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tities with near-infrared and infrared spectroscopic analyses, oxygen-reduction potential of the solutions with a ORP meter, and then molecular weight distribution by GPC-HPLC analyses. There have been observed a large difference among these kaki-tannin samples.

P663 Immunological effects of tannins through dendritic cell

Yoshimura M[1], Akiyama H[2], Kondo K[2], Sakata K[2], Matsuoka H[2], Amakura Y[1], Teshima R[2], Yoshida T[1]
[1]Matsuyama University, College of Pharmaceutical Sciences, 4 - 2 Bunkyo-cho, Matsuyama, Ehime 790 - 8578, Japan;
[2]National Institute of Health Sciences, 1 - 18 - 1 Kamiyoga, Setagaya-ku, Tokyo 158 - 8501, Japan

Ellagitannins widely distributed in medicinal plants have been demonstrated to exhibit diverse biological activities including host-mediated antitumor and immunomodulatory anti-leishmanial effects, which are associated with macrophage activation. In the present study, we examined the immunological effect of oenothein B (**1**), a macrocyclic ellagitannin dimer with potent antioxidative and antimicrobial activities, on a human dendritic cell (DC) which is one of the important antigen presenting cells. The effect of **1** on DCs was assessed by flow-cytometric analysis and morphological observation using a microscopy comparing with those of (-)-epigallocatechin gallate (EGCG, a green tea tannin). The production of cytokines in LPS stimulated DCs was also analyzed using the ELISA. EGCG and **1** induced the apoptosis of DCs and showed a significant down-regulation for the expression of cell surface molecules, CD1a and CD83, suggesting the inhibition against the maturation of DCs. Oenothein B also suppressed remarkably the production of inflammatory cytokines such as IL-1β and IL-6 in dose-dependent manner. These data may partly be able to explain the traditional use of tannin-containing medicinal plants for treatment of a variety of inflammatory diseases.

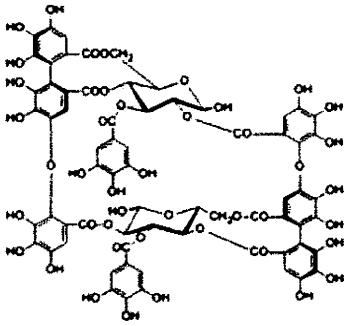

Fig. 1: Oenothein B (**1**)

P664 Polyphenols in myrtaceous plant: Polyphenolic compounds in clove and pimento and their antioxidative activities

Yoshida T, Yoshimura M, Amakura Y
Matsuyama University, College of Pharmaceutical Sciences, 4 - 2 Bunkyo-cho, Matsuyama, Ehime 790 - 8578, Japan

In our continuing study on natural polyphenolic compounds from the myrtaceous plants [1], we herein isolated and characterized two new polyphenolic glucosides (**1** and **2**) from clove (*Syzygium aromaticum*) and pimento (*Pimenta dioica*). Compound **1** was isolated together with 15 known compounds (5 hydrolysable tannins and 10 related polyphenols) from flower buds of *S. aromaticum*, and its structure was characterized as 6'-*O*-acetylisobiflorin. Structure of compound **2**, obtained from berries of *P. dioica* along with 14 known compounds (3 hydrolysable tannins and 11 related polyphenols) was elucidated as (2*S*)-3-(4-hydroxy-3-methoxyphenyl)-propane-1,2-diol 1-*O*-(6'-*O*-galloyl)-β-D-glucoside on the basis of spectral analysis (NMR, MS and $[\alpha]_D$) and chemical conversions. Additionally, antioxidative activity of their extracts and the isolated compounds was estimated by using ORAC (oxygen radical absorbance capacity) assay. Among them, eugenol (**3**) showed the most potent antioxidative activity [ORAC value: 39,270 μmol TE (= trolox equivalent)/g].

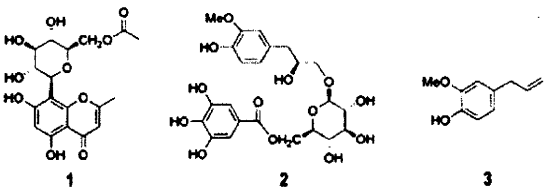

Fig. 1

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P665 Evaluation of the antioxydant capacity of grapes, pomaces and wines and their correlation with its phenolic constituents for five mediterrenean wines varieties

Ky I, Kolbas N, Teissedre P
Institut des Sciences de la Vigne et du Vin, 210 chemin de Leysotte CS 50008, 33882 Villenave d'Ornon Cedex, France

Grapes and wines are a particularly rich source of phenolic compounds which can act as antioxydants and lead to some health benefits such as a chemopreventive [1] role toward cardiovascular and degenerative diseases, including neurodegenerative pathologies and cancer. In fact, grape pomace, considered as an industrial waste, have also displayed strong antioxidant proprieties [2] and could furnish useful products as food additives. The most important factors affecting the polyphenol contents in grapes, pomaces and wines are the grapes varieties, vineyard location and wine-making process. Thus, different grape, pomace and wine from five important Vallé-du-Rhône's cultivar of red wine grape (Carignan, Counoise, Grenache, Mourvèdre and Syrah) were fully exploited. The aim of this study was to evaluate the correlation between antioxidant potential and total phenol content of grape, pomace and wine from each variety. The antioxidant potential of each sample was determined by different antioxidant test (ABTS, DPPH, FRAP SOD, ORAC) wheras the phenolic profile and content was established by HPLC-UV-Fluo in order to determine relationship between antioxidant activity and grape variety. Moreover, ratio of initial phenolic compounds from grape to wine and waste in pomace was also estimated. **References:** 1. Halpern et al. 1998, Journal of International Medical Research, 26(4), 171 - 180. 2. Torres et al. 2002 Journal of Agricultural and Food Chemistry, 50, 7548 - 7555.

P666 Oak wood ellagitannins influence on the organoleptic perception of red wine

Michel J, Jourdes M, Giordanengo T, Mourey N, Teissedre P
UMR1219-Institue des Sciences de la Vigne et du Vin, 210, chemin de Leysotte, 33882 Villenave d'Ornon Cedex, France

Some wood substances such as ellagitannins can be extracted during wine ageing in oak barrels. Recently, there has been an increasing interest in ellagitannins because they have been implicated in numerous biological properties, including antioxidant, anticancer, anti-inflammatory, antibacterial, and anti-HIV replication activities. [1,2,3] The impact of ellagitannins concentration on the organoleptic perception of red wine is still be under investigation. [4] In our research, we classified staves according to their ellagitannins level using a NIRS online procedure (Oakscan®) [5] and were able to correlate the NIRS classification with the level of ellagitannins estimated by HPLC-UV. The different types of staves were added to red wine during its ageing and the extraction and evolution of the main ellagitannins was monitored by HPLC-UV. The influence of ellagitannins levels on wine perception was estimated by a trained judge's panel. It appears that the staves classification estimated by NIRS procedure on wood was in good agreement with the total level ellagitannins extracted by organic solvents as well as the level of ellagitannins quantified in red wines aged during 4 months with the classified staves. The level of ellagitannins was estimated by quantification of ellagic acid released during hydrolysis as well as by quantification of each specific ellagitannins: vescalagin, castalagin, grandinin, roburins (A, B, C, D, E) by HPLC-UV-MS. The level of ellagitannins in red wine seems to have an impact on the roundness of the wines. Moreover, it appears that astringency and bitterness were not negatively impacted by the level of ellagitannins in wine. **References:** 1. Quideau, S., (2009), Chemistry and biology of ellagitannins: An underestimated class of

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Variational analysis of marker constituents and antioxidative potencies by preparation methods of natural antioxidants as food additives

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Yoshiaki Amakura, Morio Yoshimura, Ayako Yoshimura, Takashi Yoshida

