NATURAL PRODUCT COMMUNICATIONS

An International Journal for Communications and Reviews Covering all Aspects of Natural Products Research
Evaluation of Anti-glycation Activities of Phlorotannins in Human and Bovine Serum Albumin-glyceraldehyde Models

Shingo Sugiura$^{a,d}$, Ryosuke Taniguchi$^{a,d}$, Yoshihiko Nishioka$^b$, Ryota Iwase$^b$, Reiji Tanaka$^{a,d,f}$, Hideo Miyake$^{a,d,f}$, Tetsushi Morii$^{a,d,f}$, Mitsuyoshi Ueda$^{a,d,f}$ and Toshiyuki Shibata$^{a,d,f}$

$^a$Graduate School of Bioresources, Mie University, 1577 Kurimamachiya-cho, Tsu, Mie 514-8507, Japan
$^b$Faculty of Bioresources, Mie University, 1577 Kurimamachiya-cho, Tsu, Mie 514-8507, Japan
$^c$Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, 2-24-16 Naka-cho, Koganei, Tokyo 184-8588, Japan
$^d$Seaweed Biorefinery Research Center, Mie University, 1577 Kurimamachiya-cho, Tsu, Mie 514-8507, Japan
$^e$Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwake, Sakyo-ku, Kyoto 606-8502, Japan
$^f$Japan Science and Technology Agency, CREST, 4-1-8 Hon-cho, Kawaguchi, Saitama 332-0012, Japan

Received: June 1st, 2018; Accepted: June 22nd, 2018

The anti-glycation activities of phlorotannins contained in the Japanese Lessoniaceae (Ecklonia cava, Eck. kurome, Eck. stolonifera, Eisenia arborea, and Eis. bicyclis) were tested using serum albumin-glyceraldehyde (GA) models. In the human serum albumin (HSA)-GA model and the bovine serum albumin (BSA)-GA model, the concentrations of crude phlorotannins at 50% inhibition (IC50) of fluorescent advanced glycation end products (AGEs) formation was in the range of 0.48 to 0.70 mg/mL and 0.52 to 0.75 mg/mL, respectively. In tests using phloroglucinol and purified phlorotannins (eckol, fucofuroeckol A, phlorofucofuroeckol A, dieckol, and 8,8´-bieckol), dieckol had the highest inhibitory activity (IC50: 5.5 x 10^7 µM) against fluorescent AGEs formation in HSA-GA model and showed about 18 times inhibition compared with aminoguanidine hydrochloride of positive control. In the BSA albumin model, 8,8´-bieckol had about 27 times AGEs formation inhibitory activity (IC50: 6.2 x 10^7 µM) against aminoguanidine hydrochloride. In tests on GA scavenging activity, it was shown that compounds with phloroglucinol tetramer or higher had a scavenging rate of 70%, or more, with a reaction time of 120 minutes. These results suggest that among the phlorotannins, in particular the dimers of eckol (dieckol and 8,8´-bieckol), there are effective compounds for inhibiting the formation of AGEs derived from GA.

Keywords: Advanced glycation end products, Anti-glycation, 8,8´-Bieckol, Glyceraldehyde, Dieckol, Phlorotannins, Lessoniaceae.

Advanced glycation end products (AGEs) are a general term for structures generated by nonenzymatic reactions between proteins and reducing sugars such as glucose and fructose [1,2]. In previous studies [3-6], it has been clarified that AGEs are produced not only from the reducing sugars but also from sugar metabolic intermediates and intermediates of Maillard reactions. It is known that dicarbonyl compounds (methylglyoxal, glyoxal, and 3-deoxyglucosone) generated from autoxidation, and degradation products of glucose, have higher blood concentrations in diabetic patients than in healthy subjects [7,8]. In addition, it has been considered that the dicarbonyl compounds have high reactivity with proteins because there are two carbonyl groups in the molecule. From this scientific background, the relationship between AGEs derived from the dicarbonyl compounds and lifestyle-related diseases has been drawing attention. In recent years, according to Takeuchi et al.’s report [9], it was revealed that α-hydroxy aldehydes such as glyceraldehyde and glucose and glycolaldehyde are more reactive with proteins than dicarbonyl compounds. Among AGEs generated in vivo, it has been reported that AGEs derived from GA (GA-AGEs) accelerate intracellular oxidative stress through its binding to its receptor, and can cause strong cytotoxicity [10-12]. It is also pointed out that the GA-AGEs are involved in the onset and progression of diabetic vascular complications [9,13,14], Alzheimer’s disease [9,15,16], nonalcoholic steatohepatitis [9,17,18], hypertension [9,14], and cancer [9,19]. Therefore, suppression of GA-AGEs formation and scavenging of GA can be regarded as effective for prevention and treatment of these diseases.

