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The Antioxidative Components from *Alpinia nutans*

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Abstract

The methylene chloride extract of *Alpinia nutans* was investigated for its antioxidant constituents. 5,6-Dehydrokawain (**1**), flavokawin-B (**2**), 1,7-diphenyl-5-hydroxy-6-hepten-3-one (**3**), (−)-pinocembrin (**4**) and a mixture of stigmasterol and β-sitosterol were isolated. The antioxidant activity was measured on isolates **1–4** using ferric thiocyanate (FTC) and diphenylpicrylhydrazyl (DPPH) free radical scavenging techniques.

Keywords: *Alpinia nutans* Rosc., anti-oxidative activity, 5,6-dehydrokawain, 1,7-diphenyl-5-hydroxy-6-hepten-3-one, flavokawin-B, (−)-pinocembrin.

Introduction

The species of Zingiberacea are widely used in traditional medicines, flavouring agents and spices, as well as the source of certain dyes (Burkill, 1966). For example, the species from genera *Alpinia, Amomum, Curcuma, Costus*, *Kaempferia* and *Zingiber* are among the most often used as ingredients in traditionally prepared health supplements, tonics and ointments. Recently, we screened several species from the Zingiberaceous family for antioxidant activity using the FTC method (Habsah et al., 2000). In this study, *Alpinia nutans*, also known as 'nodding ginger', was one of the species showing high activity. The species is used as a diuretic and to control hypertension, and pharmacological study has shown that the extract was cytogenetic (Dias & Takahashi, 1994), and it causes lowering of blood pressure as well as reduction of atria contractility (Mendonca et al., 1991). The essential oils from the leaves and the rhizomes of this species contained cineol as the main constituent, in addition to several monoterpenes as well as methyl cinnamate (Gildemeister & Hoffmann, 1956; Haggag & El-Shamy, 1977).

Materials and methods

General experimental procedures

Melting points were determined on a Kofler hot-stage apparatus and were uncorrected. The UV and IR spectra were recorded on a Shimadzu UV-vis 160 and a Perkin Elmer 1650 FTIR spectrometers, respectively. ^{1}H- and ^{13}C-NMR spectra were recorded on Varian Unity 500. The spectra were interpreted by the aid of the field gradient correlation spectroscopy (FGCOSY), field gradient heteronuclear multiple bond correlation (FGHMBC) and field gradient hetronuclear quantum correlation (FGHMQC) experiments. Mass spectra were recorded on Hitachi M2000 mass spectrometer. Ionization was induced by electron impact at 70 eV. Column chromatography utilized Si gel Merck 7734 and Merck 9385 while analytical TLC utilized Merck Si gel DC-Plastikfolien 60 F_{254}.

Plant material

The rhizomes and roots of *Alpinia nutans* were harvested from the Germplasm Unit of Universiti Putra Malaysia in October, 1997. The voucher specimen (No. AN-3) has been deposited at the herbarium of the Biology Department, Universiti Putra Malaysia, Serdang, Malaysia.

Extraction and isolation

The fresh sample (8 kg) was cut into smaller pieces and air-dried under the shade. The dried material (1.5 kg) was ground

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and extracted, three times each with 3 L dichloromethane followed by 3 L methanol. The extracts were evaporated under reduced pressure to give 40 g and 60 g of dichloromethane and methanol crude extracts, respectively.

The dichloromethane extract (40 g) was subjected to column chromatography on silica gel column (petroleum ether/diethyl ether), followed by $CHCl_3$/EtOAc and finally MeOH to afford 9 fractions (A–I). Fraction G was further chromatographed on silica gel column ($CHCl_3$/EtOAc and benzene/acetone) to afford **1** after recrystallization from diethyl ether. Similarly, fraction F was also subjected to silica gel column chromatography (petroleum ether/$CHCl_3$, followed by $CHCl_3$/MeOH) to afford **3** fractions (F1–F3). Further chromatography on fraction F1 over a silica gel column (petroleum ether/EtOAc) gave three subfractions (F1/1–F1/3). Purification of fraction F1/1 on preparative TLC (petroleum ether/EtOAc; 1 : 1) and recrystallisation from petroleum ether/EtOAc (1 : 1) gave **2**. Column chromatography of fraction F1/3 on silica gel (petroleum ether/EtOAc, 9 : 1) and recrystallization from hexane/EtOAc gave compound **3**. Fraction F3 was subjected to column chromatography on silica gel (benzene/acetone) and preparative TLC to afford **4**. A mixture of stigmasterol and β-sitosterol was isolated from fraction F2 after subjecting it to column chromatography (silica gel; petroleum ether/$CHCl_3$).

Antioxidant activity assay

The assay using ferric thiocyanate (FTC) method was carried out as described previously in the modified technique (Habsah et al., 2000). The sample solutions were prepared by dissolving the test sample (4 mg or 2 mg for the final concentrations of 0.02 and 0.01% w/v, respectively) in 4 ml of 99.5% ethanol, 4.1 ml of 2.5% linoleic acid in 99.5% ethanol, 8 ml of 0.02 M phosphate buffer (pH 7.0) and 3.9 ml of water contained in a screw-cap vials (Ø 3.8 × 7.5 cm). They were then placed in an oven at 45 °C in the dark. To measure the extent of oxidation, a 0.1 ml portion of this mixture was transferred into a test-tube (Ø 1.5 × 15 cm) and 9.7 ml of 75% (v/v) ethanol were added to it, followed by 0.1 ml of 30% ammonium thiocyanate, and finally 0.1 ml of 2×10^{-2} M ferrous chloride in 3.5% hydrochloric acid. After 3 min the absorbance at 500 nm was obtained. The measurements were repeated three-times for each sample and the readings were averaged. This procedure was repeated every 24 h until 1 day after absorbance of the control reached its maximum value.

