalteu reagent at a concentration of 10% followed by the addition of 0.5 mL of sodium carbonate at a concentration of 7.5%. The concentrations ranged from 0.05 to

5,0µg / mL. Measurements of the absorbances as a function of concentration were made in spectrophotometer at 765 nm in triplicate. [14]

RESULTS AND DISCUSSION

The communities around the Caatinga biome use plants as medicinal resource, constituting a therapeutic collection of great importance, being used in many ways in forms of teas, juices, syrups and potions [15, 16]. From popular accounts and some studies on these plants woke up the interest on their beneficial health effects, being reason for their choices.

From surveys of ethanolic extracts, it was possible to identify several classes of secondary metabolites, as shown in Table 1. The presence of these metabolites give the plants their therapeutic activity. They excel in the pharmaceutical area for the beneficial effects to human health [17]. Some classes of metabolites further protect the body from oxidative stress by acting as antioxidants. Antioxidants are chemical compounds that prevent oxidative damage when reacting with free radicals [18].

Table-01.Identification of secondary metabolite classes

Species	1	2	3	4	5	6	7	8	9	10
T. ulmifolia	-	+	-	-	+	+	+	+	+	+
L.montevidensis	-	+	-	-	+	+	-	+	-	-
A. zerumbete	-	+	-	-	+	+	+	+	-	-
M. charantia	-	+	-	-	+	+	+	+	-	-
X. americana	-	+	+	-	+	+	+	-	+	+

1: Phenols, 2: Condensed tannins, 3: Pyrogallictannins ; 4: Anthocyanins and anthocyanidins; 5: Flavones, Flavonols and xanthone; 6: Flavononols ; 7: Flavonones; 8: Chalcones and auronas; 9: Leucoanthocyanidins; 10: Catechin; alkaloids. (+) presentand (-) absent.

The discovery of new natural antioxidant sources replaces the application of the synthetic ones, since they can cause damage to the organism. The study related to the use of such compounds is associated with the prevention of diseases such as cardiovascular diseases, cancer, Alzheimer's disease and aging retardation [19]. The plants which are capable of producing substances such as these have been the target of research to identify and to isolate these compounds, this high demand is related to the low efficiency of the human endogenous system in producing antioxidants, then the use of alternative substances is necessary that will meet the need of the body. Among the various natural substances that may be antioxidants the phenolic compunds are evidenced in that they are in different categories such as phenols, flavonoids and tannins [20]. Flavonoids are polyphenolic compounds produced by the plants' secondary metabolism, involved in the development and protection of plants against pathogen attack. In the human body acts mainly in the inhibition or reduction of free radicals. This function happens because its hydroxyls donate electrons [21]. Studies indicate its importance in the diet to prevent lung cancer and improving cardiovascular diseases [22, 23]. High consumption of foods rich in flavonoids provides a reduction in risks of chronic diseases such as cancer and other degenerative diseases [24]. The results of the antioxidant tests , flavonoids and total phenols are expressed in tables 02 and 03 respectively.

Table-02.Results of the antioxidant testing

Species	CI_{50} µg/mL
T. ulmifolia	126.7 ± 1.9
L. montevidensis	244.5 ± 0.4
A. zerumbete	36.3 ± 2.2
M. charantia	54.5 ±1.5
X. americana	156.4 ±0.4
BHT	7.4 ± 1.8

Table-03.Content (mg / mL) of flavonoids and total phenols

Species	Flavonoids	Phenols
T. ulmifolia	3.3 ± 0.3	19.5 ±1.2
L. montevidensis	3.2 ± 1.2	12.5 ± 3.1
A. zerumbete	0.4 ± 0.1	17.1 ±1.3
M. charantia	1.0 ± 0.2	8.7 ± 3.9
X. americana	0.6 ±0.2	10.6 ± 0.6

With plenty of application in folk medicine, species of the *Turnera* genus are indicated for the treatment of anemia, bronchitis, fever, cough, gastrointestinal disorders and respiratory diseases. Studies on the species *T. ulmifolia* collected in Natal-RN, Brazil, reported 70-90% inhibition of DPPH radical [25], which contradicts with the results obtained in this work. Regarding the *Lantana* genus, its species are rich in organic compounds such as flavonoids

and terpenoids which play pharmacological activities as nematicide, anthelmintic, antibacterial and leishmanicide. Studies with essential oil of the same species showed low potential to inhibit DPPH radical because of high IC50 value [26], this result shows an approximation to the value found for the extract in this study.