College of Pharmaceutical Sciences, Matsuyama University

Abstract

In order to propose effective preparation methods for natural antioxidant food additives, variations in the marker constituents and antioxidative potencies of extracts depending on different extraction methods were estimated. Several extracts were prepared using different solvents from the leaves of *Salvia officinalis* (Sage), fruits of *Foeniculum vulgare* (Fennel), and *Houttuynia cordata* (Dokudami) which are the raw materials of natural food additives. Their antioxidative potencies and marker ingredients were estimated based on the oxygen radical absorbance capacity (ORAC) and HPLC analysis. Among the various extracts prepared from sage, the 50% aqueous ethanolic (EtOH) extract, which contained rosmarinic acid as the major component, showed high antioxidative potency. The EtOH, aqueous EtOH and other extracts of fennel showed antioxidative activities weaker than the other tested materials. The fraction including a volatile oil of fennel showed weak antioxidative activity, therefore it was suggested that the active constituents were contained in the polar fractions. Syringin (sinapyl alcohol 4-*O*-β-D-glucoside) and quercetin 3-*O*-β-D-glucuronide were identified as the major constituents of the fennel extract. In the dokudami extracts, the 50% aqueous EtOH extract showed a high antioxidative potency, which was suggested to be responsible for the quercitrin (quercetin 3-*O*-α-L-rhamnoside) of the major constituent.

Keywords : Food additive, antioxidant agent, *Houttuynia cordata*, *Salvia officinalis*, *Foeniculum vulgare*

I Introduction

Most existing food additives,[1] which are officially registered based on the Food Sanitation Act in Japan, are natural extracts containing various ingredients. Existing food additives are those in which effective components are not always defined due to poor characterization of the ingredients in the respective raw materials. In addition, improvement of the preparation methods is still an important issue to ensure their reliability and scientific evidence. The food additives are prepared according to the procedures described in the list of the existing food additives.[2] In many cases, a couple of procedures are described for one food additive. For example, the antioxidant extracts of sage can be prepared by "extraction from the leaves of *Salvia officinalis* Linne with water, ethanol (EtOH), or hexane". It is strongly suggested that the ingredients extracted by these methods should show a different effectiveness, while scientific evidence for such a distinction has been lacking until now. Investigation of the appropriate preparation methods of food additives should facilitate their proper use. In addition, sufficient chemical analyses of the food additives should contribute to their safety assessment. Based on this situation, we have already investigated the ingredients in the antioxidant extracts of the food additive, "eucalyptus leaf extract", and reported the significant difference in the antioxidative activity by changing the extraction solvent.[3] Among the "existing food additives", we prepared several extracts from three raw materials [sage (sage extract), fennel (essential oil-removed fennel extract), and dokudami (dokudami extract)] using the preparation methods described in the list, and assessed and analyzed their antioxidative potencies and ingredient distributions. The antioxidative activity was evaluated on the basis of oxygen radical absorbance capacity (ORAC) which has been widely

Corresponding author: Yoshiaki Amakura, College of Pharmaceutical Sciences, Matsuyama University,
4-2 Bunkyo-cho, Matsuyama, Ehime 790-8578, Japan

accepted as a tool for antioxidant assessment.[4-6]

Ⅱ Materials and Methods

1. Samples and reagents

Sage (from the leaves of *Salvia officinalis*) and fennel (from the fruits of *Foeniculum vulgare*) were obtained from Nagaoka Perfumery Co., Ltd. (Osaka, Japan). Dokudami (from aerial parts of *Houttuynia cordata*) was purchased from Uchida Wakanyaku (Tokyo, Japan). Column chromatography was conducted using a Diaion HP-20 (Mitsubishi Chemical, Tokyo, Japan), MCI GEL CHP20P (75–150 μm) (Mitsubishi Chemical), Toyopearl HW-40 (fine grade) (Tosoh, Tokyo, Japan), and YMC GEL ODS-AQ (AQ12S50) (YMC, Kyoto, Japan). Anethole and *p*-anisaldehyde were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals were of analytical reagent grade.

2. Instrumentation

The ^{1}H- and ^{13}C-NMR spectra were recorded on a Brucker AVANCE500 instrument (Bruker BioSpin, Billerica, MA) (500 MHz for ^{1}H and 126 MHz for ^{13}C), and the chemical shifts are given in ppm values relative to those of the solvents [dimethyl sulfoxide-d_6 (DMSO-d_6), methanol-d_4 (MeOH-d_4) and deuterium oxide (D_2O)] on a tetramethylsilane scale. The standard pulse sequences programmed for the instrument (Avance 500) were used for the 2D measurements (COSY, HSQC, and HMBC). J_{CH} was set at 8 or 10 Hz in the HMBC. Electrospray ionization (ESI)-MS spectra were obtained using a micrOTOF-Q (Bruker Daltonics, Billerica, MA) mass spectrometer using acetonitrile as the solvent. HPLC was carried out using a Shimadzu Prominence system (Shimadzu, Kyoto, Japan) or TOSOH UV-8010 and DP-8020 (Tosoh).

The reversed-phase (RP) HPLC conditions were as follows: column, L-column ODS (5 μm, 2.1 I.D. × 150 mm) (Chemicals Evaluation and Research Institute, Tokyo, Japan); mobile phase, solvent A was 5% acetic acid and solvent B was acetonitrile (0-30 min, 0-50% B in A; 30-35 min, 50-85% B in A; 35-40 min, 85% B in A; 40-50 min, 85-90%; 50-55 min, 90-100% B in A; 55-60 min, 100% B); column temperature, 40°C; flow rate, 0.3 mL/min; detection, 200-400 nm. The preparative RP-HPLC conditions were as follows: column, YMC-Pack ODS (5 μm, 10 I.D. × 150 mm) (YMC); mobile phase, solvent A was 5% acetic acid and solvent B was acetonitrile (0-30 min, 0-50% B in A; 30-35 min, 50-85% B in A; 35-40 min, 85% B in A; 40-50 min, 85-90%; 50-55 min, 90-100% B in A; 55-60 min, 100% B); column temperature, 40°C; flow rate, 2.0 mL/min; detection, 200-400 nm. The normal-phase HPLC conditions were as follows: column, column, YMC-Pack SIL A-002 (5 μm, 150 × 4.6 mm i.d.) (YMC, Kyoto, Japan); mobile phase, *n*-hexane-MeOH-tetrahydrofuran-formic acid (55:33:11:1) and oxalic acid 450 mg/L; column temperature, room temperature; flow rate, 1.5 mL/min; detection, 280 nm. An absorbance microplate reader, SpectraMax M2 multimode reader (Molecular Devices, Sunnycale, CA), was used.

3. Extraction and isolation

3.1 Sage

The preparations of the extracts as analytical samples were as follows: The EtOH, 50% aqueous EtOH, *n*-hexane and water extracts were prepared by extracting the dried leaves of sage (2 g) with each solvent (40 mL) by sonication for 20 min at room temperature, and each extract was then submitted for HPLC analysis. The isolation·procedure was as follows: Sage (450 g) was homogenized in 80% EtOH (EtOH–H_2O 8:2) (7 L), and a concentrated solution (*ca.* 1 L) was successively extracted with *n*-hexane (2 L) and ethyl acetate (2 L) to give the respective *n*-hexane (16 g), ethyl acetate (9.7 g), and water (45 g) extracts. The ethyl acetate (1 g) and water (2 g) extracts were separately chromatographed over Diaion HP-20, MCI-GEL CHP-20P, Toyopearl HW-40 and YMC GEL ODS-AQ with aqueous MeOH to give vicenin-2 (1)[7] (4 mg), luteolin 7-*O*-β-D-glucuronide (2)[8] (6 mg), scutellarein (3)[9] (5 mg), rosmarinic acid (4)[10] (95 mg), cirsmaritin (5)[11] (4 mg), and salvigenin (6)[12] (5 mg). These compounds were identified by comparison of their spectral data with those reported in the literature. NMR and MS data of the compounds were as follows:

Vicenin-2 (Apigenin 6,8-di-*C*-β-D-glucoside) (1): ^{1}H-NMR (DMSO-d_6) δ 7.97 (2H, d, *J*=8, H-2', 6'), 6.90 (2H, d, *J*=8, H-3', 5'), 6.61 (1H, s, H-3), 4.90-4.75 (2H, m, Glc H-1, 1'), 4.00-3.00 (10H, Glc H-2-6, 2'-6'). ^{13}C-NMR δ 182.5 (C-4), 164.0 (C-2), 163.0 (C-7), 161.5 (C-4'), 159.7 (C-5), 155.5 (C-9), 129.0 (C-2', 6'), 122.0 (C-1'), 116.3 (C-3', 5'), 109.0 (C-6), 104.8 (C-10), 104.6 (C-8), 103.0 (C-3), 82.0, 81.5 (Glc C-5, 5'), 78.9, 78.8 (Glc C-3, 3'), 74.4, 73.5 (Glc C-1, 1'), 71.5, 71.3 (Glc C-2, 2'), 70.0, 69.9 (Glc C-4, 4'), 61.1, 61.0 (Glc C-6, 6'). ESI-MS *m/z* 593 $[M-H]^-$.

