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# Isolation of Hyperoside and Isoquercitrin from *Camellia sasanqua* as Antioxidant Agents

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**Abstract:** Two antioxidant active compounds were isolated from the methanol extract of *Camellia sasanqua* using various *in vitro* assays: 1,1-diphenyl-2-picrylhydrazyl (DPPH), β-carotene bleaching and reducing power assays. The ethyl acetate (EtOAc) fraction of the methanol extract had the highest DPPH radical-scavenging activity with an Inhibition Concentration ($IC_{50}$) value of 18.3±1.63 μg mL$^{-1}$. Sephadex LH-20 column chromatography was used to separate the EtOAc fraction into eight fractions (F1-F8). Antioxidant activity was significantly higher in fraction 5 with an $IC_{50}$ value 14.61±0.02 μg mL$^{-1}$. Fraction 5 was further separated by HPLC preparative with Capcellpak $C_{18}$ MG followed by the Cosmosil 5$C_{18}$-AR-II column, using a guided DPPH radical-scavenging assay. The compounds isolated were identified as: Hyperoside (1) and isoquercitrin (2) after recrystallization from ethanol, based on Mass Spectrum (MS) and Nuclear Magnetic Resonance (NMR) analyses. Their DPPH radical-scavenging activities based on the 50% scavenging concentration decreased in the following order: Isoquercitrin (21.6 mM)>hyperoside (27.5 mM). The antioxidant activities of hyperoside and isoquercitrin were 67.52±0.64 and 64.33±0.51%, respectively, in the β-carotene bleaching assay. These compounds were found to have good reducing powers (OD value: 2.5-3.8) at concentrations of 50-140 μg mL$^{-1}$, using the potassium ferricyanide reduction method. Although, these compounds are well-known, hyperoside (1) was isolated from this herb for the first time.

**Key words:** *Camellia sasanqua*, hyperoside, isoquercitrin, antioxidant activity

## INTRODUCTION

Reactive Oxygen Species (ROS) including the hydroxyl radical, superoxide radical and hydrogen peroxide are various forms of activated oxygen and areproduced by Ultraviolet Light (UV), ionizing radiation, chemical reactions and metabolic processes. Oxidative stress has been shown to be among the main causative factors for the induction of many chronic and degenerative diseases including cancer (Muramatsu *et al*., 1995), atherosclerosis (Steinberg *et al*., 1989), gastric ulcers (Das *et al*., 1997), diabetes mellitus, ischemic heart disease, cancer, ageing, immune suppression and neurodegenerative diseases (Heinecke, 2003). Damaged DNA, which may lead to mutations, has also implicated ROS in carcinogenesis (Sawa *et al*., 1999).

The most effective way to eliminate free radicals is by antioxidants. Antioxidants, both exogenous and endogenous, whether synthetic or natural, are substances that neutralize free radicals or their actions (Devasagayam *et al*., 2004). Various antioxidants such as ascorbic acid (vitamin C) and glutathione (Meister, 1994), α-tocopherol (vitamin E) (Rosen *et al*., 1995), flavonoids (Pietta, 2000) and polyphenols (Perron and Brumagim, 2009) are mainly derived from natural sources that are capable of protecting against DNA damage. The number of studies investigating naturally-occurring antioxidants for use in food, such as rutin contained in *Eugena polyantha* (Lelono and Tachibana, 2013), or medicine to replace synthetic antioxidants has increased (Gazzani *et al*., 1998).

*Camellia sasanqua* or sazanka, family Theaceae, is an evergreen plant cultivated in a number of countries as a foodstuff (Fishman and Bandyukova, 1991). Sazanka has been shown to exhibit both anti-inflammatory (Akihisa *et al*., 1998) and anti-allergic (Matsuda *et al*.,

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2010) activities. In this study, we described the isolation and identification of active antioxidant compounds from the methanol extract of *Camellia sasanqua*.

## MATERIALS AND METHODS

**Plant materials:** *Camellia sasanqua* leaves were collected from the Ehime Prefectural Greenery Center, Ehime Prefecture in October 2010 and May 2012. The leaves were kept dry and away from direct sunlight during storage. Specimens were deposited in the Laboratory of Plant Chemistry, Faculty of Agriculture, Ehime University, Japan, for further use.

