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SHORT COMMUNICATION

Antioxidant activity and a new butanolide from the primitive endemic genus *Hortonia*

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Abstract: The antioxidant activity of MeOH and dichloromethane extracts of the leaves and their alkaloid fractions of the three representative species of the endemic genus *Hortonia* were evaluated using DPPH radical scavenging bioassay. The results showed that the percentage yield of the basic compounds from all three species was very low. In general, the antioxidant activity of the leaf extracts and their acid washings was very low. It was also determined, contrary to what had been reported, that the alkaloid boldine was not found in detectable amounts in any of the acid washed fractions. Chemical investigations of the three species yielded a new butanolide, which was characterized by NMR and mass spectral data.

Keywords: Antioxidant activity, boldine, butanolide, *Hortonia angustifolia*, *Hortonia floribunda*, *Hortonia ovalifoli*.

INTRODUCTION

The chemistry and biological activity of endemic plants and plant varieties of Sri Lanka have received inadequate attention. Although Sri Lanka possesses 920 endemic plants (Dassanayake & Fosberg, 1980-1987), the progress on the evaluation of their therapeutic potential has been very slow (Bandara *et al*., 1990; Hewage *et al*., 1997; 1998). The urgency for speedy investigations was highlighted after the publication of hypoglycaemic activity of the plant *Salacia reticulata* Wight var. *diandra* by a Japanese group and the potent hypoglycaemic compounds from the aqueous extracts were patented (Yoshikawa *et al*., 1997). However, the organic extracts of *S. reticulata* have been investigated by several scientists in Sri lanka before the Japanese group (Premakumara *et al*., 1992; Gunatilaka *et al*.,1993; Tezuka *et al*.,1993; 1994; Dhanabalasingham *et al*., 1996).

In this backdrop, further investigations of the chemical and biological activity of the genus *Hortonia*, an endemic genus belonging to the family Monimiaceae Juss, whose ancestors are considered to have originated in Gondwanaland 100 -120 million years ago (Somasekaram, 1997) have been reported in this study. Interestingly, its three representative species, *H. angustifolia*, *H. floribunda* and *H. ovalifolia* exhibit similar chemistry in addition to showing a similar leaf morphology. However, a recent study based on the evaluation of species of *Hortonia* by DNA barcoding has confirmed that they are indeed three distinct species (Rajapakse *et al*., 2012). Previous studies have reported the presence of mosquito larvicidal lactones and butanolides (Ratnayake *et al*., 2001; 2008a;b), antifungal ishwarane (Ratnayake *et al*., 2008c) and cytotoxic hydrazulenones (Carr *et al*., 2012) from all three species.

It was recorded that the foliar parts of *H. floribunda* contains boldine, which slows down the development of fatty tissues (www.clarinsusa.com). Boldine, an aporphine alkaloid from *Peumus boldus* (Monimiaceae) is known for its antioxidant activity among the other health promoting pharmacological properties. It has been reported in previous studies that compounds with antioxidant activity were not isolated from all three *Hortonia* species. Hence it was decided to embark on a careful investigation of the leaf extracts of all three *Hortonia* species for antioxidant activity and for the presence of boldine. Since a preliminary investigation indicated the presence of low antioxidant potential of *H. angustifolia*, an extensive investigation of this claim for the presence of boldine (Perera *et al*., 2009) was undertaken. Herein, the results of a comprehensive study of the antioxidant activities of all three species of *Hortonia* have been reported.

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METHODS AND MATERIALS

During this study in September 2005, specimens of *H. angustifolia*, *H. floribunda* and *H. ovalifolia* were collected from Kanneliya, Hakgala and the foothills of Adam's Peak, Sri Lanka. *H. angustifolia* was collected at an altitude of 700 m, *H. floribunda* was collected in the montane forests above 1300 m, and *H. ovalifolia* above 1600 m. Voucher specimens (*H. angustifolia* PDA 526; *H. floribunda* PDA 24083, *H. ovalifolia* PDA 522) were deposited at the National Herbarium, Peradeniya, Sri Lanka.