The diphenyl-*p*-picrylhydrazyl (DPPH) radical scavenging activity was measured as described previously (Yen & Hsieh, 1997), with slight modification. The reaction mixture of 1 ml of 0.3 mm DPPH in EtOH and 0.5 ml of 1 mm sample (final volume 1.5 ml, final concentration 0.3 mm) was prepared in a screw capped bottle (Ø 1.5 × 3.5 cm) wrapped in aluminum foil and then left in the dark at room temperature for 30 min. Remaining DPPH was measured by colorimetry at 517 nm. All measurements were done in triplicate for each sample and they are averaged. Percentages of DPPH radical scavenging activity were calculated as {1 − (Optical density of sample/optical density of control) × 100}.

Results and discussion

Compounds **1**–**4** and a mixture of stigmasterol and β-sitosterol were identified based on the spectral data (UV, MS, IR, ^{1}H NMR and ^{13}C NMR) and comparison with literature values as 5,6-dehydrokawain (**1**) (Sirat et al., 1996; Itokawa et al., 1981), flavokawin-B (**2**) (Sirat et al., 1996; Itokawa et al., 1981), 1,7-diphenyl-5-hydroxy-6-hepten-3-one (**3**) (Sirat et al., 1996; Kuroyonogi et al., 1983), (−)-pinocembrin (**4**) (Sirat et al., 1996; Jung & McLaughlin, 1990) and a mixture of stigmasterol and β-sitosterol (Wright et al., 1978).

Antioxidant activity assays were conducted on all the four compounds isolated. 5,6-dehydrokawain and pinocembrin showed comparable antioxidative activity to α-tocopherol at 0.02% although they were not as active at 0.01% concentrations (Figs. 1 and 2). Both compounds showed a weak DPPH free radical scavenging activity (37.4% and 41.5%, respectively) at 0.3 mm as compared to the standards, ascorbic acid (89.5%) and BHT (86.6%) (Table 1). There has not been any report on antioxidantive study of these compounds found in the literature. 5,6-Dehydrokawain has been reported to have antiulcer effect as well as antiplatelet and antifungal activities (Tawata et al., 1996). Pinocembrin was previously reported to demonstrate *in vitro* antimicrobial activity and *in vivo* local anesthetic effects. In addition, it was also reported to be cytotoxic toward cells, to act as inhibitor to a protein

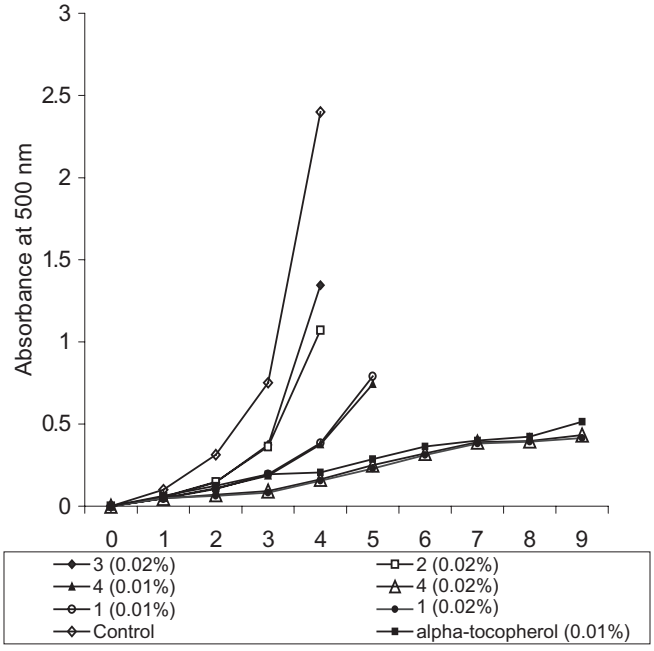

Figure 1. Antioxidant activity (FTC method) of the isolates; 5,6-dehydrokawain (**1**); flavokawin B (**2**); 1,7-diphenyl-5-hydroxy-6-hepten-3-one (**3**); (−)-pinocembrin (**4**) (0–9 days).

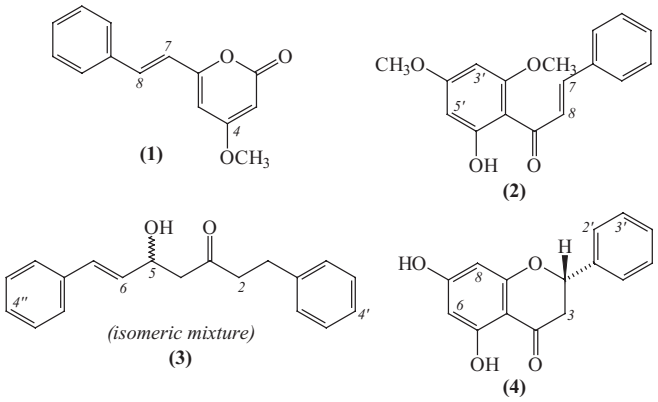

Figure 2. Structures of compounds **1**–**4** isolated from A. *nulans* Rosc.

Table 1. DPPH free radical scavenging activity of 5,6-dehydrokawain.

Compound [0.3 mM]	Free radical scavenging activity (%)
1	41.5
2	27.0
3	23.7
4	37.4
BHT	86.6
Vit.C	89.5

tyrosine kinase (Jung et al., 1990) and to the metabolism of carcinogen benzopyrene (Liu et al., 1992).

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