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Antioxidant Activity of *Vochysia discolor* Mart. Flowers

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SUMMARY. The ethanol extract of flowers of *Vochysia discolor* Mart. (Vochysiaceae) was screened to evaluate its antioxidant activity. The activity was determined by an improved assay based on the i) decolorization of the radical monocation of [2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS•+) and ii) for hypochlorous acid scavenger using 5-thio-2-nitrobenzoic acid (TNB), both monitored by colorimetric methods. The study provided for the first time information on the antioxidant activity of a species in the genus *Vochysia.* The great antioxidant activity displayed by the material suggested it should be used as a complement for the suppression of free radicals after new studies.

RESUMEN. "Actividad Antioxidante de Flores de *Vochysia discolor* Mart.". En este estudio fue evaluada la propiedad antioxidante del extracto alcohólico de flores de *Vochysia discolor* Mart. (Vochysiaceae) por los ensayos de i) decoloración del radical monocatiónico [2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS•+) y ii) supresión del ácido hipocloroso por el método del ácido 5-tio-2-nitro-benzóico (TNB). Nuestros resultados muestran, por primera vez, la existencia de propiedades antioxidantes en una especie del género *Vochysia,* hecho que, debido a la grande capacidad antioxidante observada para el material, tiene gran potencial para ser empleado como complemento en la supresión de radicales libres.

INTRODUCTION

Vochysiaceae is a tropical family of trees and shrubs, comprising eight genera and 2.000 species, half of which found in the genus *Vochysia.* In Brazil occur seven genera and 150 species [1]. The most important genera are *Callisthene* (10 species), *Qualea* (60 species), *Salvertia* (one species) and *Vochysia* (105 species) [2].

Several phytochemical studies on Vochysiaceae have been performed, specially in the genus *Vochysia.* Derivatives of the ellagic acid were reported for *Vochysia acuminata* and *V. thyrsoidea* [3]. Lopes *et al.* [4] isolated 5-deoxyflavones from leaves of *Vochysia* sp., and vochysine, a pyrrolydoflavane, was isolated from stems of *Vochysia guianensis* [5]. Moreover, the genus *Vochysia* is a rich source of terpenoids. Bartogenic and vismiaefolic acids were found in stems of *Vochysia vismiaefolia* [6], while *V. divergens* contain β-sitosterol and betulinic and sericic acids [7], divergioic acid, 24-hydroxytormentic acid and its glucopyranosyl ester [8], tormentic acid [9]. In *V. ferruginea* were reported uvaol, erythrodiol, ursolic acid, oleanolic acid, 6β-hydroxymaslinic acid, β-sitosterol-glucoside, 2α, 3β-dihydroxyurs-12-en-28-oic acid, 2α, 3β-dihydroxyolean-12-en-28-oic acid, bellericagenin A and its (28→1) β-D-glucopyranosyl ester (bellericaside A) [10].

Several *Vochysia* species have been used in the Latin America folk medicine, and a large number of reports are found describing biological activities of these plants. *V. divergens* is used against infections and asthma [11]. Compounds isolated from this species had proved to be antifungal [7], anti-allodynic [9], antibacterial [12] and

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antinociceptive actions [13]. Anti-inflammatory and analgesic activities were reported to alcoholic extracts of *V. ferruginea* [14]. Recently was reported that triterpenoids isolated from *V. pacifica* inhibit a target site of inflammatory process, a phosphodiesterase (PDE4) [15]. However, any biological activity or medicinal uses were reported to *V. discolor*.

In course of study of plants with biological activities from Brazilian rock fields ("campos rupestres"), we present in this study results of the antioxidant activity of *V. discolor* ethanol extract of flowers, popularly known as "vinheiro", a endemic shrub found in this vegetation.

MATERIALS AND METHODS

Plant Material

Flowers of *Vochysia discolor* Mart. (Vochysiaceae) were collected in november of 2003, in Diamantina-MG, Brazil. Voucher specimens were deposited in the Herbarium of UFVJM (F. Petacci s.n., DIA 398).

Extraction of material

Extraction of fresh flowers was performed by immersion in ethanol by 15 days. The procedure was repeated twice. The solution was dried under reduced pressure.