**Chemicals:** Quercetin dehydrate, potassium ferricyanide [$K_3Fe(CN)_6$], trichloroacetic acid, ferric chloride ($FeCl_3$), β-Carotene and 1,1-diphenyl-2-picrylhydrazl (DPPH) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Tween-40 was purchased from Sigma-Aldrich Co. Ltd. (Tokyo, Japan). TLC (Thin Layer Chromatography) aluminium sheet (0.25mm) Silica gel 60 $F_{254}$, 20×20 cm was obtained from Merck (Darmastadt, Germany). Sephadex LH-20 (5×50 cm, 70-230 mesh), was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden). All solvents were purchased from Wako Chemical Co. Ltd. (Osaka, Japan) and distilled before used.

**General experimental procedures:** Melting points were measured on a Yanaco micro melting point apparatus (Yanaco Co., Ltd., Kyoto, Japan) and were uncorrected. Ultraviolet (UV) spectra were measured with a Shimadzu (UV-VIS 1200 spectrophotometer (Shimadzu Corp., Kyoto, Japan). HPLC preparative was conducted with a Waters system consisting of a 600 Delta pump controller equipped with a Charatec Sample Loader SL964, Waters module column oven, UV-visible Detector 2489 and Fraction collector III(Waters, Japan). The mass spectra of compounds were obtained using TOF-MS (Waters, Japan) and high resolution FAB-MS. Nuclear Magnetic Resonance (NMR) spectra were recorded on a JEOL JNM-EX400 (JEOL, Ltd., Tokyo, Japan) at 500 and 125 MHz, respectively. Chemical shifts were expressed as δ in ppm and TMS was used as the internal standard. The coupling constant (J) was recorded in Hz.

**Sample preparations:** *Camellia sasanqua* leaves were cut into small pieces and air-dried before extraction. The dried leaves (1000 g) were extracted for 48 h at room temperature with methanol solvent. The extraction was repeated twice. The methanol extract was concentrated with a rotary evaporator under reduced pressure. The methanol extract was a dark brown semisolid. The methanol extract of *Camellia sasanqua* leaves (60 g) was suspended in methanol:water (1:4) and then partitioned with n-hexane, chloroform, ethyl acetate and n-butanol solvents to give n-hexane, chloroform, ethyl acetate and n-butanol fractions. Activity-guided isolation was conducted using 1,1-diphenyl-1-picrylhydrazl (DPPH) radical-scavenging.

**Isolation of antioxidant compounds from the ethyl acetate fraction:** The ethyl acetate fraction (60 g) was fractionated on a Sephadex LH-20 column and then eluted with $CHCl_3$:MeOH (8:2, 7:3, 6:4, 5:5, v/v). The separated fractions were first checked by the TLC plate. Spots were detected under a UV lamp (254 nm) or by spraying with bromocresol green, DPPH, concentrated sulphuric acid and p-anysaldehyde sulfuric acid before heating the TLC plate. Repeated bioassay-guided fractionation was conducted using Sephadex LH-20 and HPLC preparative.

**Structural analysis**

**Compound 1:** Light yellow crystal (ethanol); M.P.: 233-235°C; UV $\lambda_{max}$(log ε): 361 (4.38), 258 (4.46), 209 (4.67); FAB-MS m/z 465 $[M+H]^+$; TOF-MS $[M+H]^+$ 465.1040 [$C_{21}H_{20}O_{12}$ as 465.0022];$^1$H-NMR (MeOD, 500 MHz) δ = 7.98 (1H, d, J = 2.0 Hz, H-6'), 7.72 (1H, dd, J = 8.5, 2.5 Hz, H-5'), 6.99 (1H, d, J = 8.5 Hz, H-2'), 6.5 (1H, d, J = 2 Hz, H-8), 6.3 (1H, d, J = 2 Hz, H-6), 5.3 (1H, d, J = 8 Hz, H-1"), 3.98-3.6 (6H, m, H-2"-6");$^{13}$C-NMR (MeOD, 125 MHz), Table 1.

