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Antioxidative Constituents of *Hedyotis diffusa* Willd.

Dharma Permana[1], Nordin Hj. Lajis[1,*], Faridah Abas[1], A. Ghafar Othman[1], Rohaya Ahmad[1], Mariko Kitajima[2], Hiromitsu Takayama[2] and Norio Aimi[2].

[1]*Laboratory of Phytomedicines, Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
[2]*Faculty of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan*

Abstract – The antioxidative constituents isolated from *Hedyotis diffusa* were identified as quercetin 3-*O*-β-rutinoside (**1**) and quercetin 3-*O*-β-glucoside (**2**). We also isolated asperuloside (**3**) from this plant. Identification was done based on spectroscopic analysis. Quercetin 3-*O*-β-rutinoside was the stronger antioxidant than quercetin 3-*O*-β-glucoside while asperuloside was inactive.
Keywords – *Hedyotis diffusa* Willd., antioxidant activity, quercetin 3-*O*-β-rutinoside, quercetin 3-*O*-β-glucoside, asperuloside

Introduction

Hedyotis diffusa is a small straggling herb commonly found among the weed in vegetable gardens. The local Chinese community uses the aerial part of this plant, in the forms of decoction or paste, to improve blood circulation, to remedy tonsillitis, boils, appendicitis, dysentery, urethral infection, hepatitis as well as for treatment of tumour and snake bites (Sung *et al.*, 1998; Masuda *et al.*, 1981). In the preliminary screening for antioxidant activity of *Hedyotis* spesies, the *n*-butanol fraction of *Hedyotis diffusa* showed strong positive result. Earlier studies on *H. diffusa* have established the presence of cinnamoyl scandoside derivatives and asperuloside (Nishima *et al.*, 1981; Wu *et al.*, 1991); anthraquinones, ursolic acid, stigmasterol and β-sitosterol (Ho *et al.*, 1986); as well as acylated flavonol glycoside and quercetin 3-*O*-glycoside derivatives (Lu *et al.*, 2000). We describe the isolation of the antioxidative constituents from this plant.

Experimental

General experimental – Melting points were determined on Kofler hot-stage apparatus and were uncorrected. UV and IR (in mini KBr form) spectra were recorded on a Shimadzu UV-Vis 160 and a Perkin Elmer 1650 FTIR spectrometers, respectively. ^{1}H and ^{13}CNMR spectra were recorded on a JEOL JNM-A 500 spectrometer at 500 MHz (^{1}H) and 125 MHz (^{13}C), respectively. FAB-MS was obtained from JEOL HX-110A spectrometer. Column chromatography and analytical TLC utilised Merck 9385 and Merck DC-Plastikfollen 60 F_{254}, respectively.

Plant material – *H. diffusa* was obtained from cultivation in the experimental farm of UPM in March 1998. It was air dried and ground before extraction. The voucher specimen (No:06364) was lodged at the herbarium of the Biology Department, UPM.

Extraction and isolation – The ground air-dried sample of *Hedyotis diffusa* (300 g) was extracted three times with MeOH, each time by soaking in 3 L of solvent for overnight before it was decanted. The combined extracts were evaporated under reduced pressure to give a brown gum (91 g). The gum was shaken with 750 ml water:MeOH (2:1) mixture and partitioned with, $CHCl_3$, EtOAc and *n*-BuOH. Removal of the solvent from the *n*-BuOH fraction under reduced pressure gave a brownish gum (31 g). The extract (30 g) was then subjected to silica gel column chromatography and successively eluted with EtOAc followed by EtOAc:MeOH (9:1, 4:1, 3:1) mixtures and finally with MeOH to give thirty three (15 ml) fractions. The combined fractions of 5-7 was further rechromatographed on silica gel column and eluted with $CHCl_3$:MeOH (4:1) to afford asperuloside **3** (250 mg) after recrystallisation from acetone: $CHCl_3$ mixture. Fraction 8 (400 mg) was rechromatographed with EtOAc:MeOH (9:1) and recrystallised from acetone: $CHCl_3$ to afford compound **1** (8.1 mg). The combined fraction of 9-15 (617 mg) afforded compound **2** (7.4 mg) upon column chromatography on silica gel using EtOAc:MeOH (3:1) as eluent, followed by recrystallisation from acetone:

*Author for correspondence
Fax: +60 3 89423087, E-mail: nhlajis@ibs.upm.edu.my

$CHCl_3$.

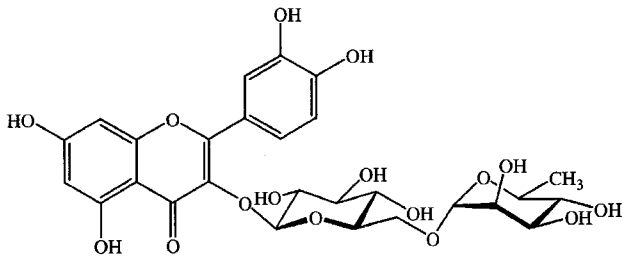
The identity of all compounds were confirmed based on the analyses of the spectral data and their comparison with those reported in the literature (Harborne *et al.*, 1975; Markham *et al.*, 1978; Wenkert and Gottlieb, 1997; Bianco *et al.*, 1978; Peng *et al.*, 1999).