Air dried and ground leaves (650 g) of each of the three species of *Hortonia* were subjected to sequential extraction with CH_2Cl_2 followed by MeOH in a bottle shaker. The basic (or alkaloidal) constituents in the CH_2Cl_2 and MeOH extracts were extracted by liquid-liquid partitioning by the incorporation of 6 N HCl. The basic constituents in the aqueous HCl layer were liberated by the addition of 1 N NaOH and extracted into CH_2Cl_2. The resulting brownish coloured crude extracts (Table 1) from CH_2Cl_2 and MeOH extracts were analyzed for alkaloids.

Air-dried and powdered leaves of *H. angustifolia* (650 g) were extracted with CH_2Cl_2 (3 x 700 mL) at 27 °C for 24 h. The combined CH_2Cl_2 extracts were concentrated *in vacuo* to obtain a black oil (37.4 g), which was subjected to medium pressure liquid chromatography (MPLC) on silica gel (eluent: step gradient from hexanes to EtOAc) to provide six fractions. The second fraction after further purification by silica gel flash chromatography (8:92 EtOAc/hexanes) yielded compound **1** (15 mg). Compound **1** was further purified by C_{18} reversed-phase HPLC (7:3 CH_3CN/H_2O).

Table 1: Percentage yield of extracted basic material

Plant extract	Weight of extract (g)	Weight of crude basic extract (g)	% Yield [a]
H. angustifolia			
CH_2Cl_2	20.1	0.065	0.32
MeOH	19.2	0.300	1.58
H. floribunda			
CH_2Cl_2	18.4	0.048	2.60
MeOH	16.2	0.249	1.53
H. ovalifolia			
CH_2Cl_2	21.7	0.077	0.35
MeOH	20.3	0.320	1.57

[a] % Yield based on plant extract

RESULTS AND DISCUSSION

The results (Table 1) showed that the percentage yield of basic compounds in general from all three species was very low. In addition, the preliminary TLC analysis of the crude CH_2Cl_2 and MeOH extracts gave a negative response against Dragendroff's reagent, indicating the absence of significant concentrations of alkaloidal constituents in the plant. Importantly, a careful ^1H NMR analyses of these crude extracts (10 mg in 1 mL of $CDCl_3$) using a VARIAN 300 MHz spectrometer failed to show the OCH_3 signals of boldine between δ_H 3 – 5. It was rationalized that if boldine was indeed present in reasonable quantities, the HCl wash would exhibit antioxidant activity. Therefore the focus of the investigation was shifted to determining the antioxidant activity of the extracts. It has been reported that a very low concentration of boldine (IC_{50} = from 5 x 10^{-6} to 15 x 10^{-6}) can protect red blood cell plasma membranes from antioxidant sensitive oxygen uptake (Speisky *et al*., 1991).

Table 2: Antioxidant activity of leaf extracts and acid washed fractions

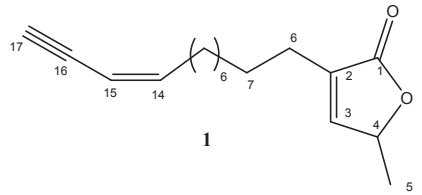
Plant	Total antioxidant activity (% AOA)[a]
H. angustifolia extracts	
MeOH	10.01 ± 0.13
CH_2Cl_2	1.43 ± 0.30
Acid extract	
MeOH	1.03 ± 0.04
CH_2Cl_2	1.52 ± 0.13
H. floribunda extracts	
MeOH	9.03 ± 0.12
CH_2Cl_2	1.83 ± 0.20
Acid extract	
MeOH	1.10 ± 0.06
CH_2Cl_2	1.42 ± 0.03
H. ovalifolia extracts	
MeOH	8.64 ± 0.22
CH_2Cl_2	1.63 ± 0.10
Acid extract	
MeOH	1.20 ± 0.12
CH_2Cl_2	1.33 ± 0.33
Ascorbic acid	78.74 ± 0.42

[a] The activities shown are the mean ± SD of 500 mg dm^{-3} (ppm) concentrations of analyte solutions (concentration of DPPH used 1 × 10^{-4} mol dm^{-3}).