**Compound 2:** Yellow crystal (ethanol); M.P. 215-216°C; UV $\lambda_{max}$ (log ε): 360 (4.18), 258 (4.28), 207 (4.53);

Table 1: $^{13}$C-NMR data for compounds 1 and 2, hyperoside isoquercitrin (MeOD, 125 MHz)

| Position | Compound 1 | Hyperoside | Compound 2 | Isoquercitrin |
|---|---|---|---|---|
| 2 | 159.3 | 159.3 | 159.3 | 159.3 |
| 3 | 136.6 | 136.6 | 136.4 | 136.4 |
| 4 | 180.3 | 180.3 | 180.3 | 180.3 |
| 5 | 163.8 | 163.8 | 163.9 | 163.9 |
| 6 | 100.7 | 100.7 | 100.7 | 100.7 |
| 7 | 167.0 | 167.0 | 166.9 | 166.9 |
| 8 | 95.5 | 95.5 | 95.5 | 95.5 |
| 9 | 159.6 | 159.6 | 159.8 | 159.8 |
| 10 | 106.4 | 106.4 | 106.5 | 106.5 |
| 1' | 123.7 | 123.7 | 123.9 | 123.9 |
| 2' | 116.9 | 116.9 | 116.8 | 116.8 |
| 3' | 146.6 | 146.6 | 146.7 | 146.7 |
| 4' | 150.8 | 150.8 | 150.7 | 150.7 |
| 5' | 118.6 | 118.6 | 118.4 | 118.4 |
| 6' | 123.7 | 123.7 | 123.9 | 123.9 |
| 1" | 106.2 | 106.2 | 105.1 | 105.1 |
| 2" | 73.9 | 73.9 | 76.5 | 76.5 |
| 3" | 75.9 | 75.9 | 78.9 | 78.9 |
| 4" | 70.8 | 70.8 | 72.0 | 72.0 |
| 5" | 78.0 | 78.0 | 79.2 | 79.2 |
| 6" | 62.8 | 62.8 | 63.4 | 63.4 |

FAB-MS m/z 465 $[M+H]^+$; TOF-MS $[M+H]^+$465.0883 [$C_{21}H_{20}O_{12}$ as 465.0022];$^1$H-NMR $\delta$ = 7.84 (1H, d, J = 2.0 Hz, H-6'), 7.72 (1H, dd, J = 8.5, 2.0 Hz, H-5'), 7.0 (1H, d, J = 8.5 Hz, H-2'), 6.53 (1H, d, J = 2 Hz, H-8), 6.34 (1H, d, J = 2 Hz, H-6), 5.4 (1H, d, J = 7.5 Hz, H-1"), 3.86-3.5 (6H, m, H-2"-6");$^{13}$ C-NMR (MeOD, 125 MHz), Table 1.

**DPPH radical-scavenging assay:** The scavenging activity of the DPPH radical was monitored according to the method described by Yen and Chen (1995). A 0.1 mL methanol solution containing between 0.4 and 2.0 mg of the extract was mixed with 2 mL of methanol and a methanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) (1 mM, 0.5 mL) was then added. The mixture was stirred for 15 sec and then left to stand at room temperature for 30 min. The absorbance of this solution was then read at 517 nm. The percent inhibitory activity was calculated from:

$$\frac{A_0-A_1}{A_0}\times 100$$

where, $A_0$ is the absorbance of the control and $A_1$ is the absorbance of the extract or standard. The 50% Inhibition Concentration ($IC_{50}$) of each compound was determined from the graph of percentage antioxidant activity against sample concentration on millimolar (mM), whereas $IC_{50}$ of methanol extract and its separated fractions were determined on micro gram per milliliter (μg $mL^{-1}$). Assays were performed in triplicate and the results were expressed as mean values±SD. Quercetin was used as a reference compound.

