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Short Communication

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Radical Scavenging Capacity of *Piper arboreum* and *Piper tuberculatum* (Piperaceae)

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SUMMARY. **In the scope of our ongoing researchers on antioxidant compounds, twenty four extracts and fractions obtained from *Piper arboreum* Aublet and *Piper tuberculatum* Jacq. (Piperaceae) were screened for radical scavenging capacity (RSC) by using DPPH colorimetric assay. The strongest activity was found in ethyl acetate fractions from the leaves of *P. arboreum* (IC_{50} = 5.70 μg/mL) and *P. tuberculatum* (IC_{50} = 8.40 μg/mL). Hydromethanol fractions of the leaves of *P. tuberculatum* and *P. arboreum* showed moderate RSC, with values of IC_{50} (μg/mL) of 11.9 and 19.2, respectively. Additionally, a brief phytochemical study of the ethyl acetate fraction of *P. arboreum* leaves affording quercetin (1) and quercitrin (2), two flavonols with antioxidant activity previously described in the literature.**

INTRODUCTION

Piperaceae is a tropical and subtropical family of herbs, shrubs, small trees, and hanging vines, representing four major genera, namely *Piper, Peperomia, Manekia* and *Zippelia,* comprising approximately 4000 species, half of which found in the genus *Piper* [1].

Several phytochemical studies on Piperaceae have been performed, specially in the genus *Piper* [2]. Derivatives of the gaudichaudianic acid were reported for *P. aduncum* and *P. gaudichaudianum* [3]. Navickiene *et al.* [4] described monoterpenes and sesquiterpenes of *P. arboreum, P. aduncum,* and *P. tuberculatum.* Moreover, the genus *Piper* is a rich source of nitrogenated compounds such as amide-type alkaloids bearing isobutyl, pyrrolidine, piperidine and dihydropyridone moities isolated from *P. hispidum, P. tuberculatum* and *P. arboreum* [5,6].

Various plants of *Piper* genus are largely used in folk medicine in several parts of the world and have been reported to accumulate secondary metabolites with diverse biological activities [7]. In some Northeast Brazil communities, *P. tuberculatum,* popularly known as "pimenta-darta", and "pimenta-longa" has been largely used as antidote for snake bite and sedative [8]. However, there are no reports on ethnomedicinal uses for *P. arboreum.*

As part of our bioprospecting program, whose main goal to discover antioxidant agents from Brazilian flora, we have screened hundreds of plants collected in São Paulo State, obtained promising results [9-11]. Among these, *P. arboreum* and *P. tuberculatum* were chosen for biological and chemical investigation and to our knowledge there are no previous reports on radical scavenging capacity.

Thus, the aim of the current study is to screen the extracts and fractions of leaves, stems, fruits and compounds from *P. arboreum* and *P. tuberculatum* for an antioxidant effect on DPPH radical.

KEY WORDS: Antioxidant, DPPH, *Piper arboreum, Piper tuberculatum,* Piperaceae, Radical Scavenging Capacity.

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MATERIALS AND METHODS

Plant material

Specimens of *P. arboreum* Aublet and *P. tuberculatum* Jacq. (Piperaceae) were cultivated from seeds under greenhouse conditions at the Institute of Chemistry, São Paulo State University, Araraquara, Brazil. Plant material was collected in May of 2006, and identified by Dr. Guillermo E. D. Paredes (Universidad Pedro Ruiz Gallo, Lambayeque, Peru). The vouchers specimens Kato-163 and Cordeiro-1936 were deposited at the herbarium of the Institute of Biosciences, São Paulo University, São Paulo, Brazil.

Extraction

Shade-dried and powdered plant material (leaves, fruits or stems) of *P. arboreum* and *P. tuberculatum* (30.0 g) were extracted with ethanol (5 x 350 mL), for three weeks at room temperature. After filtering, the solvent was evaporated under reduced pressure to yield a thick syrup, which was dispersed in methanol: water (4:1) and then successively partitioned with hexane and ethyl acetate. Samples of the ethanol extract and the hexane, ethyl acetate, and lyophilized hydromethanol fractions were tested for potential antioxidant activity.

Isolation and identification of flavonols 1 and 2

The ethyl acetate fraction of the leaves of *P. arboreum* (830 mg) was subjected to preparative Gel Permeation Chromatography (GPC) on a Sephadex LH-20 (Pharmacia®) column (155 x 6.0 cm i.d.) and eluted with methanol. Fractions (25.0 mL) were collected and checked by TLC on silica gel F254 plates (Merck®) eluted with a mixture of ethyl acetate:water:formic acid:acetic acid (100:27:11:11). Subfractions 17-23 (280 mg) were purified by repeated column chromatography (CC) with silica gel (Merck®) eluted with chloroform:methanol (3:1), furnishing quercetin (**1**; 75 mg) and quercitrin (**2**; 83 mg). The molecular structures of these compounds were identified by comparison with literature data, mainly ^{1}H and ^{13}C NMR δ values [12].

Evaluation of radical scavenging capacity (RSC)

Radical scavenging capacity (RSC) of extracts, fractions and flavonols was determined using DPPH• (2,2-diphenyl-1-picrylhidrazyl) as reagent. Solutions of samples at various concentrations (1-100 µg/mL) in ethanol were individually added to 0.6 mM DPPH in ethanol. The mixture was incubated in the dark at 25 °C for 30 minutes. Remaining DPPH• was determined colorimetrically at 531 nm by blanking against absolute ethanol. Anti-radical abilities against DPPH• were expressed by using mean values obtained from triplicates as percentage of radical reduced (inhibition %) calculated from the equation: Inhibition % = [1– (A_{sample}/A) X 100], where A is test absorbance without sample (only ethanol and DPPH•) and A_{sample} is test absorbance with samples. Concentration providing 50% inhibition (IC_{50}) was calculated form the graph plotted inhibition percentage against concentration tested. Synthetic antioxidant Trolox® was adopted as positive control [13].

