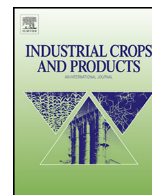
Industrial Crops and Products 76 (2015) 550–556

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop



Does rainfall affect the antioxidant capacity and production of phenolic compounds of an important medicinal species?

Thiago Antônio de Sousa Araújo[a,b,*], Valerium Thijan Nobre de Almeida e Castro[b], Lilian Grace da Silva Solon[c], Gabriel Araújo da Silva[c], Maria das Graças Almeida[c], José Galberto Martins da Costa[d], Elba Lucia Cavalcanti de Amorim[b], Ulysses Paulino Albuquerque[a]

[a] *Laboratório de Etnobiologia Aplicada e Teórica—LEA, Departamento de Biologia, Área Botânica, Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros s/n, Dois Irmãos, Recife, PE CEP: 52171–030, Brazil*

[b] *Laboratório de Produtos Naturais—LAPRONAT, Departamento de Ciências Farmacêuticas, Centro de Ciências da Saúde, Universidade Federal de Pernambuco, Universidade Federal de Pernambuco, Rua Prof. Artur de Sá S/N, Cidade Universitária, Recife, PE CEP: 50740-521, Brazil*

[c] *Laboratório Multidisciplinar—LABMULT, Departamento de Análises Clínicas e Toxicológicas, Faculdade de Farmácia, Centro de Ciências da Saúde, Universidade Federal do Rio Grande do Norte, Av: General Cordeiro de Faria s/n, Petrópolis, Natal, RN CEP: 59012-570, Brazil*

[d] *Laboratório de Pesquisa de Produtos Naturais—LPPN. Universidade Regional do Cariri, Rua Cel. Antônio Luís 1161, Pimenta, Crato, CE CEP: 63105-000, Brazil*

ARTICLE INFO

Article history:
Received 23 February 2015
Received in revised form 2 June 2015
Accepted 9 July 2015
Available online 6 August 2015

Keywords:
Dry forest
Caatinga
Oxidative stress
Water stress
DPPH
Anadenanthera colubrina
Hyperin

ABSTRACT

The variation in water availability can cause oxidative stress in plant species, which respond by producing antioxidants. Phenolic compounds are among the primary substances responsible for antioxidant activities. Thus, the goal of this study was to evaluate whether rainfall in a dry forest in Brazil affects the antioxidant activity and the major phenolic compounds in the stem bark of *Anadenanthera colubrina*, an economically important species from the semi-arid region of Brazil. Monthly samplings were performed over one year for the analysis of free radical sequestration by the DPPH method and for the identification and quantification of major phenolic compounds by HPLC. These data were correlated with each other and with the monthly rainfall. Three major phenolic compounds were identified, one of which was hyperin. The stem bark was highly capable of scavenging free radicals. The antioxidant activity and the production of the three compounds only slightly varied during the year, despite the significant differences among a few of the months studied. These differences are apparently unrelated to rainfall. No correlation was found between the major compounds and free radical scavenging. Our results suggest that rainfall was not the environmental factor responsible for monthly differences in the antioxidant activity of and concentrations of major phenolic compounds in the bark of *A. colubrina*.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Antioxidant activity has been the subject of several studies because of its relationship with several pathological processes, such as cancer, inflammation, cardiovascular disease and diabetes (Choi et al., 2002). These processes share the characteristic increase of reactive oxygen species (ROS) that cause oxidative stress. Thus, in the search for new antioxidant sources, one of the research targets is plants because they have developed their own antioxidant mechanisms to combat stressful conditions. This stress may be related to environmental factors, such as water availability, light intensity, temperature and salinity, causing the plants to adapt to these situations by producing compounds that act on the antioxidant mechanism (Jaleel et al., 2009).

Phenolic compounds, such as tannins and flavonoids, are responsible for the plant's antioxidant defense when it undergoes environmental stress or when it undergoes different morphological stages of development that cause increased ROS (Pourcel et al., 2007), aiding the chemical adaption of the plant to these conditions. This adaptation occurs via genes that activate the biosynthesis of antioxidant mechanisms, particularly secondary metabolism genes that aid in plant development (Oh et al., 2009).

* Corresponding author at: Laboratório de Etnobiologia Aplicada e Teórica—LEA, Departamento de Biologia, Área Botânica, Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros s/n, Dois Irmãos, Recife, PE CEP 52171–030, Brazil.

E-mail address: thiagocaruaru@hotmail.com (T.A. de Sousa Araújo).

http://dx.doi.org/10.1016/j.indcrop.2015.07.008
0926-6690/© 2015 Elsevier B.V. All rights reserved.

Among these metabolites, phenolic compounds are highlighted due to many studies relating them to the highest antioxidant activity of plants (Lee et al., 2004; Mccune and Johns, 2007; Ramirez et al., 2009; Zhang et al., 2010) especially for their ability to sequester free radicals (Kumari and Kakkar, 2008; Zang et al., 2010).

However, there are few studies focusing on medicinal plants and secondary metabolites acting via the antioxidant mechanism under conditions of environmental stress (Yong et al., 2006). Understanding this process is important because secondary metabolic compounds, particularly phenolic compounds, play roles in stabilizing free radicals and forming new radicals by chain-breaking (Maestri et al., 2006), among other roles, which are important functions under conditions of oxidative stress in plants and in humans.

