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RESEARCH NOTE

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Antioxidative Diarylheptanoids from the Fruits of *Alpinia oxyphylla*

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Abstract The antioxidative activity of *Alpinia oxyphylla* was investigated through measuring the radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) and inhibitory activity for linoleic acid peroxidation. Two antioxidative diarylheptanoids, yakuchinone A (**1**) and oxyphyllacinol (**2**), were isolated from the fruits of *A. oxyphylla* using thin layer chromatography (TLC) autographic assays. The DPPH scavenging activities of the compounds (IC_{50}=**1**, 57±2.1 μM; **2**, 89±3.1 μM) were lower than vitamin C (IC_{50}=51±1.1 μM), but higher than butylated hydroxytoluene (BHT, IC_{50}=99±2.2 μM). Also, inhibitory activities for linoleic acid peroxidation of the compounds (IC_{50}=**1**, 0.19±0.011 mM; **2**, 0.31±0.009 mM) were higher than those of vitamin C (IC_{50}=0.59±0.017 mM) and BHT (IC_{50}=0.52±0.014 mM). In addition the ^{13}C-NMR data of oxyphyllacinol (**2**) have been first reported in this paper.

Keywords: *Alpinia oxyphylla*, antioxidative activity, 1,1-diphenyl-2-picrylhydrazyl (DPPH), linoleic acid peroxidation, diarylheptanoid, yakuchinone A, oxyphyllacinol

Introduction

The fruits of *Alpinia oxyphylla* (Zingiberaceae) have been used as a popular remedy of indigestion, inappetence, and intestinal disorders (1). As for its pharmacological effects, anti-diuresis, anti-ulceration, anti-dementia, and anti-tumor promotional properties, protection of ischemic damage, and insecticidal effects have been reported (2-5), and its principal compounds were identified as diarylheptanoids and terpenes (6-10). In the course of screening for anti-oxidative materials from natural sources, the MeOH extracts from the fruits of *A. oxyphylla* showed very high 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity. The antioxidative diarylheptanoids, yakuchinone A (**1**) and oxyphyllacinol (**2**), were isolated from the fruits of *A. oxyphylla* through a thin layer chromatography (TLC) autographic assay method (11), which included silica gel TLC of each fractions followed by the spraying of DPPH solution. In the present paper, the antioxidative activities of the compounds were investigated through measuring radical scavenging effect on DPPH and inhibitory activity for linoleic acid peroxidation. In addition, the exact chemical shifts in the ^{13}C-nuclear magnetic resonance (NMR) of oxyphyllacinol (**2**), which have not been reported so far, were also reported.

Materials and Methods

General procedure The NMR spectra were obtained on Varian Inova AS400 NMR spectrometer (Varian, Washington DC, USA). The infrared (IR) spectra were recorded with Perkin-Elmer Spectrum One FT-IR Spectrophotometer (Perkin Elmer, Bukinghamshire, UK). Electron impact (EI) mass spectra were taken on a Jeol JMS-700 spectrometer (Jeol, Tokyo, Japan). Optical rotations were measured on a Jasco P-1020 digital polarimeter (Jasco, Tokyo, Japan). Kiesel gel 60 (70-230 mesh) and Kiesel gel 60F_{254} (Merck, Darmstadt, Germany) were used for column chromatography and TLC, respectively. Butylated hydroxytoluene (BHT, Sigma, St. Louis, MO, USA) and vitamin C (Sigma Chemical Co., Poole, UK) were used as positive control in the antioxidative activity test.

Plant material The fruits of *A. oxyphylla* were purchased from oriental medicine market in Seoul, Korea and identified by Dr. Hyeong-Kyu Lee, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon, Korea. Voucher specimen (KHU0081) is deposited at the Plant Metabolism Research Center, Kyung Hee University, Suwon, Korea.

TLC autographic assay method The $CHCl_3$-MeOH solution of each fraction was developed on silica gel TLC and then the DPPH MeOH solution (40 μg/mL) was sprayed on it. The decolorization of some spots indicated the presence of the antioxidative compounds (11).