**β-Carotene bleaching assay:** The antioxidant activities of isolated compounds were also evaluated using the β-carotene bleaching assay (Jayaprakasha *et al*., 2001). A solution of β-carotene was prepared by dissolving 2 mg of β-carotene in 10 mL of chloroform. Two milliliters of the solution was transferred into a boiling flask containing 20 mg linoleic acid and 200 mg Tween 40. Chloroform was removed using a rotary evaporator at 40°C for 5 min and 50 mL of distilled water was slowly added to the residue with vigorous agitation to form an emulsion. Aliquots of the emulsion (4.8 mL) were transferred into different test tubes containing 0.2 mL of samples in methanol at 0.5 mg $mL^{-1}$. The tubes were then shaken and incubated at 50°C in a water bath. The zero time absorbance was measured at 470 nm using a spectrophotometer as soon as the emulsion was added to each tube. Absorbance readings were then recorded at 15 min intervals until the color of the control sample changed. A blank, devoid of β-carotene, was prepared for background subtractions. Antioxidant activity was calculated using the following equation:

$$\text{Antioxidant activity (AA) (\%)} = 1-\left[\frac{(A_{x0}-A_{xe})}{A_{b0}-A_{be}}\right]\times 100$$

where, $A_{x0}$ and $A_{xe}$ were absorbances of the sample at 0 and 120 min and $A_{b0}$ and $A_{be}$ were absorbances of the control at 0 and 120 min. Assays were performed in triplicate and the results were expressed as the Mean±Standard Deviation (SD). Quercetin and ascorbic acid were used as standards.

**Reducing power assay:** The reducing power assay conducted for the isolated compounds was described previously (Oyaizu, 1986). A total of 0.5 mL of the methanol solution containing various concentrations of samples (10-200 μg $mL^{-1}$) was mixed with 2.5 mL of 0.2M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] and was then incubated at 50°C for 20 min. The mixture was centrifuged at 3000 rpm for 10 min after 2.5 mL of 10% trichloroacetic acid was added. The upper layer of the solution (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride and absorbance was then measured at 700 nm. An increase in absorbance for the reaction indicated an increase in reducing power. The assay was performed in triplicate and the results were expressed as the Mean±SD. Quercetin and ascorbic acid were used as positivecontrols.

## RESULTS AND DISCUSSION

***In vitro* free radical-scavenging activity of the soluble fractions:** The methanol extract was examined with the *in vitro* DPPH radical-scavenging assay and exhibited higher antioxidant activity ($IC_{50}$ = 20.73±1.42 μg $mL^{-1}$) than that of quercetin as the positive control ($IC_{50}$ = 10.3±0.20 μg $mL^{-1}$). The methanol extract (60 g) was then separated using solvent-solvent extraction to obtain n-hexane (8.58 g), $CHCl_3$ (6.36 g), EtOAc (11.1 g) and n-butanol (29.76 g) fractions. The antioxidant activity of each fraction and quercetin as the positive control were evaluated based on their DPPH radical-scavenging activities. The antioxidant activity of each fraction and quercetin decreased in the following order: quercetin ($IC_{50}$ = 10.3 μg $mL^{-1}$)>EtOAc fraction (18.3)>n-butanol fraction (27.7)>n-hexane fraction (119.3)>$CHCl_3$ fraction (194.6), as shown in Table 2. Antioxidant activity was higher in the EtOAc fraction than in the other fractions

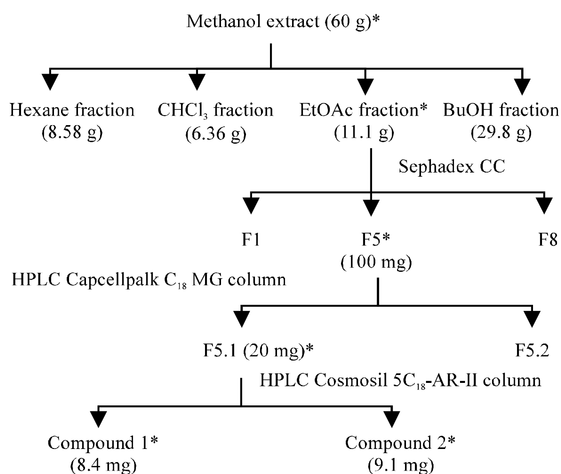


Fig. 1: Procedure for the isolation of active antioxidant compounds from the methanol extract of *Camellia sasanqua*. *Active fraction or compound

Table 2: DPPH radical-scavenging activities of the various fractions obtained after solvent-solvent extraction of the methanol extract of *Camellia sasanqua*

| Sample | $IC_{50}$ (μg $mL^{-1}$) |
|---|---|
| Methanol extract | 20.73±1.42 |
| n-hexane fraction | 119.30±2.92 |
| $CHCl_3$ fraction | 194.60±3.82 |
| EtOAc fraction | 18.30±1.63 |
| n-BuOH fraction | 27.70±1.91 |
| Quercetin | 10.30±0.20 |

which indicated that this fraction may contribute to the high antioxidant activity of its methanol extract. Therefore, the isolation and purification of antioxidant compounds from the EtOAc fraction of the methanol extract was performed.