In a preceding report [20], we isolated phlorotannins (eckol, fucofuroeckol A, phlorofucofuroeckol A, dieckol, and 8,8´-bieckol) (Figure 1) from Japanese Lessoniaceae (Ecklonia cava, Eck. kurome, Eck. stolonifera, Eisenia arborea, and Eis. bicyclis) and evaluated their anti-glycation properties in the serum albumin-methyglyoxal models. In the report [20], we clarified the following two facts: (1) phlorofucofuroeckol A and fucofuroeckol A with a benzobisbenzofuran skeleton have inhibitory activities far superior to aminoguanidine hydrochloride against the formation of fluorescent AGEs, and (2) eckols (eckol, dieckol, and 8,8´-bieckol) have higher methyglyoxal scavenging activity than aminoguanidine.
glycation activity. Since the IC₅₀ values of aminoguanidine are not clinically applied because it has several positive control in studies in the search for compounds having anti-glycation activity that inhibits the formation of AGEs and suppresses crosslinking and polymerization of proteins in vitro [22]. Aminoguanidine is not clinically applied because it has several adverse side effects on humans, but it is frequently used as a positive control in studies in the search for compounds having anti-glycation activity. Since the IC₅₀ values of aminoguanidine hydrochloride obtained in this study were 1.10 mg/mL in HSA-GA model and 1.93 mg/mL in BSA-GA model, it was found that the crude phlorotannins of *Eiks bicyclis* has anti-glycation activity of about 2.3 times and 3.7 times with respect to aminoguanidine hydrochloride. Currently, *Eck. kurome* is cultivated as a supply source of phlorotannins in Kumamoto prefecture, Japan [23]. The crude phlorotannins were prepared from both naturally occurring and cultured versions of *Eck. kurome*, and their inhibitory activities on the formation of fluorescent AGEs were evaluated. As shown in Table 1, IC₅₀ values of crude phlorotannins from cultured *Eck. kurome* in each model were almost the same as those of the natural plants of *Eck. kurome*. Therefore, as with the natural plants of Lessoniaceae, it was confirmed that crude phlorotannins of cultured *Eck. kurome* can be utilized as a natural product having an inhibitory effect against AGEs formation.

*Table 1: IC₅₀ values of crude phlorotannins from Lessoniaceae against fluorescent AGEs formation.*

<table>
<thead>
<tr>
<th>Algae</th>
<th>Specific area of origin</th>
<th>HSA-GA (mg/mL)</th>
<th>BSA-GA (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eck. cava</td>
<td>Mie</td>
<td>0.70</td>
<td>0.55</td>
</tr>
<tr>
<td>Eck. kurome</td>
<td>Fukuoka</td>
<td>0.58</td>
<td>0.55</td>
</tr>
<tr>
<td>Eck. kuro</td>
<td>Kumamoto</td>
<td>0.61</td>
<td>0.59</td>
</tr>
<tr>
<td>Cultured Eck. kurome</td>
<td>Kumamoto</td>
<td>0.52</td>
<td>0.58</td>
</tr>
<tr>
<td>Eck. stolonifera</td>
<td>Yanaguchi</td>
<td>0.54</td>
<td>0.56</td>
</tr>
<tr>
<td>Eis. arborae</td>
<td>Mie</td>
<td>0.51</td>
<td>0.61</td>
</tr>
<tr>
<td>Eis. bicyclis</td>
<td>Fukuoka</td>
<td>0.48</td>
<td>0.52</td>
</tr>
</tbody>
</table>

All the data are expressed as the mean of three independent measurements. The IC₅₀ values of aminoguanidine hydrochloride were 1.10 mg/mL in the HSA-GA model and 1.93 mg/mL in the BSA-GA model.

