HORTONIA ANGUSTIFOLIA DOES NOT CONTAIN DPPH SENSITIVE ANTIOXIDANT ALKALOIDS

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Introduction

Plants of family Monimiaceae are known to elaborate a variety of alkaloids some of which are reported to have interesting pharmacological properties. In Sri Lanka the family Monimiaceae is represented by the endemic genus *Hortonia* and the most recent revision of the family Monimiaceae by Dassanayake (1996) recognizes three species of *Hortonia*; *H. floribunda* Wight ex. Arn, *H. ovalifolia* Wight and *H. angustifolia* (Thw.) Trimen. The overall aims of this current study were to locate, isolate, identify and evaluate novel bioactive alkaloids from endemic species *Hortonia angustifolia* of family Monimiaceae. There is no reported phytochemical work on alkaloids of genus *Hortonia*.

Materials and Methods

Plant material

Leaves and stems of *Hortonia angustifolia* (Thw.) Trimen were collected from Kanneliya in the Southern Province of Sri Lanka. A voucher specimen (*H. angustifolia*: PDA 526) was deposited at the National Herbarium, Peradeniya, Sri Lanka.

Extraction and isolation

Air dried, powdered leaves and stems of *H. angustifolia* (304 g) were sequentially extracted into CH_2Cl_2 and MeOH (2 × 24 hrs) at room temperature. Evaporation of the solvents gave the respective extracts.

Alkaloid extraction

The CH_2Cl_2 extract was dissolved in $CHCl_3$ and was partitioned with 2 N HCl. The acidic portion was basified with 25 % NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ layer which was positive to alkaloidal test with Dragendroff's reagent was dried and evaporated in vacuo (35 °C) to leave a brownish coloured crude alkaloid mixture (65 mg). Alkaloid extraction from MeOH extract was carried out according to a slightly modified method of Chang *et al.*, (1998). MeOH extract was partitioned to yield $CHCl_3$ and aqueous extracts. The bases in the $CHCl_3$ layer were extracted with 2 N HCl and the acidic layer was basified with 25 % NH_4OH and then extracted with $CHCl_3$ which was dried and evaporated in vacuo to leave a brown viscous residue (300 mg).

Antioxidant assay

The test solution was prepared by mixing 40 µL aliquot of the 500 ppm methanolic plant solution with 3.00 mL of 1×10^{-4} M 1, 1-diphenyl-2-picryl-hydrazil radical (DPPH) solution. Absorbance was read immediately at 515 nm spectrophotometrically with data

being recorded continuously at 1 min intervals until the absorbance stabilized (16 min) and MeOH (3 mL) served as the blank. The absorbance of the DPPH radical without antioxidant (control) and the reference compound ascorbic acid were also measured. All the determinations were performed in three replicates and averaged. The radical scavenging activities of the tested samples, expressed as percentage antioxidant activity (% AOA) was calculated according to the formula:

$$(\%\,AOA) = [\{(A_C - A_T) \div A_C\} \times 100]$$

Where A_C = Absorbance of the control at t = 0 min and A_T = absorbance of the test solution at t = 16 min.

Results and Discussion

The preliminary TLC analysis of the crude CH_2Cl_2 and MeOH extracts gave negative response against Dragendroff's reagent exhibiting the presence of low amount of alkaloidal constituents. Extracted alkaloidal fraction (percent yield: 0.32 %, based on the plant extract) from CH_2Cl_2 extract was positive to the Dragendroff's reagent and formed red color spots which were UV active (254 nm) in the developed TLC. This confirms the presence of alkaloids in a very low amount in the crude alkaloid fraction dichloromethane extract of the endemic species *H. angustifolia*. To the best of our knowledge this is the first report on the DPPH radical scavenging activities of the plant extracts of the endemic genus *Hortonia*. Methanol extract of the *Hortonia angustifolia* (Thw.) Trimen demonstrated the highest antioxidant activity with percentage antioxidant activity (%AOA) of 10.01 % (Table 1). All the alkaloid extracts of the plant were shown to possess significantly low antioxidant activity and can be regarded as inactive to the DPPH radical scavenging assay. The antioxidant activity of the plant *Peumus boldus* has been attributed to the presence of the alkaloid boldine (Speisky *et al.*, 1994, 1991; Silva *et al.*, 2002). The significantly low antioxidant activities of the crude alkaloid extracts of *H. angustifolia* indicate the absence of boldine in the alkaloidal fractions. Previous phytochemical studies on the genus *Hortonia* has concluded that there is similar chemistry of the three species *H. angustifolia, H. floribunda* and *H. ovalifolia* suggesting that all three species contained similar compounds (Ratnayake *et al.*, 2008). Therefore, it is reasonable to assume that boldine might be absent in the other two species as well.

Table 1. The radical scavenging activities of extracts and the alkaloid fractions of *Hortonia angustifolia* (Monimiaceae) by the DPPH photometric assay

Fraction	Total Antioxidant activity (% AOA)[a]
Methanol Extract	10.01 ± 0.13
Dichloromethane Extract	1.43 ± 0.30
Crude Alkaloid :	
Methanol Extract	1.03 ± 0.04
Dichloromethane Extract	1.52 ± 0.13
Ascorbic acid	78.74 ± 0.46

a: The activities shown are the mean ± SD of 500 mg / dm^{-3} (ppm) concentration of analyte solutions (concentration of DPPH : 1×10^{-4} M)

Conclusion

Hortonia angustifolia does not contain DPPH sensitive antioxidant alkaloids.

References

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