Thus, our goal is to correlate three factors, namely, available rainfall (one of the main limiting factors in semi-arid regions) antioxidant activity by free radical scavenging and the concentrations of major phenolic compounds in *Anadenanthera colubrina* (Vell) Brenan. These factors will test the following hypotheses: (1) the antioxidant activity and/or concentrations of the major phenolic compounds of *A. colubrina* bark extracts vary throughout the year; (2) If the first hypothesis is true, then this variation is associated with rainfall; (3) There is a correlation between the concentrations of major phenolic compounds and the antioxidant power of the *A. colubrina* extracts.

2. Experimental

2.1. Sampling site

The plant material was collected in the municipality of Altinho (8°29′32″S × 36°03′03″W), Pernambuco, Northeastern Brazil. The specific sampling area was the Carão site (08°35′16.1″S × 36°05′36.1″W), a rural community located 16 km from the city center. This community is located in a Caatinga area characterized by xerophytic vegetation, the presence of deciduous plants with thorns and a semi-arid climate with a long, dry period followed by intermittent rains and a short drought period followed by torrential rains (Araújo et al., 2007; Brasil, 2011). Among the species occurring in this region, *A. colubrina* has been highlighted because studies show its therapeutic potential evidenced from the diverse diseases for which it can be used (healing injury, cancer and inflammation). In addition, the phytochemical characteristics of this species include large amounts of phenolic compounds, especially in the bark, which is the region of the plant most used in the preparation of medicines by the community (Albuquerque et al., 2007; Monteiro et al., 2006a,b; Araujo et al., 2008; Soldati and Albuquerque, 2010).

2.2. Sampling the plant material

Monthly collections were taken from the bark of 12 individual *A. colubrina* samples with a mean diameter at breast height of 41.16 cm for 12 months (in 2012), totaling 144 samples, to evaluate the antioxidant activity and the contents of the major phenolic compounds. Time samples ranged between 9:30 h and 13:00 h to reduce the effect of circadian variation (Tausz et al., 2003).

2.3. Extract preparation

The collected material was cut into smaller fragments to facilitate drying, dried under shade at approximately 26 °C for three days, ground in a knife mill and homogenized by sieving the particles (16 Mesh or 1.00 mm). There was very little incrustation on the knives, indicating low moisture. The dried and ground powder was subjected to maceration for six days at a ratio of 1:20 w/v using 80% methanol. After maceration, the material was filtered and evaporated under reduced pressure to obtain the dried extract (Araújo et al., 2012).

2.4. Antioxidant analysis

The DPPH (1,1-diphenyl-2-picrylhydrazyl) assay was used to measure the antioxidant activity according to the protocol adapted from Sousa et al. (2007). Aliquots of the dried extracts were diluted to six concentrations ranging from 100 to 10 mg/mL. From each concentration, 0.5 mL was removed, and 3 mL of a methanol solution containing up to 100 μM DPPH free radical was added. After 30 min, the UV absorbance of the resulting solutions was recorded at λ 517 nm. Analyses were performed in triplicate, and duplicate readings were obtained for each concentration. Ascorbic acid was used as a positive control at concentrations ranging from 5 to 50 mg/μL.

To determine the effective concentration, the percentage of remaining DPPH (% $DPPH_{REM}$) was calculated according to the following equation:

$$\%DPPH_{REM} = \frac{[DPPH]\,T = t}{[DPPH]\,T = 0} \times 100$$

where $[DPPH]T = t$ is the DPPH concentration in the medium after the reaction with extract or with the control and $[DPPH]T = 0$ is the initial DPPH concentration (40 μg/mL).

The $DPPH_{REM}$ values were used to construct a graph for the EC_{50} calculation in which concentrations of the positive standard or the samples were the independent variable and the percentage of remaining DPPH was the dependent variable. The EC_{50} was determined from an exponential curve obtained from the graph.

2.5. Quantification of phenolic and flavonoid compounds by HPLC-PDA

High performance liquid chromatography (HPLC) was performed with a Varian ProStar HPLC system equipped with quaternary pump (Model ProStar 240), PDA detector (ProStar model 335), and autoinjector along with Varian Galaxie Chromatography Data System software, version 1.9.302.530. Reversed-phase chromatographic analyses were performed under gradient conditions using a C18 column (Phenomenex, 4.6 mm × 100 mm) packed with 5 μm diameter particles. The mobile phase was water containing 0.1% formic acid (solvent A) and acetonitrile (solvent B) with a flow rate of 1.3 mL/min (Aznar et al., 2011).

Gallate of (−)-epigallocatechin with 95% purity and hyperin with 96% purity, both from Sigma–Aldrich, were used as reference materials for the characterization of the major compounds. The solvents used in the analysis were HPLC grade, and Milli-Q water (Millipore Corporation, Bedford, MA) was used for the mobile preparation.

These analyzes were performed every two months with the same dry extract used to test the antioxidant activity, totaling six samplings during the year, beginning with February and ending in December.

For sample preparation, the dried bark extracts collected over these months were dissolved in methanol at 5 mg/mL. After filtration with a 0.22 μM Allcrom syringe filter, 9 μL of each sample was injected into the HPLC system. The relative amount of phenolic compounds in the extracts was calculated considering the spectrum similarity, and the results are expressed as equivalents of epigallocatechin gallate (ppm) and the hyperin extract (ppm).

As the sample is a crude plant extract and not a single substance, the HPLC baseline is not smooth, as is commonly expected. Moreover, as we used a gradient system, solvent exchange during the process promotes noise at the baseline (see Snyder et al., 2010)

Download English Version:

https://daneshyari.com/en/article/6375693

Download Persian Version:

https://daneshyari.com/article/6375693

Daneshyari.com