*Table 2: IC₅₀ values of phloroglucinol and isolated phlorotannins against fluorescent AGEs formation.*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>HSA-GA (µM)</th>
<th>BSA-GA (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phloroglucinol</td>
<td>3.8 x 10⁻⁴</td>
<td>3.8 x 10⁻⁴</td>
</tr>
<tr>
<td>Eckol</td>
<td>1.1 x 10⁻⁴</td>
<td>1.3 x 10⁻⁴</td>
</tr>
<tr>
<td>Fucofuroeckol A</td>
<td>7.4 x 10⁻⁵</td>
<td>1.4 x 10⁻⁵</td>
</tr>
<tr>
<td>Phlorofucofuroeckol A</td>
<td>7.3 x 10⁻⁵</td>
<td>1.4 x 10⁻⁵</td>
</tr>
<tr>
<td>Dieckol</td>
<td>5.5 x 10⁻⁵</td>
<td>8.7 x 10⁻⁵</td>
</tr>
<tr>
<td>8,8'-dieckol</td>
<td>5.7 x 10⁻⁵</td>
<td>6.2 x 10⁻⁵</td>
</tr>
</tbody>
</table>

All the data are expressed as the mean of three independent measurements. The IC₅₀ values of aminoguanidine hydrochloride were 1.0 x 10⁻⁵ µM in the HSA-GA model and 1.7 x 10⁻⁵ µM in the BSA-GA model.

In order to further analyze the inhibitory activity of phlorotannins against fluorescent AGEs formation in the albumin-GA models, tests were carried out using phloroglucinol and five kinds of isolated compounds (eckol, fucofuroeckol A, phlorofucofuroeckol A, dieckol, and 8,8'-dieckol). Similar to the results in the albumin-methylglyoxal models obtained in the preceding report [20], phloroglucinol and the isolated eckols inhibited the formation of fluorescent AGEs in a concentration-dependent manner in both serum albumin-AGEs models (data not shown). As a result of calculating the IC₅₀ value, dieckol and 8,8'-dieckol showed the effective activities in both models (Table 2). In the HSA-GA model, the IC₅₀ value of dieckol was 5.5 x 10⁻⁵ µM (Table 2), which was found to have about 18 times activity compared with aminoguanidine hydrochloride. The IC₅₀ value of 8,8'-dieckol obtained in the BSA-GA model was 6.2 x 10⁻⁵ µM (Table 2), and it had about 27 times inhibitory activity with respect to aminoguanidine hydrochloride. Even compounds with the lowest inhibition of AGEs also had activity about 9.1 times (eckol) in the HSA-GA model and about 12 times (fucofuroeckol A and phlorofucofuroeckol A) in the BSA-GA model as compared with aminoguanidine hydrochloride. Therefore, it was strongly suggested that the phlorotannins contained in Lessoniaceae, in particular dimers of eckol (dieckol and 8,8'-dieckol) have very excellent inhibitory activity on the formation of AGEs derived from GA.

GA is thought to be caused by three pathways: the glycolytic pathway, the polyol pathway, and the fructose metabolic pathway in vivo [24]. It has also been shown that GA-AGEs are produced more rapidly in vivo than other AGEs such as AGEs derived from glucose and AGEs derived from methylglyoxal [4,25,26]. Therefore, it is considered that the scavenging of GA may contribute to the reduction of glycation stress. As a result of experiments at a concentration of 5 mg/mL, each type of crude phlorotannins prepared from the five kinds of Lessoniaceae scavenged GA over time (data not shown). The GA scavenging rate at the reaction time of 120 minutes was in the range of 62.3% (Eck. stolonifera) to 78.2% (Eck. cava). Furthermore, GA scavenging activity was measured for phloroglucinol and five kinds of eckols, and the data obtained are shown in Figure 2. Except for eckol, the tested compounds scavenged GA is a roughly linear manner over time (Figure 2). The GA scavenging rate at a reaction time of 120 minutes was 63.8% for phloroglucinol, 58.1% for eckol, 77.3% for fucofuroeckol A, 70.0% for phlorofucofuroeckol A, 73.9% for dieckol, 75.0% for 8,8'-dieckol, and that of aminoguanidine hydrochloride was 86.2% (Figure 2). Although the GA scavenging activity of tested compounds was lower than aminoguanidine hydrochloride, it was revealed that compounds having phloroglucinol tetramer or higher had a scavenging activity of approximately 70%, or more.
proliferation, and bioavailability. Using phloroglucinol and the isolated compounds, their effect on the growth of HeLa (cancer cell line) and 3T3-L1 (normal cell line) was evaluated by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay (Figure 3). As a result of adding each compound to the medium at a concentration of 1 to 100 μg/mL, the survival rate of both cell lines was approximately 85%, or more (Figure 3). As a result of testing at concentrations of 50 and 100 μg/mL, each compound showed inhibitory activity in the range of about 10 to 20% against formation of fluorescent AGEs. Therefore, it was suggested that phloroglucinol and phlorotannins may not exhibit cytotoxicity at concentrations that exert anti-glycation properties.

