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Antioxidant activity of prenylated hydroquinone and benzoic acid derivatives from *Piper crassinervium* Kunth

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Dedicated to Professor Rod Croteau on the occasion of his 60th birthday.

Abstract

Growing evidence suggests that RNOS (reactive nitrogen and oxygen species) are involved in the damage of biomolecules, contributing to the aetiology of several human diseases. Thus, the demand for antioxidants has stimulated the search for new compounds with potential use in this field. The in vitro antioxidant potential of prenylated hydroquinones and prenylated 4-hydroxy-benzoic acids from fruits of *P. crassinervium* was evaluated in terms of their capacity to suppress both DPPH (2,2-diphenyl-1-picrylhydrazyl) radical and chemiluminescence produced from luminol, using 2,2′-azo-bis(2-amidinopropane) (ABAP) as a peroxyl radical source. The inhibition of lipid peroxidation was assessed using liposomes from phosphatidylcholine as a membrane model. The prenylated hydroquinones had higher antioxidant activity than the benzoic acids and, among the hydroquinones, the *E* isomer was more efficient than the *Z* isomer.

Keywords: *Piper crassinervium*; Piperaceae; Prenylated hydroquinones; Prenylated benzoic acids; Antioxidants; Lipoperoxidation; DPPH; Chemiluminscence

1. Introduction

Hydroquinones bearing prenyl moieties are natural products common to marine organisms such as Ascidiaceae (*Aplidium* sp.) and brown algae (Howard et al., 1979; Fadli et al., 1991) and have rarely been found in other organisms. The derivatives isolated from leaves of *Piper crassinervium* are among the few cases of their occurrence in land plants (Danelutte et al., 2003; Lago et al., 2004). The biological activities for such compounds include antitumoral, antileukemic and mitosis inhibition (Muller et al., 1985a,b). Furthermore they have analgesic, relaxant (De Pasquale et al., 1991) and antioxidant effects (Cotele et al., 1991).

Piper species have also been described to contain structurally similar compounds to prenylated benzoic acids, and in the case of *P. aduncum* (Baldoqui et al., 1999) antimicrobial and molluscicidal activities were evaluated (Okunade et al., 1997; Orjala et al., 1993), *P. arieianum, P. tabogatum* and *P. dilatatum* also contain further prenylated benzoic acids with antifungal activity (Green et al., 1991; Roussis et al., 1990; Terreaux et al., 1998).

The antioxidant potential of the compounds from *P. crassinervium* (**1**–**6**) were evaluated through the capacity to inhibit the luminescence from luminol induced by 2,2′-azo-bis (2-amidinopropane) (ABAP) radical liberation. Additionally, the ability to scavenge 1,1-diphenyl-2-picryl-hydrazyl (DPPH), a stable radical was analyzed, as well. Finally, protection against lipoperoxidation was evaluated using Fe^{3+}/EDTA and ascorbic acid induced-peroxidation in liposome from phosphatidylcholine.

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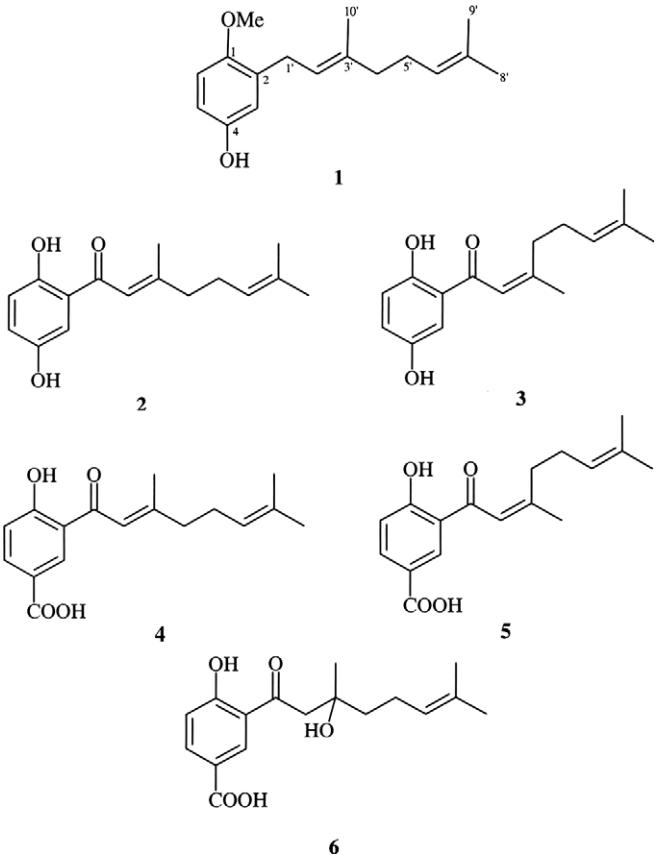