**Isolation of antioxidant active compounds from the EtOAc fraction:** The isolation procedures for active compounds are shown in Fig. 1. Fractionation of the methanol extract resulted n-hexane, $CHCl_3$, EtOAc and n-butanol fractions (14.3, 10.6, 18.5 and 49.6%, respectively). A portion of the EtOAc fraction (11.1 g) was subjected to column chromatography using Sephadex LH-20 and then eluted with the mixture solvents of $CHCl_3$:MeOH to give eight fractions. Each fraction was subjected to the *in vitro* DPPH radical-scavenging assay. Fraction 5 (F5, 100 mg) was the most active with an $IC_{50}$ value of 14.61±0.02 μg $mL^{-1}$ and yield value of 15.2%.

Fraction 5 was then separated by preparative HPLC using the Capcellpak C-18 MG S-5 μL column (60% MeOH by 0.5% HCOOH) to obtain 2 fractions (F5.1 and F5.2). F5.1 (20 mg) was the most active fraction with an $IC_{50}$ value of 14.02±0.03 μg $mL^{-1}$ and yield value of 7.9%. F5.1 was then separated using preparative HPLC with the Cosmosil 5C-18-AR-II column ($H_2O$:MeOH:THF = 8:2:1) to give compounds 1 ($R_t$ 22.69 min, yellow powder, 8.4 mg) and 2 ($R_t$ 24.66 min, yellow powder, 9.1 mg).

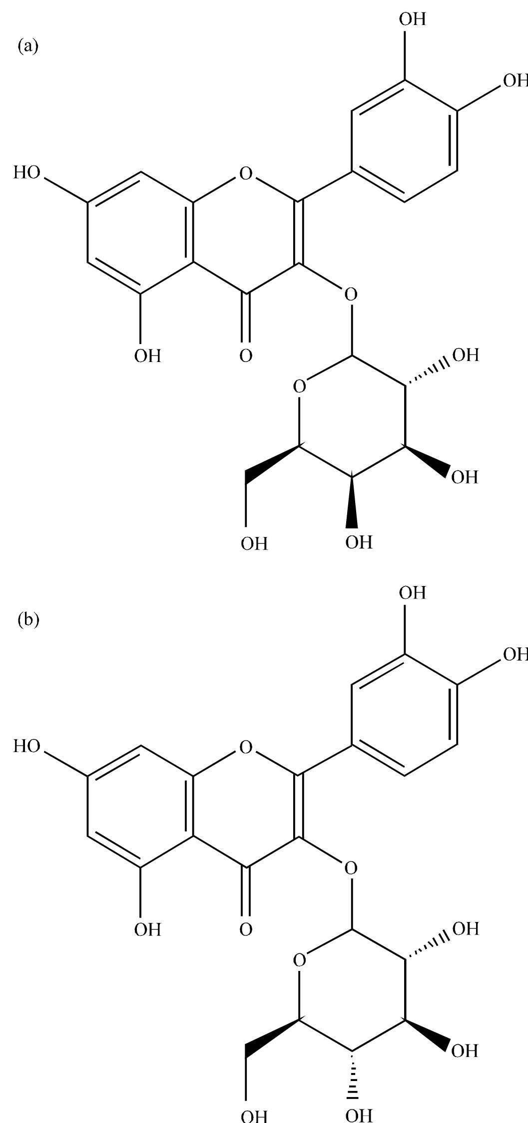


Fig. 2(a-b): Antioxidant compounds isolated from the leaves of *Camellia sasanqua* (a) Hyperoside (1) and (b) Isoquercitrin (2)

The identification of compounds 1 and 2 as the active compounds, as described in Fig. 2, was elucidated using TOF-MS, FAB-MS and nuclear magnetic resonance analyses after the compounds were recrystallized from ethanol with an authentic standard.