RESULTS AND DISCUSSION

Human cells are constantly exposed to reactive oxygen radical generated by a number of biotic and abiotic factors such as irradiation, atmospheric and food pollutants or byproducts of metabolic processes. When the exposure overwhelms endogenous preventive systems, cells are exposed to a potentially harmful load of oxidants, leading to various free-radical-induced noxious effects [14].

In this context, free radicals are implicated in the pathogenesis of various human diseases such as myocardial and cerebral ischemia, arteriosclerosis, diabetes, rheumatoid arthritis, inflammation, cancer-initiation and aging processes [15]. Therefore, there is growing interest in free radical scavengers having the potential as protective agents against that diseases [16-18].

In the present work, the ethanol extract and three fractions (hexane, ethyl acetate and hydromethanolic) of the fruits, leaves and stems of *P. arboreum* and *P. tuberculatum* were evaluated for RSC and the results are shown in Table 1.

In general, ethanol extracts obtained from leaves exhibited stronger RSC than did those from fruits and stems. The low-polarity fractions obtained by extraction with hexane proved to be inactive, since the concentration at which this extract showed activity was over 100 µg/mL. Hydromethanol fractions were less effective than ethyl acetate fractions, suggesting that the potential antioxidant compounds were in the medium-polarity fractions. The ethyl acetate fractions of the leaves of *P. arboreum* and *P. tuberculatum* exhibited the best activities against DPPH radical, with values of IC_{50} (µg/mL) of 5.70 and 11.4 µg/mL, respectively, and most ethyl acetate fractions displayed an activity considered moderate (IC_{50} < 30 µg/mL). Hy-

Plant part or compounds	Extract or fractions tested	*Piper arboreum*	*Piper tuberculatum*
Fruits			
	ethanol	51.8 ± 1.2	50.3 ± 1.1
	hexane	> 100	> 100
	ethyl acetate	14.4 ± 0.4	24.6 ± 0.7
	hydromethanol	> 100	28.3 ± 1.0
Leaves			
	ethanol	16.1 ± 0.6	21.3 ± 1.4
	hexane	> 100	> 100
	ethyl acetate	5.70 ± 0.4	8.40 ± 0.6
	hydromethanol	19.2 ± 0.1	11.9 ± 0.5
Stems			
	ethanol	> 100	> 100
	hexane	> 100	> 100
	ethyl acetate	29.9 ± 0.8	23.4 ± 0.9
	hydromethanol	> 100	> 100
quercetin (**1**)	–	2.88 ± 0.1	–
quercitrin (**2**)	–	3.18 ± 0.1	–
Trolox®	–	4.72 ± 0.4	–

Table 1. Scavenger effect of *Piper arboreum* and *Piper tuberculatum* on DPPH radicals expressed by IC_{50} ($\mu g.mL^{-1}$).

dromethanol fractions of the leaves of *P. tuberculatum* and *P. arboreum* showed moderate RSC, with values of IC_{50} (µg/mL) of 11.9 and 19.2, respectively.

Higher plants are known to provide a diverse range of secondary metabolites [19]. In recent years, flavonoids have been widely recognized as a major class of secondary metabolites with antioxidant properties due to their ability to scavenge free radicals [20].

Additionally, phytochemical fractionation of *P. arboreum* stems furnished two flavonoids responsible for the observed RSC of ethyl acetate fraction, which were identified as quercetin (**1**) and quercitrin (**2**), exhibiting IC_{50} values of 2.88 and 3.18 µg/mL, respectively (Fig. 1).

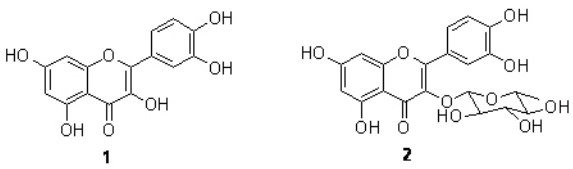

Figure 1. Molecular structures of flavonols isolated from the ethyl acetate fraction of *Piper arboreum* leaves.

Compounds **1** and **2** are flavonols (or 3-hydroxy-flavone derivatives) of widespread occurrence in nature whose medicinal properties have been extensively demonstrated in the literature, especially the radical scavenger capacity [21]. Their antioxidant properties have been attributed to their capacity to scavenge free radicals generated in aqueous phase, increasing the resistance of macromolecules against oxidation [22]. Moreover, the electroactivity of quercetin and their 3-*O*-ramnosyl derivative is due to presence of structural features such as ortho-dihydroxy groups (catechol group on ring B), α,β-unsaturated carbonyl moiety and α and β-hydroxyketone groups, which are responsible for enhancement of the radicalar stabilization after the initial oxidation steps [23].

CONCLUSIONS

It may be concluded from the results of this study that *P. arboreum* and *P. tuberculatum* has potential antioxidant activity based on scavenging DPPH radical. Moreover, ethyl acetate fractions of the leaves of *P. arboreum* could be an important source of potent radical scavengers, useful for developing of novel antioxidant

agents. In view of these findings, further chemical and pharmacological investigations to identify others secondary metabolites and to evaluate the potential of this *Piper* species as an antioxidants *in vivo* are recommended.

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