Luteolin 7-*O*-β-D-glucuronide (2): ^{1}H-NMR (DMSO-d_6) δ 7.38 (1H, dd, *J*=2.5, 8.5, H-6'), 7.35 (1H, d, *J*=2.5, H-2'), 6.76 (1H, dd, *J*=2.5, 8.5, H-5'), 6.74 (1H, *J*=2, H-8), 6.40 (1H, d, *J*=2, H-6), 5.00 (1H, d, *J*=7.5, GlcUA H-1), 3.50 (1H, d, *J*=9.5, GlcUA H-5), 3.28 (1H, t, *J*=9.5, GlcUA H-3), 3.25 (1H, brt, *J*=9.5, GlcUA H-2), 3.18 (1H, brt, *J*=9.5, GlcUA H-4). ^{13}C-NMR δ 181.6 (C-4), 171.6 (GlcUA C-6), 164.8 (C-2), 162.9 (C-7), 161.0 (C-5), 156.9 (C-9), 147.4 (C-4'), 146.4 (C-3'), 119.4 (C-1), 119.4 (C-6'), 115.9 (C-5'), 112.5 (C-2'), 105.1 (C-10), 102.1 (C-3), 99.7 (GlcUA C-1), 99.5 (C-8), 94.5 (C-6), 76.6 (GlcUA C-3), 73.6 (GlcUA C-5), 73.0 (GlcUA C-2), 72.0 (GlcUA C-4). ESI-MS *m/z* 461 $[M-H]^-$.

Scutellarein (6-Hydroxy apigenin) (3): ^{1}H-NMR (DMSO-d_6) δ 7.91 (2H, d, *J*=9, H-2', 6'), 6.95 (2H, d, *J*=9, H-3', 5'), 6.92, 6.80 (each 1H, s, H-3, 8). ^{13}C-NMR δ 181.6 (C-4), 163.5 (C-2), 161.0 (C-4'), 153.3 (C-9), 149.6 (C-7), 147.0 (C-5), 129.0 (C-6), 128.3 (C-2', 6'), 121.5 (C-1'), 116.2 (C-3', 5'), 104.0 (C-10), 102.2 (C-3), 93.8 (C-8). ESI-MS *m/z* 285 [M–H]$^-$.

Rosmarinic acid (4): ^{1}H-NMR (CD_3OD) δ 7.55 (1H, d, *J*=15.5, H-7), 7.03 (1H, d, *J*=1.5, H-2), 6.93 (1H, dd, *J*=2, 8, H-6), 6.75 (1H, d, *J*=2, H-2'), 6.70 (1H, d, *J*=8, H-5'), 6.61 (1H, dd, *J*=2, 8, H-6'), 6.24 (1H, d, *J*=15.5, H-8), 5.19 (1H, dd, *J*=4, 8, H-8'), 3.10, (1H, dd, *J*=8, 14.5, H-7'), 3.01 (1H, dd, *J*=4, 14.5, H-7'). ^{13}C-NMR δ 173.5 (C-9'), 168.4 (C-9), 149.6 (C-4), 147.7 (C-7), 146.7 (C-3), 146.0 (C-3'), 145.1 (C-4'), 129.3 (C-1'), 127.6 (C-1), 123.1 (C-6), 121.8 (C-6'), 117.6 (C-2'), 116.5 (C-5'), 116.3 (C-5), 115.2 (C-2), 114.4 (C-8), 74.7 (C-8'), 37.8 (C-7'). ESI-MS *m/z* 359 [M–H]$^-$.

Cirsimaritin (5,4'-Dihydroxy-6,7-dimethoxyflavone) (5): ^{1}H-NMR (DMSO-d_6) δ 7.96 (2H, d, *J*=9, H-2', 6'), 6.94 (2H, d, *J*=9, H-3', 5'), 6.93, 6.85 (each 1H, s, H-3, 8), 3.93, 3.74 (each 3H, s, -OMe). ^{13}C-NMR δ 182.2 (C-4), 164.1 (C-2), 161.3 (C-4'), 158.63 (C-7), 152.6 (C-9), 152.3 (C-5), 128.5 (C-2', 6'), 121.0 (C-1'), 116.0 (C-3', 5'), 105.1 (C-10), 102.7 (C-3), 91.8 (C-8), 60.0, 56.4 (each 3H, -OMe). ESI-MS *m/z* 313 [M–H]$^-$.

Salvigenin (5-Hydroxy-6,7,4'-trimethoxyflavone) (6): ^{1}H-NMR ($CDCl_3$) δ 7.84 (2H, d, *J*=9, H-2', 6'), 7.04 (2H, d, *J*=9, H-3', 5'), 6.60, 6.55 (each 1H, s, H-3, 8), 3.97, 3.93, 3.90 (each 3H, -OMe). ^{13}C-NMR δ 182.9 (C-4), 164.5 (C-2), 163.0 (C-4'), 159.1 (C-7), 153.4 (2C, C-5, 9), 131.9 (C-6), 128.5 (2C, C-2', 6'), 123.9 (C-1'), 115.0 (C-3', 5'), 106.5 (C-10), 104.6 (C-3), 90.8 (C-8), 61.3, 56.8, 55.5 (each 3H, -OMe). ESI-MS *m/z* 327 [M–H]$^-$.

3.2 Fennel

Extracts for the analytical samples were prepared as follows: The EtOH and 50% aqueous EtOH extracts were prepared by extracting fennel (2 g) with each solvent (40 mL) by sonication for 20 min at room temperature. Steam distillation of the fennel (500 g) produced on essential oil (4.6 g) and oil-free extracts (38.3 g). Each extract was submitted for HPLC analysis.

Components of the fennel were isolated by the following large-scale experiment: The fruits of the fennel (400 g) were homogenized in 80% EtOH (EtOH–H_2O 8:2) (4 L), and a concentrated solution (*ca.* 0.5 L) was successively extracted with *n*-hexane and ethyl acetate (3 L each) to give the respective *n*-hexane (12.9 g), ethyl acetate (1.8 g), and water (32 g) extracts. The *n*-hexane (2 g), ethyl acetate (1 g), and water (10 g) extracts were separately subjected to a combination of chromatography over Diaion HP-20, MCI-GEL CHP-20P, Toyopearl HW-40 and YMC GEL ODS-AQ with aqueous MeOH to give 3-*O*-caffeoylquinic acid (chlorogenic acid) (7)[13, 14] (32 mg), sinapyl alcohol 4,3'-di-*O*-β-D-glucoside (8)[15, 16] (18 mg), syringin (sinapyl alcohol 4-*O*-β-D-glucoside) (9)[15] (126 mg), quercetin 3-*O*-β-D-glucuronide (10)[17] (54 mg), *p*-anisaldehyde (11)[18] (11 mg), and anethole (12)[19] (125 mg). NMR and MS data of these compounds were as follows:

3-*O*-Caffeoylquinic acid (Chlorogenic acid) (7): ^{1}H-NMR (CD_3OD) δ 7.55 (1H, d, *J*=16, H-7'), 7.04 (1H, d, *J*=2, H-2'), 6.95 (1H, dd, *J*=2, 8, H-6'), 6.77 (1H, d, *J*=8, H-5'), 6.25 (1H, d, *J*=16, H-8'), 5.33 (1H, m, H-3), 4.26 (1H, m, H-5), 3.71 (1H, dd, *J*=3, 8.5, H-4), 1.98-2.20 (4H, m, H-2, 6). ESI-MS *m/z* 353 [M–H]$^-$.