To our knowledge, there is no report on the evaluation of the anti-glycation properties of plant extracts or natural products in the serum albumin-GA models. The Lessoniaceae used in this study are suggested that phloroglucinol and phlorotannins may not exhibit anti-glycation properties if treated with the same algae used as a foodstuff in Japan and Korea. The results obtained in the preceding [20] and present studies strongly support that phlorotannins are superior anti-glycation substances derived from natural plants and that they may contribute to both alleviation of symptoms and prevention of onset of diseases caused by AGEs generated in vivo.

Experimental

Materials: For samples of brown algae (Ecklonia cava Kjellman, Eck. kareome Okamura, Eck. stolonifera Okamura, Eisenia arborea Areschoug, and Eis. bicyclus Kjellman), the algal plants used in the preceding report [20] were used. The cultured Eck. kareome was purchased from the Fisheries Cooperative Association of Amakusa in Kumamoto Prefecture, Japan, in 2015. The algae used for the extraction of phlorotannins were washed with filtered seawater, air-dried, and pulverized via pulverizing mill (ABS-W, Osaka Chemical). The algal powders were stored at -30°C until use. Aminoguanidine hydrochloride, HSA, and GA were purchased from Wako Pure Chemical Industries. BSA was obtained from Sigma-Aldrich. 1,3-Cyclohexanedione was purchased from Tokyo Chemical Industry. All other reagents used in this study were of analytical grade.

Extraction and purification of phlorotannins: Extraction of phlorotannins from algal powder was prepared according to the method described in the previous report [23]. Phlorotannins were purified by column chromatography and preparative HPLC using the same conditions described in the previous reports [27,28]. Each of the obtained phlorotannins (eckol, fucofuroeckol A, phlorofucofuroeckol A, dieckol, and 8,8´-bieckol) was confirmed to have a purity of 98% or more by three-dimensional HPLC (SPD-M10AV, Shimadzu) with an Inertsil ODS-3 column (4.6 mm i.d. x 250 mm, GL Science) [28]. The identification of the purified phlorotannins was carried out using LC/ESI/MS with the analysis condition reported in the preceding study [23]. The purified phlorotannins were stored at -30°C until used as samples.

Serum albumin-GA assay: Sample, GA (400 mM), and albumin (HSA or BSA, 20 mg/mL) solutions were prepared separately by dissolving in a 100 mM phosphate buffer (pH 7.4). The measurement was carried out using a 96-well black plate (FLUOTRAC600, Greiner) and a microplate reader (Infinite 200, Tecan). The sample solution (40 µL), GA solution (10 µL), and albumin solution (50 µL) were added to the well in the plate. Measurement was carried out with fluorescence intensity at an excitation of 370 nm and an emission of 440 nm, and the obtained value was taken as a control value. After incubation at 37°C for 24 hours, the fluorescence intensities of each well were measured under the same measurement conditions. For blank wells, a 100 mM phosphate buffer was used instead of a sample solution. Aminoguanidine hydrochloride was used as a positive control. The inhibition rate (%) of fluorescent AGEs formation was calculated using the following formula:

Inhibition rate (%) = {1 – [(fluorescence intensity of sample after incubation for 24 hours – fluorescence intensity of control of sample) / (fluorescence intensity of blank after incubation for 24 hours – fluorescence intensity of control of blank)]} x 100.

The IC50 value was calculated from the logarithmic function obtained by plotting the inhibitory rate of fluorescent AGEs formation against the sample concentration.