A variety of compounds that characterize therapeutical activities are known to the *Alpina* genus. Currently studied for its properties against cancer, Gram positive and Gram negative bacterial genuses and some fungi classes. One of its most evidenced pharmacological properties is the antioxidant activity detected in various species. In this study the species *A. zerumbet* showed low IC_{50} value, revealing to have a good antioxidant activity. In other works with the the plant's rhizome it was found to be a great inhibitor of the DPPH radical [27].

The species *X. americana* is widely used in fighting infections, injuries and lack of air. Its healing action can be justified by some substances such as tannins found in the extract of the leaves reported by Ogunleye and ibitoy (2003) [28]. Its fruits are rich in vitamin C which makes it important in combating disease manifestations [29]. Studies with the methanol extract showed significant value to the kidnapping of the DPPH radical [30], similar to the result for the ethanol extract studied here.

Previous studies with *M. charantia* extract showed significant activity for scavenging free radicals and the hepatoprotective effect that is related to its antioxidant activity [31]. Considering these and other activities such as antibacterial, antifungal and gastroprotective can assign them the presence of the following phytochemicals: flavonoids, alkaloids, phenols and tannins identified by Pereira *et al* (2010) [32, 33]. Its fruits are potentially toxic being great molluscicides, its extract prevents the development of gastrointestinal nematode larvae and prevents larval motility [34,35]. Its extract is rich in charantin, a compound which was identified as an antidiabetic, this activity has yet to be proven, as in tests with male mice showed no significant results [36].

CONCLUSION

These findings emphasize the influence of popular knowledge about the choice of species for therapeutic treatments. However, the scientific information is essential for verification of their activities. In this study species were identified with significant antioxidant activity. This result is related to the presence of phytochemicals classes such as phenols and flavonoids that actively participate in biological actions. These were identified in all species. Considering the importance of this study can be said that the use of natural products derived from Caatinga species as antioxidants is genuine, thereby this work offers complementary scientific contribution of five species of the Caatinga biome.

Acknowledgement
The authors are grateful to the Brazilian Agencies CNPq and FUNCAP for financial support.

REFERENCES

[1] Mlp Bianchi; Lmg Antunes. *Rev. Nutr*., **1999**, 12, (2), 123-130.
[2] U. P Albuquerque; Pm Medeiros; Als Almeida; Jm Monteiro; Emfl Neto; Jg Melo; Jp Santos. J. *Ethnopharmacol*.,**2007**, 114, (3), 325-354.
[3] Er Andrade; Fa Melo-Sterza; Mm Seneda; Aa Alfieri. *Rev. Bras. Reprod. Anim*., **2010**, 34, (2), 79-85.
[4] R Braga. Plantas Do Nordeste, Especialmente Do Ceará. 3ed., Esam **1976**, 435-436.
[5] Ma Antônio; Arms Brito. *J. Ethnopharmacol*.,**1998**, 61, (3), 215-228.
[6] Mj Deena; Je Thoppil. *Fitoterapia*.,**2000**, 71, (4), 453-455.
[7] Eo Sousa; Fs Barreto; Ffg Rodrigues; Jgm Costa. *Rev. Bras. Biociências*.,**2010**, 9, (1), 1-5.
[8] Jh Leal-Cardoso; Mr Moreira; Gm Pinto Cruz; Sm Morais; Ms Lahlou; An Coelho Souza. *Phytomedicine*.,**2004**, 11, (6), 549-553.
[9] Jk Grover; Sp Yadav. *J. Ethnopharmacol*.,**2004**, 93, (1), 123-132.
[10] Tb Mariana; Ae Amanda; Rl Januária; Pr Karina; Ct Gustavo; Jrn Pedro, *Rev. Bras. Farm*., **2008**, 89, (2), 164-167.
[11] Fja Matos, Introdução A Fitoquímica Experimental. 2.Ed. Edições Ufc, Fortaleza, **1997**, 141.
[12] Ll Mensor; Fs Menezes; Gg, Leitão; As Reis; Tc Santos; Cs Coube; Sg Leitão. P. *Research*., **2001**, 15, (2), 127-130.
[13] Mi Kosalec; S Bakmaz; S Pepeliniak; S Vladimir-Knezevic. A. *Pharmaceutica*., **2004**, 54, 65-72.
[14] Vl Singleton; Rorthofer; Rm Lamuela-Raventós. *Meth.Enzymol*, San Diego., **1999,** 299, 152- 178.
[15] Ts Silva; Emx Freire. *Rev. Bras. Pl. Med*., **2010**, 12, (4), 427- 435.
[16] Jja Alves; Ma Araújo; Ss Nascimento. *Rev. Caatinga*.,**2009**, 22, (3), 126-135.
[17] Rj Pereira; Mg Cardoso. J. B. *Biodiversity*.,**2012**, 3, (4), 146-152.