Sinapyl alcohol 4,3'-di-*O*-β-D-glucoside (8): ^{1}H-NMR (CD_3OD) δ 6.76 (2H, s, H-2, 6), 6.60 (1H, brd, *J*=16, H-1'), 6.32 (1H, dt, *J*=5.5, 16, H-2'), 4.86 (1H, d, *J*=7.5, Glc H-1), 4.45, 4.29 (each 1H, m, H-3'), 4.36 (1H, *d*, J=8, Glc H-1'), 3.86 (6H, s, -OMe), 3.78 (2H, dd, *J*=1.5, 12, Glc H-6, 6'), 3.66 (2H, dd, *J*=5, 12, Glc H-6, 6'), 3.50-3.20 (8H, Glc H-2-5, 2'-5'). ^{13}C-NMR δ 154.4 (2C, C-3, 5), 136.0 (C-4), 135.0 (C-1), 133.4 (C-1'), 126.8 (C-2'), 105.6 (2C, C-2, 6), 105.3 (Glc C-1), 103.5 (Glc C-1'), 78.3, 77.8 (2C), 75.7, 75.4, 75.1, 71.4, 71.3 (Glc C-2-5, 2'-5'), 62.5 (Glc C-6, 6'), 57.1 (-OMe). ESI-MS *m/z* 533 [M–H]$^-$.

Syringin (sinapyl alcohol 4-*O*-β-D-glucoside) (9): ^{1}H-NMR (CD_3OD) δ 6.74 (2H, s, H-2, 6), 6.55 (1H, brd, *J*=15.5, H-1'), 6.32 (1H, dt, *J*=5.5, 15.5, H-2'), 4.86 (1H, d, *J*=7.5, Glc H-1), 4.21 (2H, dd, *J*=1.5, 5.5, H-3'), 3.85 (6H, s, -OMe), 3.78 (1H, dd, *J*=1.5, 12, Glc H-6), 3.66 (1H, dd, *J*=5, 12, Glc H-6), 3.48 (1H, dd, *J*=7.5, 9, Glc H-2), 3.41 (2H, m, Glc H-3, 4). ^{13}C-NMR δ 154.4 (2C, C-3, 5), 135.9 (C-4), 135.3 (C-1), 131.3 (C-1'), 130.0 (C-2'), 105.5 (2C, C-2, 6), 105.3 (Glc C-1), 78.3 (Glc C-5), 77.8 (Glc C-3), 75.7 (Glc C-2), 71.3 (Glc C-4), 63.6 (C-3'), 62.6 (Glc C-6), 57.0 (-OMe). ESI-MS *m/z* 371 [M–H]$^-$.

Quercetin 3-*O*-β-D-glucuronide (10): ^{1}H-NMR (CD_3OD) δ 7.72 (1H, d, *J*=2.5, H-2'), 7.58 (1H, dd, *J*=2.5, 8.5, H-6'), 6.84 (1H, d, *J*=8.5, H-5'), 6.38 (1H, *J*=2, H-8), 6.19 (1H, d, *J*=2, H-6), 5.33 (1H, d, *J*=7.5, GlcUA H-1), 3.70 (1H, d, *J*=9.5, GlcUA H-5), 3.58 (1H, t, *J*=9.5, GlcUA H-4), 3.52 (1H, t, *J*=9.5, GlcUA H-3), 3.48 (1H, dd, *J*=7.5, 9.5, GlcUA H-2). ^{13}C-NMR δ 179.2 (C-4), 172.1 (C-6'), 166.0 (C-7), 163.0 (C-5), 159.0 (C-2), 158.5 (C-9), 150.0 (C-4'), 145.9 (C-3'), 136.0 (C-3), 123.2 (C-6'), 122.8 (C-1'), 117.6 (C-2'), 116.1 (C-5'), 105.6 (C-10), 104.4 (GlcUA C-1), 100.0 (C-6), 94.8 (C-8), 77.8 (GlcUA C-3, 5), 75.5 (GlcUA C-2), 73.1 (GlcUA C-4). ESI-MS *m/z* 477 [M–H]$^-$.

p-Anisaldehyde (11): ^{1}H-NMR ($CDCl_3$) δ 9.81 (1H, s, H-7), 7.80 (2H, d, *J*=8.5, H-2, 6), 6.99 (2H, d, *J*=8.5, H-3, 5), 3.84 (3H, s, -OMe). ^{13}C-NMR δ 190.5 (CHO), 165.2 (C-1), 131.0 (2C, C-3, 5), 129.5 (C-4), 114.2 (2C, C-2, 6), 55.2 (-OMe).

Anethole (12): ^{1}H-NMR ($CDCl_3$) δ 7.25 (2H, d, *J*=8.5, H-2, 6), 6.84 (2H, d, *J*=8.5, H-3, 5), 6.35 (1H, dd, *J*=1.5, 15, H-7), 6.10 (1H, *J*=6, 15, H-8), 3.79 (3H, s, -OMe), 1.88 (3H, dd, *J*=1.5, 6, -Me). 13C-NMR δ 158.6 (C-1), 130.8 (C-4), 130.4 (C-7), 126.8 (2C, C-3, 5), 123.5 (C-8), 113.8 (C-2, 6), 55.2 (-OMe), 18.2 (-Me).

3.3 Dokudami

The EtOH and 50% aqueous EtOH extracts were prepared by extracting the leaves of dokudami (2 g) with each solvent (40 mL) by sonication for 20 min at room temperature. In a separate experiment, the aerial parts of dokudami (450 g) were homogenized in 80% EtOH (EtOH–H_2O 8:2) (4 L), and a concentrated solution (*ca.* 0.5 L) was successively extracted with *n*-hexane (1.5 L) and ethyl acetate (1.5 L) to give the respective *n*-hexane (3.5 g), ethyl acetate (5.7 g), and water (25.6 g) extracts. A part of the ethyl acetate (1 g) and water (5 g) extracts were separately chromatographed over Diaion HP-20, MCI-GEL CHP-20P, Toyopearl HW-40, and YMC GEL ODS-AQ with aqueous MeOH and preparative HPLC to afford 5-*O*-caffeoylquinic acid (neochlorogenic acid) (13)[13, 14] (15 mg), 3-*O*-caffeoylquinic acid (chlorogenic acid) (7)[13, 14] (20 mg), 4-*O*-caffeoylquinic acid (cryptochlorogenic acid) (14)[13, 14] (5 mg), quercetin 3-*O*-α-L-rhamnoside 7-*O*-β-D-glucoside[19] (15) (12 mg), rutin (16)[20] (4 mg), hyperin (quercetin 3-*O*-β-D-galactoside) (17)[21] (5 mg), isoquercitrin (quercetin 3-*O*-β-D-glucoside) (18)[21] (6 mg), quercitrin (quercetin 3-*O*-α-L-rhamnoside) (19)[22] (55 mg), kaempferol 3-*O*-α-L-rhamnoside (20)[22] (5 mg), and quercetin (21)[21] (2 mg). NMR and MS data of these compounds were as follows:

5-*O*-Caffeoylquinic acid (Neochlorogenic acid) (13): ^{1}H-NMR (CD_3OD) δ 7.58 (1H, d, *J*=16, H-7'), 7.05 (1H, d, *J*=2, H-2'), 6.93 (1H, dd, *J*=2, 8, H-6'), 6.75 (1H, d, *J*=8, H-5'), 6.29 (1H, d, *J*=16, H-8'), 5.30 (1H, m, H-5), 4.12 (1H, m, H-3), 3.60 (1H, m, H-4), 1.89-2.20 (4H, m, H-2, 6). ^{13}C-NMR δ 178.3 (C-7), 169.0 (C-9'), 149.4 (C-4'), 146.8 (C-7'), 146.8 (C-3'), 127.9 (C-1'), 122.9 (C-6'), 116.4 (C-5'), 115.8 (C-8'), 115.1 (C-2'), 75.4 (C-1), 74.8 (C-4), 73.0 (C-3), 68.3 (C-5), 41.5 (C-6), 38.7 (C-2). ESI-MS *m/z* 353 [M–H]$^-$.