GA-scavenging assay: The GA-scavenging activity of phlorotannins was measured using a modification of the derivatization method of GA by Usui et al [29]. Twenty-five mM GA was prepared using a 200 mM phosphate buffer (pH 7.4). The crude phlorotannins extracted from each brown algae were dissolved in the phosphate buffer to a concentration of 5 mg/mL. Purified phlorotannins, phloroglucinol, and aminoguanidine hydrochloride were dissolved in the phosphate buffer to a concentration of 25 mM each. 1,3-Cyclohexanedione (0.25 g) was dissolved in a mixture consisting of ammonium acetate (10 g), acetic acid (5 mL), and ultrapure water (50 mL), and it was used as a derivatization reagent. Each sample solution (50 µL) and a GA solution (50 µL) were mixed in well of a 96-well microplate (BioLite, Thermo Scientific) and incubated at 37°C for 30, 60, 90, and 120 minutes, respectively. After incubation, the derivatization solution was measured at 370 nm using a microplate reader (Infinite 200, Tecan). For the blank test, the phosphate buffer was used instead of a GA solution. Aminoguanidine hydrochloride was used as a positive control. The scavenging rate of GA was calculated using the following formula:

Scavenging rate of GA (%) = {1 – [concentration of GA remaining in the reaction solution (mM) / 25]} x 100.

Cytotoxicity assay: MTT (Dojindo) was used as an indicator of cell viability. Briefly, cell lines (HeLa or 3T3-L1) were cultured in 96-
well microplates (BioLite, Thermo Scientific) at a density of 5 x 10^3 cells per well. After 24 hours cultivation in a CO₂ incubator (CPE-2601, Hirasawa) with 5% CO₂ at 37°C, the cell lines were washed with fresh medium (Dulbecco’s modified Eagle’s medium with glucose and pyruvate, containing 10% fetal bovine serum and 1% antibiotic-antimycotic, Gibco) and then treated with each sample solution (10 µL) for 24 hours in the incubator. Sample solutions were prepared by dissolving in Dulbecco’s phosphate buffered saline (DPBS) without calcium and magnesium (Gibco). For blank control wells, DPBS was used instead of a sample solution. The cell lines were then re-washed with the medium, treated with 10% of MTT solution, and cultured for 4 hours in the incubator at 37°C. MTT solution was prepared by dissolving MTT (25 mg) in the following formula:

\[
\text{Cell viability} \% = \left( \frac{\text{absorbance of sample well} - \text{absorbance of blank well}}{\text{absorbance of control well} - \text{absorbance of blank well}} \right) \times 100
\]

Acknowledgments - This work was financially supported by the Japan Science and Technology Agency, CREST.

References

Anti-Melanogenic Effect of Chestnut Spike Extract through Downregulation of Tyrosinase-Related Proteins and Activation of ERK 1/2
Jung-Hee Byeon, Md Badrul Alam, Ki-Chan Kim, Sangsun Heo, Ji-young Lim, Yoon-Gyung Kwon, Peijun Zhao, Yeong-Ho Cha, Hee-jeong Choi and Sang-Han Lee

Analysis of the Volatile Components of Pouteria sapota (Sapote Mamey) Fruit by HS-SPME-GC-MS
Candelario Rodríguez, Armando A. Durant-Archibold, Ana Santana, Enrique Murillo and Carlos M. Franco Abuin

An Analysis of Volatile Components of the Liverworts Dumortiera hirsuta subsp. hirsuta and Dumortiera hirsuta subsp. nepalensis (Dumontieraceae) from Panama and Taxonomic Observations on the Species
Armando A. Durant-Archibold, Noris Salazar Allen, Anette Garrido, Jose Gudino Ledezma and Mahabir P. Gupta

Terpenes and n-Alkanes in Needles of Pinus cembra
Biljana Nikolić, Marina Todosićević, Mihajlo Ratknić, Iris Đorđević, Jovana Stanković, Mirjana Cvetković, PetarD. Marin and Vele Tešević

Morphologic and Essential oil Profiles of Three Species from Asteraceae
Melda Dolarslan and Tugba Gurkok

Composition and Chemical Variability of Needle and Berry Oils from Corsican Juniperus communis var. communis
Joséphine Ottaviani, Ange Bighelli, Joseph Casanova and Félix Tomi