**Structural determination of the isolated compounds:** Bioassay-guided fractionation of the EtOAc fraction

afforded compound 1 and 2 which were isolated as a yellow powder and testing with p-anysaldehyde sulfuric acid after heating on the TLC plate indicated the presence of glycoside.

TOF-MS analysis of compounds 1 and 2 revealed their molecular weights $[M+H]^+$ to be 465.1040 and 465.0883, respectively. FAB-MS analysis of both compounds exhibited a molecular weight $FAB^+$ of 465. The $^1$H-NMR (500 MHz, MeOD) spectrum of compound 1showed the presence of quercetin as aglycone including 2 proton signals of an A ring [δ 6.5 (1H, d, J = 2 Hz, H-8) and 6.3 (1H, d, J = 2 Hz, H-6)] and 3 proton signals of a B ring at δ 7.98 (1H, d, J = 2.0 Hz, H-6'), 7.72 (1H, dd, J = 8.5, 2.5 Hz, H-5') and 6.99 (1H, d, J = 8.5 Hz, H-2'). The $^1$H-NMR (500 MHz, MeOD) spectrum of compound 2 showed 2 proton signals of an A ring [δ 6.53 (1H, d, J = 2 Hz, H-8) and 6.34 (1H, d, J = 2 Hz, H-6)] and 3 proton signals of a B ring at δ 7.84 (1H, d, J = 2.0 Hz, H-6'), 7.72 (1H, dd, J = 8.5, 2.0 Hz, H-5') and 7.0 (1H, d, J = 8.5 Hz, H-2'). Therefore, the aglycones of compounds 1 and 2 were assigned to quercetin. The coupling constants (J), signal splitting patterns and chemical shifts in the proton and carbon signals of the sugar moieties suggested that the sugars were galactose and glucose. Therefore, the compounds were assigned as quercetin 3-O-β-D-galactopyranoside and quercetin 3-O-β-D-glucopyranoside which was confirmed with standard samples using HPLC as shown in Fig. 5. Compound 1 had a Retention time (Rt) of 22.69 min while the hyperoside standard sample had a Rt of 22.69 min. Compound 2 had a Rt of 24.66 min and the isoquercitrin standard sample hada Rt of 24.66 min. $^1$H-NMR and $^{13}$C-NMR analyses of the hyperoside and isoquercitrin standard samples revealed similarities in the coupling constants (J), signal splitting patterns and chemical shifts in the proton and carbon signals with the isolated compounds (Table 1). Based on UV-vis., MS, $^1$H-NMR and $^{13}$C-NMR analyses, compound 1 and 2 were identified as quercetin 3-O-β-D-galactopyranoside (hyperoside) and quercetin 3-O-β-D-glucopyranoside (isoquercitrin), respectively (as shown in Fig. 1). The spectral characteristics of these compounds were found to be identical with those in the literature (Cho *et al*., 2008; Lee *et al*., 2011). Although, these compounds are well-known, compound 1 was isolated from this herb for the first time, whereas compound 2 was previously found in *C. sasanqua*, as reported by Fishman and Bandyukova (1991).

**_In vitro_ antioxidant activities of the isolated compounds:** The DPPH radical-scavenging activities of compounds 1 and 2 isolated from the EtOAc fraction are shown in Table 3. Both compound 1 and 2 exhibited high scavenging activities toward the DPPH radical with $IC_{50}$ values of 27.5 and 21.6 mM, respectively, higher than that of ascorbic acid and quercetin with an $IC_{50}$ value of 27.8 and 32.2 mM, respectively.

Table 3: DPPH radical-scavenging activities of the compounds isolated from *Camellia sasanqua*

| Sample | $IC_{50}$ (mM) |
|---|---|
| Compound 1 | 27.5±0.13 |
| Compound 2 | 21.6±0.11 |
| Ascorbic acid | 27.8±0.11 |
| Quercetin | 32.2±0.20 |

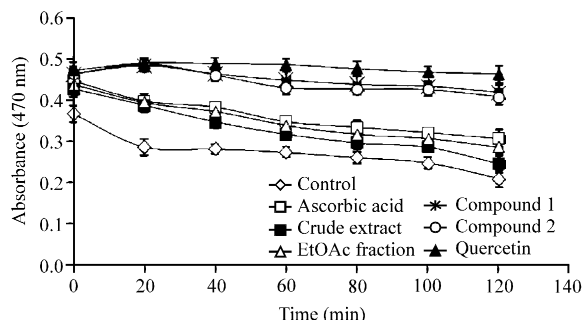


Fig. 3: Antioxidant activities of compounds 1 and 2 from *Camellia sasanqua* at 140 μg $mL^{-1}$ in the β-carotene bleaching assay. Values are shown as the Mean±SD of three parallel measurements (n = 3)