[18] Mal Couto; Sg Canniatti-Brazaca. C. T. *Alimentos*.,**2010**, 30, (1), 15-19.
[19] Sm Morais; Esb Cavalcanti; Smo Costa; La Aguiar. *Rev. Bras. Farmacognosia*.,**2009**, 19, (1), 315-320.
[20] Rsa Rhuanna; Gaa Vanessa; Mfs Egidi; Ls Alexandre; Rsg Nathália; Msm Thaisa; Ios Larissa; Fs Aldenir. Rev. Semente., **2011**, 6, (6), 240-249.
[21] Wc Dornas; Tt Oliveira; Rg Rodrigues-Das-Dores; Af Santos; Tj Nagem. Rev. C. Farm. Bás. Aplicada.,**2007**, 28, (3), 241-249.
[22] C Albert; M Núria; *Rev. Esp. Nutri. Hum. Dietética*.,**2012**, 16, (4), 143-153.
[23] Em Erin; Wh Murray. *Can. J. Cardio*., **2010**, 26, (1), 17-21.
[24] P Lorenza; M Ornella; A Anna. *Food Res. Internac*., **2013**, 54, (1), 1084-1095.
[25] Njn Brito; Ja López; Ma Nascimento; Jbm Macêdo; Ga Silva; Cn Oliveira; Aajb Rezende; Neto; A Schwarz; Mg Almeida. *Foodchem. Toxicol*.,**2012**, 50,(12),4340-4347.
[26] Eo Sousa; Jbt Rocha; Lm Barros; Arc Barros; Jgm Costa. *Ind. Crops. Products*.,**2012**, 43, 517-522.
[27] T Masuda; Y Oyama; Y Inaba; Y To ; T Arata; Y Takeda; Y Nakamoto; H Kuninaga; S Nishizato; A Nonaka. J. Jap. Soc. Food S. Technol-Nippon Shokuhin Kagaku Kogaku Kaishi.,**2002**, 49 (10), 652-61.
[28] Ds Ogunleye; S. F. *Ibitoye & Trop. J. Pharmmaceutical Research*., **2003**, 2, (2), 239-241.
[29] Gg Silva; Pa Souza; Pld Morais; Ec Santos; Rd Moura; Jb Meneses. *Rev. Bras. Frut*. **2008**, 30, (2), 311-314.
[30] Va Maikai; Pi Kobo; Bvo Maikai. *Rev. Afric. J. Biotechnology*., **2010**, 9 (45), 7744-7746.
[31] A Rezaeizadeh; Abz Zuky; Mabdollahi; Ym Goh; Mm Noordin; M Hamid; Ti Azmi. *Rev. Afric. J. Biotechnology*.,**2011**, 10, (24), 4932-4940.
[32] Bs Pereira; Dcs Nunes-Pinheiro; Akp Vasconcelos; Adn Pinheiro; Pa Rodrigues. *Rev. Bras. Pl. Med*., **2010**, 12, (3), 311-316.
[33] Ap Sharanabasappa; Bp Saraswati. *Int. J. Morphol*., **2011**, 29 (4), 1212-1218.
[34] U Aparna; Ks Vinay; Ks Dinesh. *Rev. Inst. Med. Trop*., **2013**, 55 (4), 251-259.
[35] Ln Cordeiro; Acr Athayde; Vlr Vilela; Jgm Costa; Wa Silva; Mm Araujo; Og Rodrigues. *Rev. Pl. Med*., **2010**, 12, (4), 421-426.
[36] W Hsien-Yi; K Wei-Chih; C Tain-Junn; Y Sung-Hsun; C Liang-Hao; C Jiumn-Jye. *Food. Chem. Toxicol*. **2014**, 69, 347-356.