Antioxidant activity assay using ferric thiocyanate method – This assay was carried out as described in the modified method of Kikuzaki and Nakatani (1993). A mixture of 2 mg of samples (final concentration 0.01% w/v) in 4 ml of 99.5% ethanol, 4.1 ml of 2.51% linoleic acid in 99.5% ethanol, 8.0 ml of 0.02 M phosphate buffer (pH 7.0) and 3.9 ml of distilled water contained in screw-cap vial (Φ38×75 mm) was placed in an oven at 40°C in the dark. To measure the extent of antioxidant activity, 0.1 ml of the reaction mixture was transferred to a test tube (Φ13×150 mm) and to it, 9.7 ml of 75 % (v/v) aqueous ethanol followed by 0.1 ml of 30% aqueous ammonium thiocyanate and 0.1 ml of 0.02 M ferrous chloride in 3.5% hydrochloric acid was added. Three min after the addition of ferrous chloride to the reaction mixture, the absorbance was measured at 500 nm. The measurement was taken every 24 h until one day after absorbance of the control reached its maximum value.

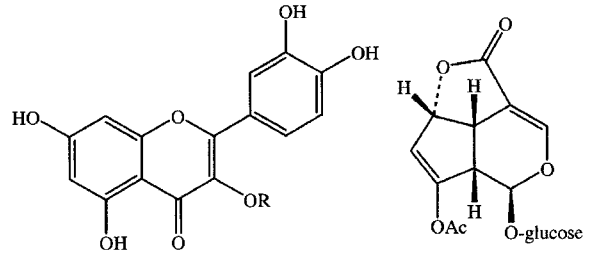
Antioxidant activity assay using DPPH method – The potential antioxidant activity of the isolated compounds was assessed on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The method of Tagashira and Ohtake (1998) was employed with slight modification. A test-sample solution (200 μl in MeOH) was added to 4 ml of 50 μM methanolic diphenylpicrylhydrazyl (DPPH) solution contained in a 10 ml test tube. After vortexing for a brief period, the mixture was incubated in the dark at room temperature for 30 min and the absorbance was measured at 517 nm. The difference in absorbance between the test-sample and the control (MeOH) was expressed as percent inhibition and considered as the activity. All test analyses were run in triplicates and averaged. Ascorbic acid and α-tocopherol were used as positive controls.

Results and Discussion

Methanol extraction of the air dried sample of *Hedyotis diffusa* followed by solvent partitioning and purification of *n*-butanol fraction resulted in isolation of three compounds, quercetin 3-*O*-β-rutinoside **1**, quercetin 3-*O*-β-glucoside (isoquercitrin) **2** and asperuloside **3**. Structure determination was accomplished based on analysis of NMR spectral data and comparison with NMR reported data. The presence of quercetin 3-*O*-β-glucoside has been known but the isolation of quercetin 3-*O*-β-rutinoside from *H. diffusa* has never been reported previously.



(1) Quercetin 3-*O*-β-rutinoside



(2) Quercetin 3-*O*-β-glucoside (R = glucose) (3) Asperuloside

Fig. 1. The flavonoid-glycosides and asperuloside isolated from *H. diffusa.*

Table 1. Percent inhibition of DPPH scavenging effects of compounds isolated from *H. diffusa* and ascorbic acid and α-tocopherol

Compound (100 μg/ml)	% Inhibition
Quercetin 3-*O*-β-glucoside **(2)**	88.6
Quercetin 3-*O*-β-rutinoside **(1)**	87.4
Asperuloside **(3)**	6.23
Ascorbic acid	93.2
α-Tocopherol	90.6

Measurement of antioxidant activity using ferric thiocyanate (FTC) and DPPH radical scavenging techniques were conducted on the isolated compounds in comparison with α- tocopherol as standard as well as with ascorbic acid for DPPH. The results of antioxidative measurement using FTC method showed that quercetin 3-*O*-β-rutinoside was the most active as compared to quercetin 3-*O*-β-glucoside and α- tocopherol whereas asperuloside was found to be inactive (Figure 2).

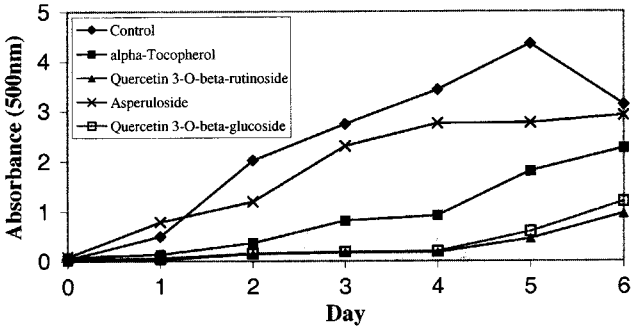


Fig. 2. Ferric thiocyanate antioxidant activity of compounds isolated from *H. diffusa* and α-tocopherol.

The activity measured using DPPH technique showed that quercetin 3-*O*-β-rutinoside and quercetin 3-*O*-β-glucoside were slightly less active than Vitamin C and α- tocopherol as radical scavengers (Table 1). This result supports the medicinal use of this species as tonic for health maintenance.

Acknowledgements

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