Extraction and isolation of antioxidative compounds The Dried *A. oxyphylla* fruits (1 kg) were extracted at room temperature with EtOH (10 L × 2). The aqueous suspension of the extracts (1.5 L) was partitioned with EtOAc (1.5 L × 2) and *n*-BuOH (1 L × 2), successively. The EtOAc extract (34.5 g) was applied to silica gel column chromatography (c.c.) (5×12 cm) and eluted with *n*-hexane-EtOAc (8:1, 5:1, and 4:1, each 900, 600, and 500 mL) monitoring by TLC to produce 17 fractions (AOE-1–AOE-17). Each fraction was tested for DPPH scavenging activity as the above procedure. The eleventh fraction (AOE-11, 0.7 g) was further purified by c.c. on silica gel (3×13 cm) using *n*-hexane-EtOAc-$CHCl_3$ (8:3:3,

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700 mL) for elution to yield compound **1** [209.5 mg, Rf : 0.53 on silica gel TLC in *n*-hexane-EtOAc (2:3)] (6-8). The 15th fraction (AOE-15, 0.5 g) was subjected to silica gel column (3×15 cm) eluted with *n*-hexane-EtOAc (5:1, 0.5 L) to afford 5 fractions (AOE-15-1–ARE-15-5). The obtained fraction containing active component (AOE-15-1, 0.2 g) was applied to silica gel c.c. (3×15 cm) eluted with *n*-hexane-EtOAc-$CHCl_3$ (8:3:3, 0.4 L) to ultimately produce a purified compound **2** [26 mg, Rf : 0.40 on silica gel TLC in *n*-hexane-EtOAc (2:3)] (8).

Compound **1**, yellowish oil; $IR_\nu(CHCl_3)$ max (1/cm): 3410, 1708, 1600; EIMS *m/z* (relative intensity %): 312 ($[M]^+$, 34), 296 (44), 194 (4), 179 (14), 151 (14), 137 (100), 131 (22), 91 (40); HR-EIMS for *m/z* 312, Found 312.1726, Cald. for $C_{20}H_{24}O_3$ 312.1725; ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 7.23-7.27 (2H, m, H-2", 6"), 7.12-7.16 (2H, m, H-3", 5"), 6.81 (1H, d, *J*=7.8 Hz, H-5'), 6.65 (1H, d, *J*=7.8 Hz, H-6'), 6.64 (1H, br. dd, *J*=7.8, 8.0 Hz, H-4"), 5.67 (1H, s, H-2'), 3.81 (3H, s, OCH_3), 2.80 (2H, t, *J*=7.6 Hz, H-1), 2.67 (2H, t, *J*=5.6Hz, H-7), 2.58 (2H, t, *J*=7.6 Hz, H-2), 2.37 (2H, t, *J*=6.8 Hz, H-4), 1.59-1.55 (4H, m, H-5, 6); ^{13}C-NMR (100 MHz, $CDCl_3$, δ_C): 210.6(C-3), 146.7(C-3'), 144.1(C-4'), 142.4(C-1"), 133.3(C-1'), 128.6 (C-2", 6"), 128.5(C-3", 5"), 126.0(C-4"), 121.0(C-6'), 114.6(C-5'), 111.4(C-2'), 56.1($O\underline{C}H_3$), 44.8(C-2), 43.1(C-4), 35.9(C-7), 31.2(C-6), 29.7(C-1), 23.6(C-5).

Compound **2**, colorless oil; $[\alpha]_D^{25}$ +2.5°(*c*=1.09, MeOH); IR_ν(KBr) max (1/cm): 3384, 2933, 2857, 1602, 1515, 1453, 1271, 1034, 700; EIMS *m/z* (relative intensity %): 314 ($[M]^+$, 12), 296 (52), 137 (100), 131 (26), 91 (42); HR-EIMS for *m/z* 314, Found 314.1886, Cald. for $C_{20}H_{26}O_3$ 314.1882; ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 7.24-7.29 (2H, m, H-2", H-6"), 7.15-7.19 (2H, m, H-3", 5"), 6.82 (1H, d, *J*=8.0 Hz, H-5'), 6.66-6.69 (2H, m, H-4", 6'), 5.56 (1H, s, H-2'), 3.85 (3H, s, O-$C\underline{H}_3$), 3.62 (1H, dddd, *J*=9.3, 9.3, 3.9, 3.9 Hz, H-3), 2.71 (1H, ddd, *J*=13.6, 9.1, 6.0 Hz, H-1a), 2.61 (2H, t, *J*=7.8 Hz, H-7), 2.58 (1H, ddd, *J*=13.6, 8.4, 7.8 Hz, H-1b), 1.25-1.76 (8H, m); ^{13}C-NMR (100 MHz, $CDCl_3$, δ_C): 146.6(C-3'), 143.8(C-4'), 142.7(C-1"), 134.2(C-1'), 128.5(C-2", 6"), 128.4(C-3", 5"), 125.8 (C-4"), 121.0(C-6'), 114.5(C-5'), 111.2(C-2'), 71.6(C-3), 56.1($O\underline{C}H_3$), 39.6(C-2), 37.7(C-4), 36.2(C-7), 32.1(C-6), 31.8(C-1), 25.6(C-5).