Antifungal and Insecticidal Properties of Juniperus thurifera Leaves
Meryem El Jemli, Naima Khattabi, Khadija Lachger, Driss Touati, Yousra El Jemli, Ilias Marmouzi, El Mahdi Wakrim, Sahia Cherrah and Katim Alaoui

Antimicrobial Activity of two Mentha Species Essential Oil and its Dependence on Different Origin and Chemical Diversity
Mária Pľuchtová, Teresa Gervasi, Qada Benamer, Vito Pellizzeri, Daniela Grufová, Luca Campone, Vincent Sedláček and Nicola Cicero

Seasonal Study of Methyleugenol Chemotype of Ocimum campechianum Essential Oil and Its Fungicidal and Antioxidant Activities

Evaluation of Antipneumonic Effect of Philippine Essential Oils Using Broth Microdilution Volatilization Method and Their Lung Fibroblasts Toxicity

Accounts/Reviews

The Roles of Natural Compounds in Epigenetics
Yanhong Yang, Zuohua Chi, Ruiping Gao and Zili Lei

Secondary Metabolites, Dietary Fiber and Conjugated Fatty Acids as Functional Food Ingredients Against Overweight and Obesity
Kamila Kasprzak, Karolina Wojtunik-Kulesza, Tomasz Oniszczuk, Maciej Kuboń and Anna Oniszczuk

The Sulfated Polysaccharides of Brown Algae and Products of Their Enzymatic Transformation as Potential Vaccine Adjuvants
Tatyana A. Kuznetsova, Elena V. Persiyanova, Svetlana P. Ermakova, Maxim Yu. Khotimchenko and Natalya N. Besednova
Natural Product Communications
2018
Volume 13, Number 8

Contents

Original Paper    Page

Synthesis and Cytotoxic Evaluation of Artemisinin Derivatives Containing an Aminopropanol Group
Le Nhat Thuy Giang, Doan Duy Tien, Dang Thi Tuyet Anh, Nguyen Tien Dung, Ngo Hanh Thuong, Luc Quang Tan,
Nguyen Ha Thanh, Le Thi Tu Anh, Nguyen Van Tuyen and Phan Van Kiem 919

Biotransformation of Bicyclic Sesqui- and Diterpene 1,2-dials and Their Derivatives by the Fungus, Aspergillus niger
Yoshinori Asakawa, Masako Sekita and Toshihiko Hashimoto 923

Synthesis of Ester-linked Taxol-oligosaccharide Conjugate and Its Drug Delivery System Using Bio-nanocapsules and
Hybrid-bio-nanocapsules
Hiroki Hamada, Shouta Okada, Noriyoshi Masuoka, Yuya Fujitaka, Kei Shimoda, Shouta Doi and Katsuhiko Mikuni 933

Monoaminergic Involvement in Decreased Locomotor Activity of Mice Treated with α and β-amyrin from Protium heptaphyllum
Gislei F. Aragão, Manoel O. de Moraes Filho, Paulo N. Bandeira, Antônio P. Frota Junior, Yasmin Ingrid S. de Oliveira,
Claudina F. Alves Balacô and Maria Elisabete A. de Moraes 935

Cytotoxic Evaluation of Compounds Isolated from the Aerial Parts of Hedypotis pilulifera and Methanol Extract of Inonotus obliquus
Hoai Thi Nguyen, Duc Viet Ho, Phu Dinh Quynh Nguyen, Hung Quoc Vo, Thao Thi Do and Ain Raal 939

Production of the Anticancer Compound Withaforin A from Genetically Transformed Hairly Root Cultures of Withania Somnifera
Zeynab Yousefian, Behnaz Hosseini, Hassan Rezadoost, Javier Palazón and Mohammad Hossein Mirjalili 943

New Oxygenated Steroid from the Marine-Derived Fungus Aspergillus flavus
Meng-Yue Yang, Jian-Kun Yang, Jin-Kai Yang, Lian-Dong Hu, Hua-Ji e Z hu and Fei Cao 949

Sulfated Glycosides from the Sea Cucumbers Block Ca2+ Flow in Murine Neuroblastoma Cells
Evgeny A. Pisyagin, Ekaterina S. Menchinskaya, Dmitry L. Aminin, Sergey A. Avilov and Alexandra S. Silchenko 953