In the β-carotene bleaching assay, the crude extract and EtOAc fraction of *C. sasanqua* were tested with the isolated compounds and were compared with ascorbic acid and quercetin as positive controls. A gradual decrease in antioxidant activity at 470 nm was observed using the β-carotene-linoleic model assay as shown in Fig. 3. The initial β-carotenes of compounds 1 and 2 exhibited antioxidant activities of 67.52±0.64 and 64.33±0.51%, respectively, after 120 min of the assay which were lower than that of quercetin (93.63±0.31%), whereas, ascorbic acid was only 11.46±0.70%. The crude extract exhibited the lowest antioxidant activity (4.46±0.52%) and antioxidant activity was higher in the EtOAc fraction (10.83±1.01%).

The reducing capabilities of the isolated compounds relative to those of quercetin and ascorbic acid are shown in Fig. 4. The reducing powers of compounds 1 and 2 increased with the amount of samples (OD value: 2.5-3.8) from 50-140 μg $mL^{-1}$, similar to quercetin and ascorbic acid. Furthermore, compounds 1 and 2 were more active than the crude extract and EtOAc fraction.

According to the antioxidant activity assays performed, compounds 1 and 2 had higher antioxidant activities than that of quercetin and ascorbic acid as positive controls on a molar basis. This results in

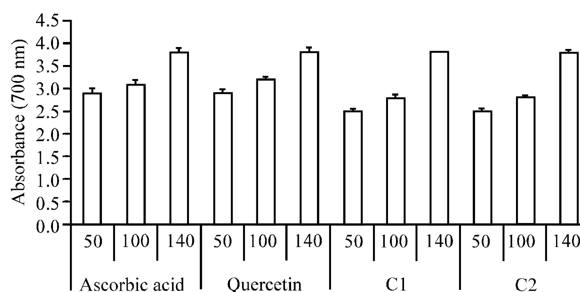


Fig. 4: Antioxidant activities of compounds 1 and 2 from *Camellia sasanqua* in the reducing power assay. Ascorbic acid and quercetin were used as positive controls. Data are presented as the Mean±SD of triplicate measurements