4-*O*-Caffeoylquinic acid (Cryptochlorogenic acid) (14): ^{1}H-NMR (CD_3OD) δ 7.63 (1H, d, *J*=16, H-7'), 7.05 (1H, d, *J*=2, H-2'), 6.96 (1H, dd, *J*=2, 8, H-6'), 6.77 (1H, d, *J*=8, H-5'), 6.35 (1H, d, *J*=16, H-8'), 4.79 (1H, dd, *J*=3, 9, H-4), 4.27 (2H, m, H-3, 5), 2.20 (1H, ddd, *J*=3, 5, 13, H-6), 2.17 (1H, dd, *J*=4, 14, H-2), 2.06 (1H, ddd, *J*=3, 4, 14, H-2), 2.00 (1H, ddd, *J*=11, 13, H-6). ^{13}C-NMR δ 177.6 (C-7), 169.0 (C-9'), 149.6 (C-4'), 147.1 (C-7'), 146.8 (C-3'), 127.9 (C-1'), 123.0 (C-6'), 116.5 (C-5'), 115.4 (C-8'), 115.2 (C-2'), 79.3 (C-4), 76.7 (C-1), 69.6 (C-3), 65.6 (C-5), 42.6 (C-6), 38.6 (C-2). ESI-MS *m/z* 353 [M–H]$^-$.

Quercetin 3-*O*-α-L-rhamnoside 7-*O*-β-D-glucoside (15): ^{1}H-NMR (CD_3OD) δ 7.36 (1H, d, *J*=2, H-2'), 7.33 (1H, dd, *J*=2, 8.5, H-6'), 6.90 (1H, d, *J*=8.5, H-5'), 6.73 (1H, *J*=2, H-8), 6.48 (1H, d, *J*=2, H-6), 5.37 (1H, d, *J*=1.5, Rha H-1), 5.05 (1H, d, *J*=7.5, Glc H-1), 4.21 (1H, m, Rha H-2), 3.92 (1H, dd, *J*=2, 12, Glc H-6), 3.75 (1H, dd, *J*=3.5, 9.5, Rha H-3), 3.72-3.35 (8H, m, Rha H-3- 5, Glc H-2-6), 0.94 (3H, d, *J*=6.5, Rha H-6). ^{13}C-NMR δ 179.9 (C-4), 164.7 (C-7), 162.9 (C-5), 159.9 (C-2), 158.1 (C-9), 150.0 (C-4'), 146.5 (C-3'), 136.5 (C-3), 123.0 (C-6'), 122.8 (C-1'), 117.0 (C-2'), 116.4 (C-5'), 107.7 (C-10), 103.5 (Rha C-1), 101.6 (Glc C-1), 100.8 (C-6), 95.8 (C-8), 78.4 (Glc C-5), 77.9, 74.7, 73.3, 72.1, 72.0, 71.9, 71.3 (Rha C-2-5, Glc C-2-4), 62.5 (Glc C-6), 17.7 (Rha C-6). ESI-MS *m/z* 609 [M–H]$^-$.

Rutin (Quercetin 3-*O*-α-L-rhamnosyl-(1→6)-β-D-glucoside) (16): ^{1}H-NMR [DMSO-d_6+D_2O (*ca.* 1%)] δ 7.54 (1H, dd, *J*=2, 8, H-6'), 7.52 (1H, d, *J*=2, H-2'), 6.84 (1H, d, *J*=8.5, H-5'), 6.39 (1H, *J*=2, H-8), 6.20 (1H, d, *J*=2, H-6), 5.33 (1H, d, *J*=7.5, Glc H-1), 4.38 (1H, brs, Rha H-1), 3.71-3.05 (10H, m, Glc H-2-6, Rha H-2-5), 0.98 (3H, *J*=6.5, Rha H-6). ^{13}C-NMR δ 177.4 (C-4), 164.1 (C-7), 161.2 (C-5), 156.5 (C-2, 9), 148.4 (C-4'), 144.7 (C-3'), 133.3 (C-3), 121.9 (C-6'), 121.2 (C-1'), 116.3 (C-2'), 115.2 (C-5'), 104.0 (C-10), 101.2 (Glc C-1), 100.7 (Rha H-1), 98.7 (C-6), 93.6 (C-8), 76.4 (Glc C-3), 75.9 (Glc C-5), 74.1 (Glc C-2), 71.8 (Rha C-4), 70.6 (Rha C-3), 70.4 (Rha C-2), 70.0 (Glc C-4), 68.2 (Rha C-5), 67.0 (Glc C-6), 17.7 (Rha C-6). ESI-MS *m/z* 609 [M–H]$^-$.

Hyperin (Quercetin 3-*O*-β-D-galactoside) (17): ^{1}H-NMR (CD_3OD) δ 7.82 (1H, d, *J*=2, H-2'), 7.58 (1H, dd, *J*=2, 8.5, H-6'), 6.84 (1H, d, *J*=8.5, H-5'), 6.39 (1H, *J*=2, H-8), 6.19 (1H, d, *J*=2, H-6), 5.14 (1H, d, *J*=8, Gal H-1), 3.84 (1H, d, *J*=2.5, Gal H-4), 3.80 (1H, dd, *J*=8, 9.5, Gal H-2), 3.63 (1H, dd, *J*=6, 11.5, Gal H-6), 3.53-3.56 (2H, m, Gal H-3, 6), 3.46 (1H, t, *J*=6.5, Gal H-5). ^{13}C-NMR δ 177.4 (C-4), 164.1 (C-7), 161.2 (C-5), 156.2 (C-2, 9), 148.4 (C-4'), 144.7 (C-3'), 133.5 (C-3), 121.9 (C-6'), 121.0 (C-1'), 115.9 (C-5'), 115.1 (C-2'), 103.9 (C-10), 101.8 (Gal C-1), 98.6 (C-6), 93.4 (C-8), 75.8 (Gal C-5), 73.2 (Gal C-3), 71.2 (Gal C-2), 67.9 (Gal C-4), 60.1 (Gal C-6). ESI-MS *m/z* 463 [M–H]$^-$.

Isoquercitrin (Quercetin 3-*O*-β-D-glucoside) (18): ^{1}H-NMR (CD_3OD) δ 7.69 (1H, d, *J*=2, H-2'), 7.57 (1H, dd, *J*=2, 8.5, H-6'), 6.85 (1H, d, *J*=8.5, H-5'), 6.37 (1H, *J*=2, H-8), 6.19 (1H, d, *J*=2, H-6), 5.22 (1H, d, *J*=8, Glc H-1), 3.70 (1H, d, *J*=5.5, Glc H-6), 3.56 (1H, dd, *J*=5.5, 12, Glc H-6), 3.47, 3.41 (each 1H, t, *J*=10, Glc H-3, 4), 3.33 (1H, dd, *J*=7.5, 10, Glc H-2), 3.21 (1H, m, Glc H-5). ESI-MS *m/z* 463 [M–H]$^-$.