The evaluation of DPPH radical scavenge activity DPPH radical scavenging activity was measured by the procedure of Fugita *et al.* (12). The sample solution (100 μL) was added to 2 mL of DPPH EtOH solution (40 μg/mL) and kept at room temperature for 30 min. Absorbance of the solution was measured at 517 nm. Antioxidative activity was expressed as electron donating ability (EDA, %), calculated as (Ac-As) × 100/Ac, where Ac is the absorbance of the solution without test material and As is the absorbance of the solution with test material. The experiments were carried out in triplicate.

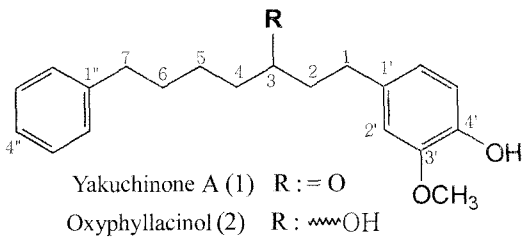
The evaluation of inhibition activity for linoleic acid peroxidation A lipid peroxidation was induced by 2,2'-Azobis (2-amidinopropane)-dihydrochloride (AAPH) (13). Two-hundred μL of linoleic acid solution (1 mg/mL) and 50 μL of sample solution (1 mg/mL) were mixed with AAPH (50 mM) and incubated at 37 for 1 hr. To the reaction mixture, 15% sodium dodecyl sulfate, 1% 2-thiobarbituric acid (TBA) and 120 μL of acetic acid glacial were added and heated at 85°C for 30 min. Absorbance was measured at 540 nm. The inhibition activity (%) was calculated as (Ac-As) × 100/(Ac-AB), where Ac is the absorbance of the solution without test material, As is the absorbance of the solution with test material, and AB is the absorbance of the blank with just test material and AAPH.

Results and Discussion

The ethanolic extract obtained from the fruits of *A. oxyphylla* was fractionated through solvent fractionation and column chromatography. The obtained fractions were evaluated for DPPH radical scavenging activity using TLC-spray method (11). Finally two antioxidative diarylheptanoids, compound **1** and **2**, were isolated with the yields of 0.0070 and 0.0009%, respectively.

Compound **1** was identified as yakuchinone A (Fig. 1) by interpretation of spectral data and comparison with those described in the literature (6-8).

When compound **2** was developed on silica gel TLC, its spot showed neither UV absorbency at 254 or 365 nm nor colorization by spraying 10% H_2SO_4 solution and heating. In this study, compound **2** was detected by the loss of violet color caused by DPPH scavenging activity on silica gel TLC. Compound **2** showed peak at *m/z* 314 ($[M]^+$, $C_{20}H_{24}O_3$) in the EI/MS spectrum and the prominent bands at 3384 (O-H), 1034 (O-C), and 1602 (C=C) 1/cm in the IR spectrum (KBr). NMR data of compound **2** were almost identical with those of yakuchinone A (**1**) with the exception of some signals owing to C-3 and its vicinity. In the ^{1}H-NMR spectrum of compound **2** ($CDCl_3$), the aromatic protons of rings A and B (δ7.27-5.67, 7.15-7.19, 6.82, 6.66-6.69, and 5.56) and a methoxy [δ3.85(3H, s)] were also observed as yakuchinone A (**1**). But an oxygenated methine proton was newly observed at δ3.62 (1H), which was absent in yakuchinone A (**1**), indicated that a ketone of yakuchinone A (**1**) was reduced into a hydroxyl group in compound **2**. In the ^{13}C-NMR spectrum of compound **2**, most of the signals were similar to those of yakuchinone A (**1**) with the exception of oxygenated methine carbon (δ_C71.6). For the reduction of compound **2**, a ketone into a hydroxyl, C-2 and C-4 signals of compound **2** were shifted to high magnetic field (δ_C 44.8 → 37.7, 43.1 → 36.2). The above evidences showed that compound **2** must be 1-(4'-hydroxy-3'-methoxyphenyl)-7-phenyl-3-heptanol, oxyphyllacinol (Fig. 1) (8).