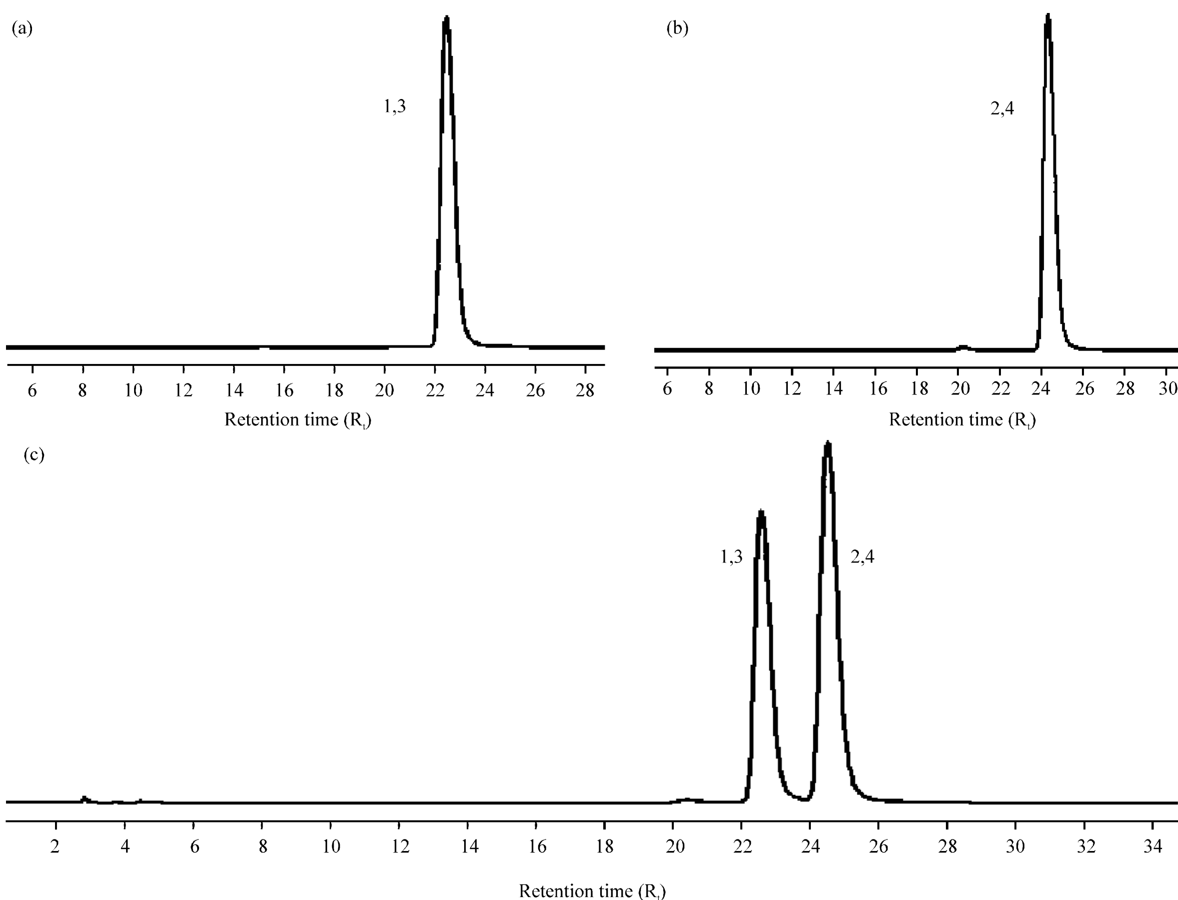


Fig. 5(a-c): HPLC profiles of (1) Compound 1, (2) Compound 2, (3) Hyperoside and (4) Isoquercitrin. (1) Compound 1 and (3) hyperoside $R_t$ 22.69 min, (2) Compound 2 and (4) isoquercitrin $R_t$ 24.66 min

accordance with Onodera *et al*. (2006) mentioned that the activity of hyperoside and isoquercitrin isolated from *Camellia japonica* were higher than that of quercetin and ascorbic acid. Seeram and Nair (2002) reported that the amount of sugar moiety plays a role in antioxidant activity, with activity decreasing with an increase in the amount of the sugar moiety. However, the antioxidant activity of hyperoside and isoquercitrin were higher than that of quercetin in this report indicating the insignificant contribution of the sugar moiety to the activity.

The use of multiple methods is necessary to accurately assess antioxidant activity because the specificities and sensitivities of the antioxidant substances may differ slightly. As a compound with

well-known antioxidant activity, the β-carotene bleaching test of ascorbic acid did not demonstrate the same antioxidant activity as that for compounds 1 and 2 with the DPPH radical-scavenging assay. The β-carotene bleaching test has been found to indicate relatively higher antioxidant activities for non polar compounds. However, the DPPH method was shown to be independent of substrate polarity (Kulisic *et al*., 2004; Koleva *et al*., 2002; Pekkarinen *et al*., 1999; Yamaguchi *et al*., 1998).

As flavonoids, compounds 1 and 2 containing the catechol moiety showed significantly higher DPPH radical-scavenging activities. The catechol structure has previously been shown to contribute to the antioxidant effect observed in flavonoids and phenolic compounds (Cho *et al*., 2008; Cao *et al*., 1997; Van Acker *et al*., 1996). Phenolic hydroxyl groups at the 3- and 4- positions of the flavonoid B ring were reported to play a key role in radical scavenging, whereas the hydroxyl group at the 7-position of the flavonoid A ring had no effect (Rice-Evans *et al*., 1996).

## CONCLUSION

Hyperoside and isoquercitrin were identified as the compounds responsible for the antioxidant activity of *Camellia sasanqua* leaves based on DPPH radical-scavenging, β-carotene bleaching and reducing power assays. This antioxidant activity can be used as a source of natural antioxidants.

## ACKNOWLEDGMENTS

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