New Sesquiterpene Pyridine Alkaloids from Hippocratea excelsa
Megumi Furukawa, Masakatsu Furukawa, Mitsuko Makino, Taketo Uchiyama, Yasuo Fujimoto and Keiichi Matsuzaki 957

Flavonoids from Millettia leucantha and Their Cytotoxicity
Uraiwan Sriphana, Chavi Yenjai, Siriporn Tungnoi, Jongjai Srirapa and Tohptyrooji Hashimoto 961

Inhibitory Effect of Pelargonidin on Secretory Group IIA Phospholipase A2
In-Chul Lee and Jong-Sup Bae 963

Skin Anti-aging Assays of Prouanthocyanadin Rich Red Rice Extract, Oryzanol and Other Phenolic Compounds
Supachai Yodkeeree, Chai Yenjai, Siriporn Tungnoi, Jongjai Srirapa and Aumporn Junsongduang 967

Identification of Plant Origin of Propolis from Thailand Stingless Bees by Comparative Analysis
Eriko Ishizu, Shou Maeda, Shunya Matsuzaki and Mayumi Miseki 973

Leaves of Eugenia brasiliensis Used as a Folk Medicine Contain Cyclooxygenase Enzyme and Lipid Peroxidation Inhibitory
Compounds
Alessandra C. Dametto, Nivaldo Boralle, Chuan-Rui Zhang, Dulce H. S. Silva and Muraleedharan G. Nair 977

Difficulties to Determine the Absolute Configuration of Guaiaretic Acid
Alfredo R. Ortega, Eleuterio Burgueño-Tapia and Pedro Joseph-Nathan 981

Comparison of Chemical Constituents in Magnoliae Officinalis Cortex Processed by “Sweating” and “Non Sweating”
based on Ultra Fast Liquid Chromatography-Triple Quadrupole-Time of Flight Mass Spectrometry and Gas
Chromatography-Triple Quadrupole Mass Spectrometry Combined with Multivariate Statistical Analysis
Hoai Thi Nguyen, Duc Viet Ho, Phu Dinh Quynh Nguyen, Hung Quoc Vo, Thao Thi Do and Ain Raal 987

Potent α-Glucosidase Inhibitors from the Roots of Aruncus sylvestris
Zhang-Peng Li, Meng Que, Wen-Yuan Gao and Yan-Fang Su 993

Cytotoxic Compounds from the Seeds of Sophora alopecuroides
Ping Song, Hao Chen, Zhanqiang Wen, Yibing Lv, Shihao Deng and Xinzhou Yang 997

Antibacterial and Antibiofilm Effects of Zanthoxylum bungeanum Leaves against Staphylococcus aureus
Shi-Yuan Chang, Kai Xiao, Jia-Qi Zhang, Kai Zhong, Elena Grosu, Zhen Gao, Yan-Ping Wu and Hong Gao 1001

Evaluation of Anti-glycation Activities of Phlorotannins in Human and Bovine Serum Albumin-glyceraldehyde Models
Shingo Sugirua, Ryosuke Taniguchi, Yoshihiko Nishioka, Ryota Iwase, Reiji Tanaka, Hideo Miyake, Tetsushi Mori,
Mitsuyoshi Ueda and Toshiyuki Shibata 1007

Stereoselective Total Synthesis of 1,4-Dideoxy-1,4-imino-L-ribitol by an Intramolecular Ring Opening of Epoxide with a
Tethered Amide
Dhudmal Chaya N, Dhanraj O Biradar, Maddipatla V. Satyanarayana and Basi V Subba Reddy 1011

Impact of Melittin on Microalgae Cell Wall: A Monolayer Study
Magda Vargas-Perez, Gerardo Sierra-García, Hugo Luna Olvera, Abelardo Chavez-Montes and Azucena Gonzalez-Horta 1013

Phytochemical Profile and Anti-lipase Activity of Balkan Endemic Jurinea tarz-ferdinandii
Antoaneta Trendafilova, Milka Todorova, Nikolina Kutova and Maya Guncheva 1017

The Chaenomeles sinensis Extract has the Potential to Exhibit Antioxidant Activity or Attenuate Liver Damage
Young-Ji Choi, Young-Moo Choo, Seung-II Jeong, Kang-Yeol Yu and Jiyoung Kim 1021

Continued inside backcover