Quercitrin (Quercetin 3-*O*-α-L-rhamnoside) (19): ^{1}H-NMR (CD_3OD) δ 7.32 (1H, d, *J*=2, H-2'), 7.29 (1H, dd, *J*=2, 8.5, H-6'), 6.89 (1H, d, *J*=8.5, H-5'), 6.34 (1H, *J*=2, H-8), 6.17 (1H, d, *J*=2, H-6), 5.34 (1H, d, *J*=1.5, Rha H-1), 4.21 (1H, m, Rha H-2), 3.74 (1H, dd, *J*=3.5, 9.5, Rha H-3), 3.42-3.30 (2H, m, Rha H-4, 5), 0.93 (3H, d, *J*=6.5, Rha H-6). ESI-MS *m/z* 447 [M–H]$^-$.

Kaempferol 3-*O*-α-L-rhamnoside (20): ^{1}H-NMR (CD_3OD) δ 7.76 (2H, d, *J*=8.5, H-2', 6'), 6.93 (2H, d, *J*=8.5, H-3', 5'), 6.36 (1H, *J*=2, H-8), 6.20 (1H, d, *J*=2, H-6), 5.37 (1H, d, *J*=1.5, Rha H-1), 4.21 (1H, m, Rha H-2), 3.69 (1H, dd, *J*=3.5, 9.5, Rha H-3), 3.42-3.30 (2H, m, Rha H-4, 5), 0.92 (3H, d, *J*=6.5, Rha H-6). ESI-MS *m/z* 431 [M–H]$^-$.

Quercetin (21): ^{1}H-NMR (CD_3OD) δ 7.65 (1H, d, *J*=2, H-2'), 7.62 (1H, dd, *J*=2, 8.5, H-6'), 6.87 (1H, d, *J*=8.5, H-5'), 6.37 (1H, *J*=2, H-8), 6.17 (1H, d, *J*=2, H-6). ESI-MS *m/z* 301 [M–H]$^-$.

4. Antioxidative assay

The antioxidant activity was estimated by the ORAC method.[3] Briefly, the ORAC assay was performed in 75 mM phosphate buffer (pH 7.4) with a final reaction volume of 200 μL. Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) (Sigma-Aldrich) (20 μL) and fluorescein (Sigma-Aldrich) (120 μL; final concentration 70 nM) solutions were pipetted into each well of a 96-well microplate. The mixture was pre-incubated in a microplate reader for 15 min at 37°C. A solution of 2,2'-azobis(2-amidinopropane) dihydrochloride (Wako, Osaka, Japan) (60 μL: final concentration, 12 mM) was rapidly added to the microplate, and after shaking for 15 s, the fluorescence was recorded every min for 90 min at the excitation and emission wavelengths of 485 and 528 nm, respectively. The area under the curve (AUC) was determined, and the net AUC was calculated by subtracting the AUC of the blank (phosphate buffer only) from that of the sample. The ORAC values were expressed as trolox equivalents (μmol TE/g) using the calibration curve generated in each assay.

Ⅲ Results and Discussion

1. Sage

The HPLC chromatograms of the EtOH, 50% EtOH, *n*-hexane, and water extracts of sage are shown in Fig. 1 (a-d). To characterize the HPLC peaks of these extracts, the 80% EtOH extract was prepared and fractionated into *n*-hexane-, ethyl acetate-, and water-soluble fractions, which are shown in Fig. 1 (e-h). After separation and purification of each fraction, six compounds in total were identified based on the spectral data (MS and NMR). They are vicenin-2 (1), luteolin 7-*O*-β-D-glucoside (2), scutellarein (3), rosmarinic acid (4), cirsmaritin (5), and salvigenin (6).

The structural formulas of these compounds are shown in Fig. 2. Based on an HPLC comparison of the isolated standards, the main peaks at the retention times of around 22 and 24 minutes on the HPLC chromatograms of the 50% EtOH and water extracts were identified as 2, 3, and 4 [Fig. 1 (a, d)]. These peaks were also detected for the ethanol extract, although at lower levels than those in the other two extracts (50% EtOH and water extracts). On the other hand, these peaks were not detected in the *n*-hexane extract [Fig. 1 (c)].

To compare the difference among the antioxidative activity depending on the extraction conditions, the radical scavenging activities of the EtOH, 50% aqueous EtOH, *n*-hexane, and water extracts were assessed using the ORAC values as indicators. As shown in Fig. 3 (a), the 50% aqueous EtOH extract was demonstrated to exhibit the highest antioxidative activity, and next was the water extract. The antioxidative potency of the 50% aqueous EtOH extract was 2-fold higher

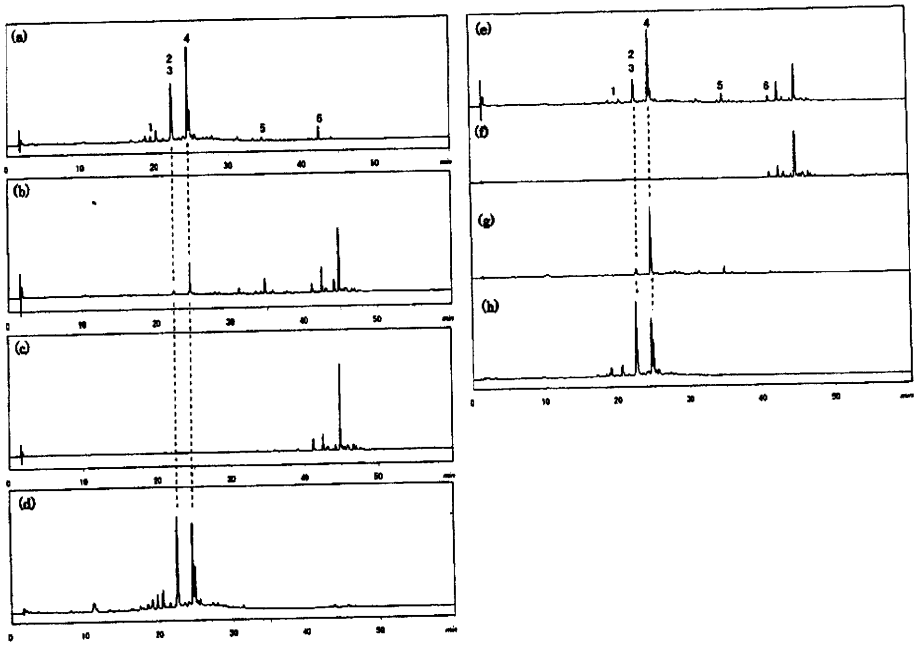

Fig. 1. RP-HPLC profiles of sage extracts

a, 50% EtOH extract; b, EtOH extract; c, *n*-Hexane extract; d, Water extract. e, 80%EtOH extract; f, 80%EtOH extract-*n*-Hexane fraction; g, 80%EtOH extract-Ethyl acetate fraction; h, 80%EtOH extract-Water fraction.

1, vicenin-2; 2, luteolin 7-*O*-β-D-glucuronide; 3, scutellarein; 4, rosmarinic acid; 5, cirsmaritin; 6, salvigenin.