Interestingly, it was observed that while the leaf MeOH extract of the plants exhibited moderate antioxidant activity (Table 2) in the DDPH radical scavenging assay (Lee *et al*., 2004), the acid wash of the plant extracts, which would have contained boldine if it was present in isolable quantities, showed negligible activity. Based on the foregoing, it is reasonable to assume that the leaves of the three species of the genus *Hortonia* do not contain boldine at levels detectable by ^{1}H NMR or revealed by antioxidant activity.

In addition to reporting the lack of alkaloids with antioxidant activity such as boldine, we also describe the isolation of a new butenolide from the leaves of *H. angustifolia*.

The structures of a series of butenolides (Ratnayake *et al*., 2001) and butanolides (Ratnayake *et al*., 2008a;b) from extracts of the same three species of *Hortonia* have been reported previously. While butenolide **1** was different from those reported previously it was noted that the molecular formula of $C_{17}H_{24}O_2$ consistent with an $[M + Na]^+$ ion at *m/z* 283.1673 in the HRESIMS, differed from that of (2*E*,3*R*,4*R*,9'*Z*)-2-(dodec-9'-en-11'-ynylidene)-3-hydroxy-4-methylbutanolide (Ratnayake *et al*., 2008a) by the loss of an oxygen atom. Additionally, the ^{1}H and ^{13}C NMR spectra of **1** (Table 3) revealed resonances that could be attributed to the presence of two residues, a 2(α)-alkyl-4(γ)-methyl-α,β-unsaturated β-lactone moiety (butenolide functionality) [δ 174.0 (C-1); 134.1 (C-2); 7.28 (H-3), 150.9 (C-3); 5.03 (H-4), 78.1 (C-4); 1.35 (H-5), 19.5 (C-5)] and a double bond [δ 6.04 (H-14), 146.4 (C-14); 5.48 (H-15), 109.2 (C-15)] conjugated to a terminal ethynyl group [δ 81.1 (C-16); 3.58 (H-17), 83.4 (C-17)], both of which had been reported previously in the butenolide and butanolide compounds (Ratnayake *et al*., 2001; 2008a). Naturally occurring five membered ring compounds continue to be of interest as synthetic targets (Piers & Karunaratne, 1983). Examination of the 2D NMR data for **1** (Table 3) confirmed these assignments and showed that the α,β-unsaturated γ-lactone was connected at the α position (C-2) to the $\Delta^{14,15}$ olefin through an alkyl chain, which had to contain 8 methylene carbons to satisfy the molecular formula of **1**. The configuration of the $\Delta^{14,15}$ double bond was determined to be *Z* based on both the coupling constant (J = 10.9 Hz) and a strong NOESY correlation between H-14 (δ 6.04) and H-15 (δ 5.48). Compound **1** was isolated from the CH_2Cl_2 extracts of the other two species, *H. floribunda* and *H. ovalifolia* under the same chromatographic conditions (as described above) in comparable yields.



1

Figure 1: The structure of compound **1**

Table 3: NMR Data for compound (**1**)

Position	δ_C	δ_H (J in Hz)	COSY	HMBC
1	174.0			
2	134.1			
3	150.9	7.28, q (1.7)	H-4, H-5, H-6	C-1, C-2, C-4, C-5, C-6
4	78.1	5.03, qq (6.8, 1.7)	H-3, H-5	C-1, C-2, C-3, C-5
5	19.5	1.35, d (6.8)	H-3, H-4	C-3, C-4
6	25.8	2.22, tt (7.7, 1.5)	H-3, H-7	C-1, C-2, C-3, C-7, C-8
7	28.2	1.55, m	H-6, H-8	C-2, C-6, C-8
8 - 11	29.5 - 30.0 [b]	1.33, br. s		
12	29.5 [b]	1.42, m	H-11, H-13	C-11, C-13, C-14
13	30.9	2.32, dq (7.4, 1.1)	H-12, H-14, H-15	C-11, C-12, C-14, C-15, C-16
14	146.4	6.04, dt (10.9, 7.6)	H-13, H-15	C-12, C-13, C-15, C-16
15	109.2	5.48, ddt (10.9, 2.4, 1.3)	H-13, H-14, H-17	C-13, C-14, C-16, C-17
16	81.1			
17	83.4	3.58, d (2.4)	H-15	C-14, C-15, C-16

[a] Spectra collected in acetone-d_6 at 600 MHz

[b] Signals are interchangeable

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