ABTS Radical Cation Decolorization Assay

An aqueous mixture of ABTS (7 mM) and potassium persulfate (2.45 mM) was incubated in the dark at room temperature for 12-16 h. The product, ABTS•+, was diluted in 50 mM phosphate buffer pH 7.4 at 25 °C, to optimal reading of absorbance ± 0.7. The reduction of ABTS•+ by solutions of *V. discolor* extract was monitored spectrophotometrically by decreasing of absorbance at 734 nm. This decolorizing assay was also applicable in the antioxidant cysteine [16]. The antioxidant capacity is given as percent (%) ABTS scavenging, calculated as [(optical density of control - optical density of extract) / (optical density of control) x 100].

Hypochlorous acid scavenger

The absorbance of 5-thio-2-nitrobenzoic acid (TNB) at 412 nm was measured before (control assay) and after the addition of HOCl to TNB in phosphate buffer (pH 7.4 25ºC). The decrease in absorbance at 412 nm is correlated with the concentration of HOCl. The solutions of *V. discolor* were incubated at 25 °C in 50 mM phosphate buffer pH 7.4 containing 35 µM HOCl for 5 min. Then 48 µM TNB (prepared freshly by reduction of DTNB (5,5'-dithiobis(2-nitrobenzoic acid) [17] was added and absorbance was measured. The scavenging of HOCl by *V. discolor* was measured by the reduction of absorbance at 412 nm using the control (the same procedure without solutions of *V. discolor*) as reference. The results were expressed as HOCl remainder concentration.

RESULTS AND DISCUSSION

Free radicals and reactive oxygen species (ROS) are characterized by the presence of atoms that have at least one unpaired electron, thus making them unstable and highly reactive and implicated in numerous pathological events including inflammation, metabolic disorders, cellular aging, artherosclerosis and carcinogenesis.

In the present work, the ethanol extract of flowers of *V. discolor* exhibited scavenging activity. Tables 1 and 2 shows the results of tests using ABTS•+ and HOCl scavenger assays, respectively. The antioxidant activity of *V. discolor* extract was initially tested by measuring their capacity to scavenge ABTS•+ (Table 1). This radical have been widely used to evaluate the antioxidant properties of natural products. Was observed a high inhibition of ABTS•+ at low concentrations of extract, with IC_{50} values approximately 12.2 µg/mL to *V. discolor* extract and 28.0 µg/mL to cysteine, evidencing a high antioxidant action of this plant.

Hypochlorous acid is a strong ROS produced in organisms by oxidation of Cl^- ions at sites of inflammation by the neutrophil enzyme myeloperoxidase. Table 2 shows results of HOCl antioxidant assay. Low concentrations used in this

Ethanolic Extract of *V. discolor* (µg/mL)	**(%) #**
5	24.10 ± 3.17
10	40.03 ± 2.37
20	65.67 ± 2.50
Cysteine	
12	22 ± 0.02
24	38 ± 0.03
48	69 ± 0.01

Table 1. Reduction of the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) cation radical (ABTS•+) in the presence of ethanolic extract of *V. discolor* and cysteine. # Values are expressed means ± SD of inhibition, of three replicate analysis.

Ethanolic Extract of *V. discolor* (µg/mL)	HOCl (µM) #
0	35.00 ± 0.00
2.5	16.00 ± 1.46
10	6.20 ± 0.70
20	2.57 ± 0.53

Table 2. Scavenger of HOCl by ethanolic extract of *V. discolor*. # Values are expressed as means ± SD, of three replicate analysis.

assay (2.5 µg/mL) resulted in a fall of approximately 54% in the HOCl concentration, and at large concentration (20.0 µg/mL) there was fall approximately 92%, showed strong HOCl scavenger action by extract.

CONCLUSIONS

The ethanolic extract of *V. discolor* flowers showed antioxidant activity based on scavenging of ABTS•+ radical and HOCl. Our data suggested that *V. discolor* bears important antioxidant activities. However, further studies are needed to evaluate the potential of this plant as an antioxidant in vivo, as well as the phytochemical investigation to isolate compounds responsible for this effect.

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