Yakuchinone A (1) R : = O

Oxyphyllacinol (2) R : ⁓OH

Fig. 1. Chemical structure of compound **1** and **2** from the fruits of *Alpinia oxyphylla*.

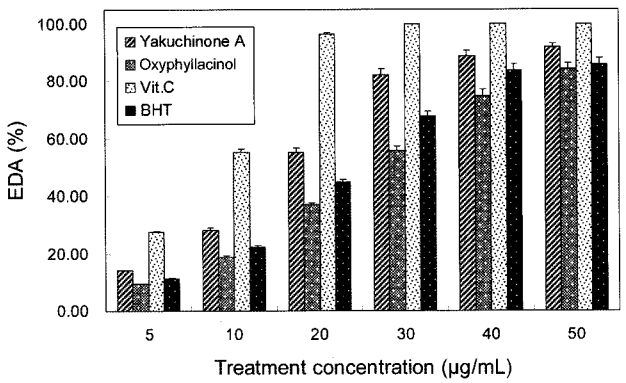


Fig. 2. Electron donating ability (EDA) of compounds isolated from the fruits of *Alpinia oxyphylla* and other commercial antioxidants. The results are the means±SE of EDA values obtained from triplicate experiments.

The exact assignment of chemical shifts in the ^{1}H- and ^{13}C-NMR of both compounds was fulfilled by using 2-D NMR experiments including ^{1}H-^{1}H COSY, gHSQC, gHMBC, and NOESY. Especially, the ^{13}C-NMR data of oxyphyllacinol (**2**) have never been reported so far.

The antioxidant activities of the two compounds were investigated by both the DPPH radical scavenging system and linoleic peroxidation system using TBA. DPPH radical scavenging activities of yakuchinone A (**1**) and oxyphyllacinol (**2**) increased in a concentration-dependent manner as shown in Fig. 2. Compared to commercial antioxidants, yakuchinone A (**1**) (IC_{50} = 57±2.1 μM) and oxyphyllacinol (**2**) (IC_{50} = 89±3.1 μM) showed lower activities than vitamin-C (IC_{50} = 51±1.1 μM) but higher than BHT (IC_{50} = 99±2.2 μM). However, the inhibitory effects of yakuchinone A (**1**) (IC_{50} = 0.19±0.011 mM) and oxyphyllacinol (**2**) (IC_{50} = 0.31±0.009 mM) for linoleic acid peroxidation showed higher activities than those of vitanim-C (IC_{50} = 0.59±0.017 mM) and BHT (IC_{50} = 0.52± 0.014 mM) (Table 1).

Although antioxidant activity of several phenolic compounds including flavonoids, phenylpropanoids, and tannins etc. have been reported till now, that of diarylheptanoid has been reported in this study for the first time.

Antioxidants have been reported to play a major role in ameliorating peroxidative damage, induced by free radicals and xenobiotics, to membranes and tissues (14). Therefore, if continual study for several antioxidant systems using reactive oxygen species, super radical anions, hydrogen peroxide radicals, living cells, and organisms showed some significant results, the diaryl-heptanoids isolated from *A. oxyphylla* could be used as preventive and/or therapeutic agents for carcinogenesis, the aging process, and cardiovascular diseases such as ischemia-reperfusion injury and hypercholesterolemic atherosclerosis (15-17).

Acknowledgments

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Table 1. IC_{50} values in the inhibition activity of the compounds from the fruits of *Alpinia oxyphylla* and other commercial antioxidants on linoleic acid peroxidation[1)]

	Yakuchinone A	Oxyphyllacinol	Vitamin C	BHT
IC_{50} value (mM)	0.19±0.011	0.31±0.009	0.59±0.017	0.52±0.014

[1)]The results are the means±SE of IC_{50} values obtained from triplicate experiments.

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