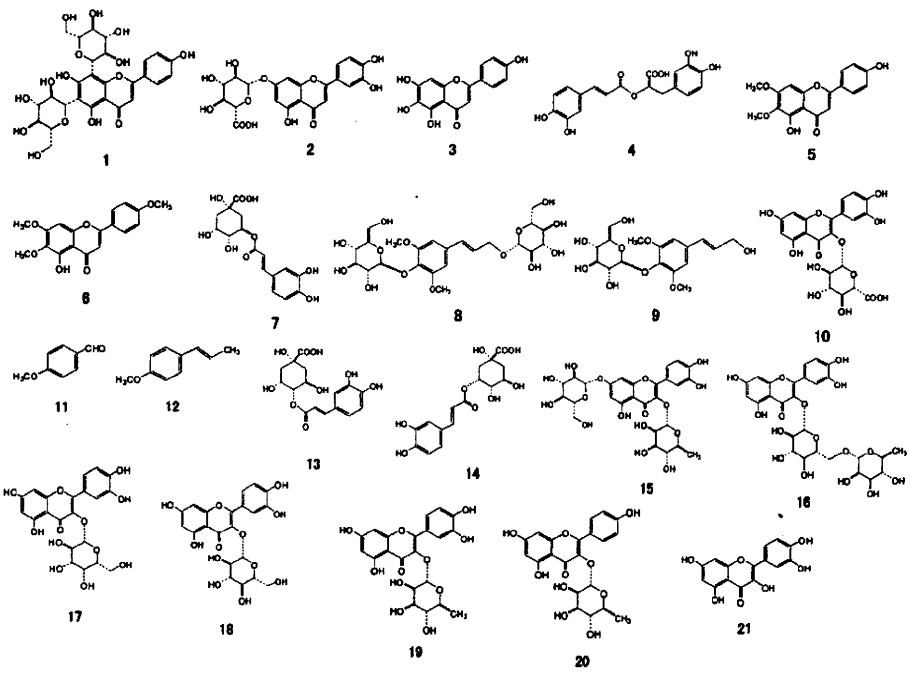

Fig. 2. Structures of compounds 1-21

than that of the EtOH extract. Among the ORAC values for the *n*-hexane, ethyl acetate, and water fractions of 80% EtOH extract [Fig. 1 (e-h)], the ethyl acetate fraction had the highest antioxidative activity, and next was the water fraction.

Rosmarinic acid (4) was detected as the main ingredient in both HPLC chromatograms of the 50% aqueous EtOH and water extracts with the higher antioxidative activity, suggesting its contribution to the activity. Actually, the ORAC value of 4 was more potent than epigallocatechin gallate (EGCG), which is a typical tea catechin, as shown in Fig. 4. This finding is in agreement with the previous report on 4 as an antioxidative ingredient of sage.[22] In addition to 4, flavonoids, which are well demonstrated as antioxidants[22] were also contained in these active extracts. On the other hand, the *n*-hexane fraction, which indicated the presence of diterpenes, such as carnosol and carnosic acid, and the absence of 4, had a low antioxidative activity.

2. Fennel

The main HPLC peaks of the 50% aqueous EtOH and EtOH extracts, steam distillate and aqueous fraction of the steam-distillation of fennel were assigned by comparison with the HPLC of the components obtained from large-scale experiments of the 80% ethanol extracts, as shown in Fig. 5 (a-d). Figure 5 (e-h) shows the HPLC chromatograms of the 80% EtOH extract and its *n*-hexane and ethyl acetate, and water soluble-fractions used for the separation and purification of the components. The structures of the isolated compounds [chlorogenic acid (7), sinapyl alcohol 4,3'-di-*O*-β-D-glucoside

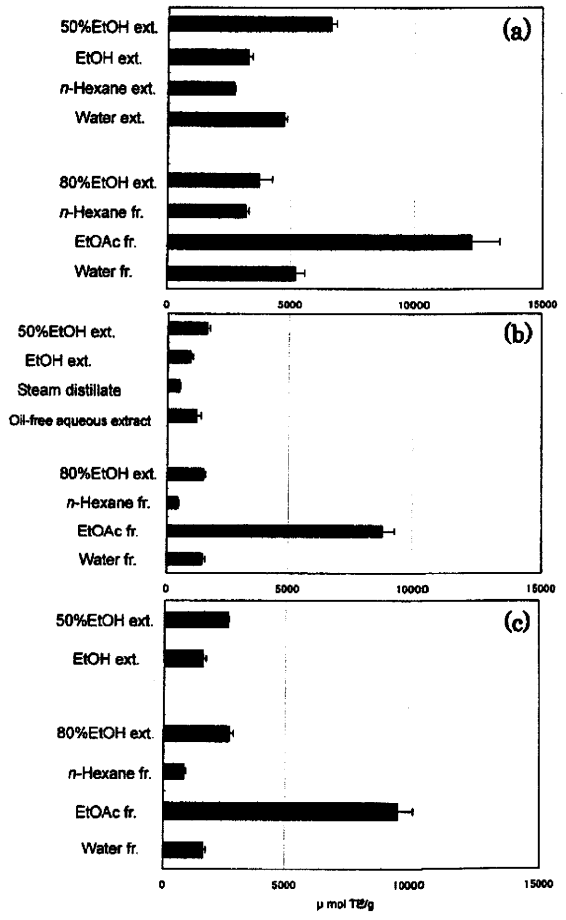

Fig. 3. ORAC values of each extract and fraction
a, sage; b, fennel; c, dokudami. TE, Trolox equivalent.

(8), syringin (9), quercetin 3-*O*-β-D-glucuronide (10), *p*-anisaldehyde (11), and anethole (12)] are shown in Fig. 2.

As shown in Fig. 3 (b), the fennel extracts exhibited low antioxidative activity. Of the four extracts, the 50% aqueous EtOH extract and oil-free aqueous extracts showed a more potent antioxidative activity than the other extracts. These active extracts all showed the presence of 8, 9, and 10 as shown in their HPLC chromatograms, indicating that these components should contribute to the antioxidative activity.

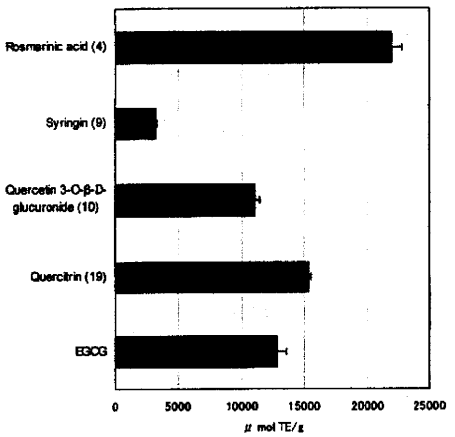

Fig. 4. ORAC values of identified compounds
EGCG, epigallocatechin gallate

Actually, when comparing the antioxidative activity among the fractions (*n*-hexane, ethyl acetate, and water fractions) of the 80% EtOH extract, the weakest activity was exhibited by the *n*-hexane fraction, in which 9 and 10 were not detected in HPLC [Fig. 5 (f)]. Although 9 was previously reported[24] as a main antioxidative ingredient of fennel, its ORAC value was lower than that of 10 (Fig. 4).

Thus, the extraction efficiencies for the main antioxidative ingredients of fennel can be improved by preparing an extract using a water-containing solvent. The steam distillate showed almost negligible activity. This study also supported the validity of the "essential oil-removed fennel extract" as an existing food additive.

3. Dokudami

Analytical samples of dokudami (*Houttuynia cordata*) for evaluation of the proper extracting conditions were prepared using two solvents, i.e., EtOH and 50% aqueous EtOH. Their HPLCs are shown in Fig. 6 (a, b). The standard samples for characterization of the HPLC peaks were obtained as described in section II-3.3. The 10 isolated compounds [chlorogenic acid (7), 4-*O*-caffeoylquinic acid (13), 5-*O*-caffeoylquinic acid (14), quercetin 3-*O*-α-L-rhamnoside 7-*O*-β-D-glucoside (15), rutin (16), hyperin (17), isoquercitrin (18), quercitrin (19), kaempferol 3-*O*-α-L-rhamnoside (20), and quercetin (21)] are shown in Fig. 2. The main peak in each HPLC was identified