2. Results and discussion

2.1. Characterization of the compounds

The compounds **2–6** were previously isolated and characterized as potent antifungal agents and were identified by direct comparison with authentic standards (Danelutte et al., 2003; Lago et al., 2004). Compound **1** was isolated as a pale yellow amorphous solid. Its molecular formula was determined as $C_{17}H_{24}O_2$ by ^{13}C NMR spectroscopy and HREIMS, which showed a molecular ion peak at *m/z* 260.1780. The ^{13}C NMR spectrum revealed 17 carbon signals, 10 of which were sp^2 carbons [δ 111.5 (CH), 112.5 (CH), 116.6 (CH), 121.9 (CH), 124.3 (CH), 131.6 (C), 131.4 (C), 136.5 (C), 149.3 (C), 151.6 (C)], and seven aliphatic carbons [δ 56.1 (CH_3), 39.8 (CH_2), 28.0 (CH_2), 26.7 (CH_2), 25.2 (CH_3), 17.7 (CH_3), 16.1 (CH_3)]. These data suggested a geranyl-hydroquinone derivative as for compounds **2** and **3**, previously isolated from leaves of *P. crassinervium* (Danelutte et al., 2003; Baldoqui et al., 1999). The ^1H NMR spectrum exhibited three aromatic proton resonances at δ 6.62 (*dd*, $J = 8.5$ and 3.5 Hz), 6.65 (*d*, $J = 3.5$ Hz) and 6.72 (*d*, $J = 8.5$ Hz), which were indicative of a 1,2,4-trisubstituted aromatic ring, and two broad-triplets at δ 5.27 ($J = 7.2$ Hz) and 5.09 ($J = 6.8$ Hz) assignable to olefinic protons in the side-chain. This spectrum also showed two broad-singlets at δ 1.58 (3 H), 1.67 (6 H), characteristic of methyl groups in a double bond, and a singlet at δ 3.76 (3H) of an aromatic methoxyl group. The doublet at δ 3.26 ($J = 7.2$ Hz), associated to the absence of carbonyl groups absorptions as revealed by IR spectrum, indicated the presence of a geranyl moiety linked to the aromatic ring and not an oxo-geranyl moiety, as observed in compounds **2–6** previously isolated from leaves of *P. crassinervium* (Lago et al., 2004).

The positions of the substituents were confirmed through analysis of the HMBC spectrum. The correlation between the methoxyl signal at δ 3.76 (s) and the carbon at δ 151.6 (C-1) associated to the cross-peaks between δ 3.26 (H-1′) and δ 151.6 (C-1), 131.6 (C-2), 116.6 (C-3), 149.3 (C-4), and 136.5 (C-3′), indicated that the methoxyl group and geranyl moiety were connected to C-1 and C-2 positions in the aromatic ring, respectively. Other important correlations are given in Table 1. Therefore, from

Table 1
^1H and ^{13}C NMR data ($CDCl_3$) for the prenylated hydroquinone **1** from *P. crassinervium*

No.	C[a]	H	DQ-COSY	HMBC (H → C)
1	151.6 (C)	–	–	–
2	131.6 (C)	–	–	–
3	116.6 (CH)	6.65 *d* (3.5)	H-5	C-1, C-5, C-1′
4	149.3 (C)	–	–	–
5	112.5 (CH)	6.62 *dd* (8.5, 3.5)	H-3, H-6	C-1, C-3
6	111.5 (CH)	6.72 *d* (8.5)	H-5	C-2, C-4
1′	39.8 (CH_2)	3.26 *d* (7.2)	H-2′	C-1, C-2, C-3, C-2′, C-3′
2′	121.9 (CH)	5.27 *br. t* (7.2)	H-1′, H-10′	C-2, C-1′, C-4′, C-10′
3′	136.5 (C)	–	–	–
4′	28.0 (CH_2)	2.02 *m*	H-5′, H-10′	C-2′, C-3′, C-6′, C-10′
5′	26.7 (CH_2)	2.11 *m*	H-4′, H-6′	C-3′, C-6′, C-7′
6′	124.3 (CH)	5.09 *br.t* (6.8)	H-5′, H-9′	C-5′, C-8′, C-9′
7′	131.4 (C)	–	–	–
8′	25.2 (CH_3)	1.58 *br.s*	H-9′	C-6′, C-7′, C-9′
9′	17.7 (CH_3)	1.67 *br.s*	H-6′, H-8′	C-6′, C-7′, C-8′
10′	16.1 (CH_3)	1.67 *br.s*	H-2′	C-2′, C-3′, C-4′
OMe	56.1 (CH_3)	3.76 *s*	–	C-1

[a] Multiplicity determined by DEPT 135°.