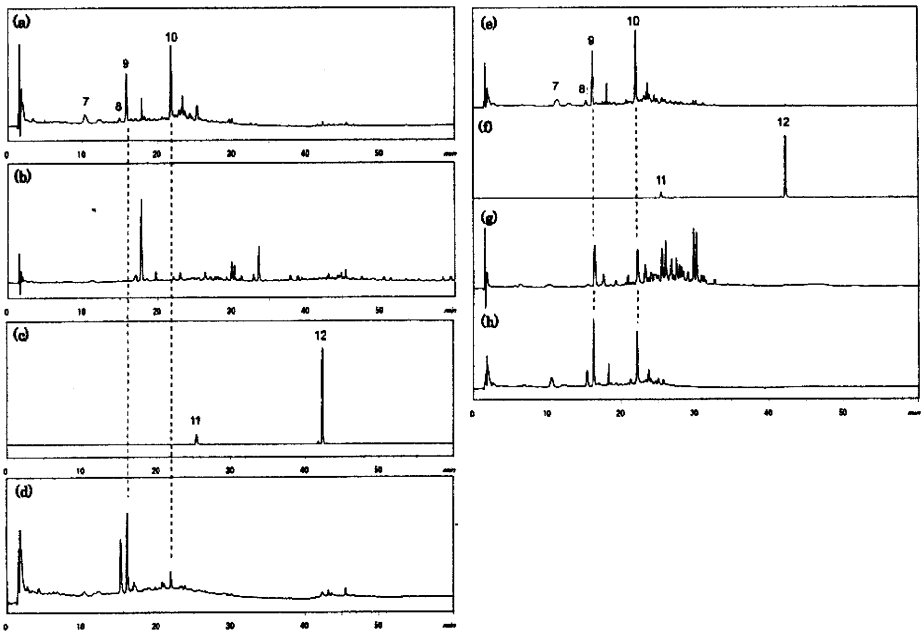

Fig. 5. RP-HPLC profiles of fennel extracts
a, 50% EtOH extract; b, EtOH extract; c, Steam distillate; d, Oil-removed extract; e, 80%EtOH extract; f, 80%EtOH extract-*n*-Hexane fraction; g, 80%EtOH extract-Ethyl acetate fraction; h, 80%EtOH extract-Water fraction.
7, chlorogenic acid; 8, sinapyl alcohol 4,3'-di-*O*-β-D-glucoside; 9, syringin; 10, quercetin 3-*O*-β-D-glucuronide; 11, *p*-anisaldehydes; 12, anethole.

as 19 [Fig. 6 (a, b)]. In addition, the other commonly detected peaks were assigned to the flavonols, 15, 16, 17, 18, 20, and 21. The caffeic acid derivatives 7, 13, and 14 were detected in the 50% aqueous EtOH extract at a higher level. This result reflects the fact that these compounds are extremely soluble in water as revealed in the HPLC of the water fraction of the 80% EtOH extract fractions [Fig. 6 (c-f)].

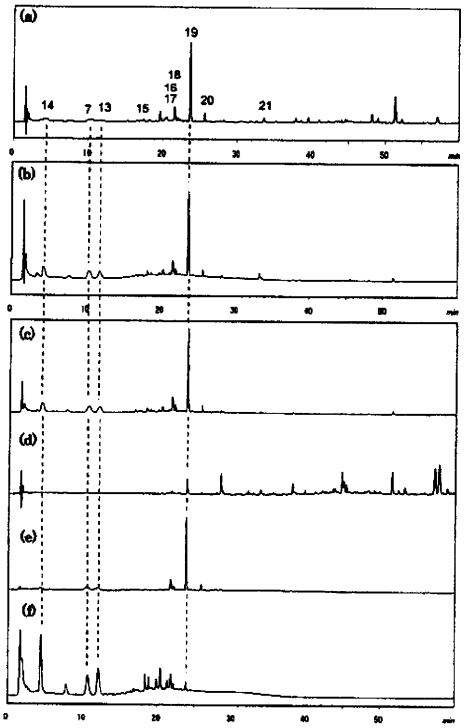

Fig. 6. RP-HPLC profiles of dokudami extracts
a, EtOH extract; b, 50%EtOH extract; c, 80%EtOH extract; d, 80%EtOH extract-*n*-Hexane fraction; e, 80%EtOH extract-Ethyl acetate fraction; f, 80%EtOH extraction-Water fraction.
7, chlorogenic acid; 13, 4-*O*-caffeoylquinic acid; 14, 5-*O*-caffeoylquinic acid; 15, quercetin 3-*O*-α-L-rhamnoside 7-*O*-β-D-glucoside; 16, rutin; 17, hyperin; 18, isoquercitrin; 19, quercitrin; 20, kaempferol 3-*O*-α-L-rhamnoside; 21, quercetin.

The EtOH and 50% aqueous EtOH extracts showed moderate antioxidative activities as shown in Fig. 3 (c), and the potency of the latter was about 1.5-fold higher than the former. To clarify the relationship between the polarity of the ingredients and activity, the ORAC values were similarly measured for the *n*-hexane-, ethyl acetate-, and water-soluble fractions obtained by partition of the 80% ethanol extract. As a result, a remarkable activity was exhibited by the ethyl acetate fraction which contained 19 as the main component [Fig.6 (e)]. This is consistent with a high ORAC value for 19 (Fig. 4). Thus 19 was suggested to be a useful marker for this antioxidant additive, as previously reported.[25)]

Ⅳ Conclusion

We examined the variation in the antioxidative activity and ingredient composition based on the different preparation methods among three materials (fennel, sage, and dokudami) which are registered as official natural antioxidant food additives. In the case of sage, the highest antioxidative activity was exhibited by the 50% EtOH extract containing rosmarinic acid (4) as the main ingredient.

In fennel, the antioxidative efficacy of three extracts, the EtOH and 50% aqueous EtOH extracts, and steam distillate was compared to that of the oil-removed fennel extract which is officially defined as the existing food additive in the list. Among the extracts, the 50% EtOH extract showed a higher antioxidative activity than the other extracts, and syringin (9) and quercetin 3-*O*-β-D-glucuronide (10) were assignable to the marker contributors. On the other hand, the antioxidative activity of the essential oil-removed fennel extract also displayed a similar activity, that substantiated its validity for being listed as an existing food additives. The main ingredients of the essential oil-removed extract were 9 and sinapyl alcohol 4,3'-di-*O*-β-D-glucoside (8).

As for dokudami, the 50% EtOH extract similarly showed a higher antioxidative activity than the EtOH extract. This extract contained quercitrin (19) as the main ingredient together with other minor flavonols including isoquercitrin (18). As these flavonols are well demonstrated as natural antioxidants, it was suggested that 19, but not 18 should be defined as the active ingredients for this antioxidant food additive.

The data obtained in this study might be useful for improving in the preparation of these three natural food additives and characterization of their antioxidative marker substances.

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論　　文

既存添加物酸化防止剤の製法による抗酸化能および主要成分の変動解析

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天倉吉章、好村守生、吉村文子、吉田隆志

松山大学薬学部

キーワード：食品添加物、酸化防止剤、ドクダミ、セージ、ウイキョウ

概　要

天然酸化防止剤の効果的な調製法を提案する目的で、数製法により調製した抽出物について主要成分と抗酸化能の変動解析を実施した。まず、添加物原料であるセージの葉（セージ抽出物）、ウイキョウの果実（精油除去ウイキョウ抽出物）およびドクダミ（ドクダミ抽出物）について数種の抽出物を調製し、各抽出物の抗酸化能（ORAC）とHPLC分析を行った。その結果、セージ抽出物については，50%エタノール抽出物が高抗酸化活性を示した。抽出物の抗酸化活性画分の含有成分を分析したところ、rosmarinic acidが主成分および有効成分として同定された。ウイキョウ抽出物については、全体的に抗酸化能は顕著ではなかった。精油を含む画分は抗酸化活性をほとんど示さず、極性画分に有効成分の含有が示唆されたため成分分析をしたところ、syringin (sinapyl alcohol 4-*O*-β-D-glucoside) およびquercetin 3-*O*-β-D-glucuronideが主成分として検出され、それらが高い抗酸化能を示すことを明らかにした。ドクダミ抽出物については、強い抗酸化性エキスを得るには、50%エタノール抽出法が適していること、およびその活性評価のためのマーカーとしては、quercitrin (quercetin 3-*O*-α-L-rhamnoside) が最適であることを明らかにした。

連絡先：〒790-8578　愛媛県松山市文京区4-2　松山大学薬学部　天倉吉章