Research Article

Cytotoxicity and Pharmacogenomics of Medicinal Plants from Traditional Korean Medicine

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Received 25 March 2013; Accepted 24 April 2013

Academic Editor: Sookyung Lee

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Aim. The present study was designed to investigate the cytotoxicity of a panel of 280 Korean medicinal plants belonging to 73 families and 198 species against human CCRF-CEM leukemia cells. Selected phytochemicals were investigated in more detail for their mode of action. Methods. The resazurin assay was used to determine cytotoxicity of the plant extracts. Microarray-based mRNA expression profiling, COMPARE, and hierarchical cluster analyses were applied to identify which genes correlate with sensitivity or resistance to selected phytochemicals of the Korean plants. Results. The results of the resazurin assay showed that cytotoxicity extracts tested at 10 μg/mL from 13 samples inhibited proliferation more than 50% (IC50 < 10 μg/mL) and the most active plants are Sedum middendorffianum (15.33%) and Lycoris radiata (17.61%). Out of 13 selected phytochemicals from these plants, hopeaphenol and deoxyxynarciclasine were the most cytotoxic ones. Genes from various functional groups (transcriptional or translational regulation, signal transduction, cellular proliferation, intracellular trafficking, RNA metabolism, endoplasmic/sarcoplasmic reticulum function, etc.) were significantly correlated with response of tumor cell lines to these two compounds. Conclusion. The results provide evidence on the possible use of selected Korean medicinal plants and chemical constituents derived from them for the treatment of tumors.

1. Introduction

Since ancient times, humans have derived many benefits from medicinal plants. A variety of different medicinal plants have traditionally been used in Asian cultures as medicinal plants to treat cancers [1]. The pharmacological screening of plants is an important mean for the discovery of new, safe, and effective drugs. Over 50,000 plants, possess therapeutic virtues in the world, and about 80% of human use herbal medicines at least once in their life [2, 3]. Medicinal plants through the multiplicities of their chemical constituents are important for the discovery of new substances active against tumors and other cancers. Screenings of medicinal plants used as anticancer drugs have provided modern medicine with effective cytotoxic pharmaceuticals. More than 60% of the approved anticancer drugs in the United State of America (from 1983 to 1994) were from natural origin [4, 5].

Traditional Korean medicine is widely used in Korea and is the primary health care system for more than 20% of the population [6, 7]. As previously emphasized in a regional demographic survey, about 30–40% of the Korean population had used complementary and alternative medicine (including traditional Korean medicine) within a 5-year period [6, 8].

In the pharmacopoeia of many countries worldwide including Korea, there is still a serious lack of information on
the use of medicinal plants in the treatment of cancer. However, it has been recommended that ethnopharmacological usages such as for immune and skin disorders, inflammatory, infectious, parasitic, and viral diseases should be taken into account when selecting plants used to treat cancer, since these reflect disease states bearing relevance to cancer or cancerlike symptoms [9, 10]. In general, leukemia cells are often more sensitive to cytotoxic drugs than adherent cancer cells, making them a suitable tool for primary bioactivity screenings. Hence, the present work was designed to investigate the cytotoxicity of a panel of 280 Korean medicinal plants against human CCRF-CEM leukemia cells. Based on the bioactivity of the extracts, selected phytochemicals of active plants were analyzed in more detail. Genes determining sensitivity or resistance to selected compounds were identified by microarray-based mRNA expression profiles and hierarchical cluster analyses in a panel of tumor cell lines of the National Cancer Institute, USA.

2. Material and Methods

2.1. Plant Material and Extraction. Medicinal plants used in the present work were collected at different localities of the Republic of Korea and provided by Prof. Ik-Soo Lee (College of Pharmacy, Chonnam National University, Kwangju, South Korea). The plants were identified at the National herbarium, where voucher specimens were deposited under the references numbers (see Supplementary material available online at http://dx.doi.org/10.1155/2013/341724). The extraction of the air-dried and powdered plant material was conducted using methanol (HPLC grade) with either ASE 300 (Dionex) or a sonicator (Branson Ultrasonics) at 50°C. The extracts were then conserved at 4°C until further use.

2.2. Cell Culture. Human CCRF-CEM leukemia cells were obtained from Professor Axel Sauerbrey (University of Jena, Jena, Germany). Cells were maintained in RPMI 1640 containing 100 units/mL penicillin and 100 mg/mL streptomycin and supplemented with heat-inactivated 10% fetal bovine serum (FBS), in a humidified environment at 37°C with 5% CO$_2$. Doxorubicin ≥ 98.0% (Sigma-Aldrich, Schnelldorf, Germany) was used as a positive (cytotoxic) control.

2.3. Resazurin Cell Growth Inhibition Assay. Alammar Blue or Resazurin (Promega, Mannheim, Germany) reduction assay [11] was used to assess the cytotoxicity of the studied samples. The assay tests cellular viability and mitochondrial function. Briefly, aliquots of $5 \times 10^5$ cells/mL were seeded in 96-well plates, and extracts were added immediately. After 24 h incubation, 20 µL resazurin 0.01% w/v solution was added to each well and the plates were incubated at 37°C for 1-2 h. Fluorescence was measured on an automated 96-well Infinite M2000 Pro plate reader (Tecan, Crailsheim, Germany) using an excitation wavelength of 544 nm and an emission wavelength of 590 nm. Doxorubicin was used as positive control. The concentration of DMSO was kept at or below 0.1% in all experiments. Each assay was done at least three times, with three replicates each. All samples were tested at a single concentration of 10 µg/mL.

2.4. COMPARE and Hierarchical Cluster Analysis of mRNA Microarray Data. The mRNA microarray hybridization of
Table 1: Korean plants with cytotoxic activity.

| Plants (and family)                                      | Traditional uses                                                                 | Part used            | Previously reported activity of the plant                                                                 | Reported chemical constituents                                                                 |
|---------------------------------------------------------|----------------------------------------------------------------------------------|----------------------|--------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|
| *Alnus japonica* Steudel (Betulaceae)                    | In oriental traditional medicine as remedies for fever, hemorrhage, diarrhea, and alcoholism [43] | Stems-stem bark     | Hepatoprotective and antioxidant activities [44], antiviral activity against the influenza virus [45] | 1,7-Bis-(3,4-dihydroxyphenyl)-3-hydroxyheptane-5-O-β-D-xylopyranoside; 1,7-bis-(3,4-dihydroxyphenyl)-heptane-3-O-β-D-glucopyranosyl(1→3)-β-D-xylopyranoside; 1,7-bis-(3,4-dihydroxyphenyl)-heptane-3-O-β-D-apiofuranosyl(1→6)-β-D-glucopyranoside; 1,7-bis-(3,4-dihydroxyphenyl)-heptane-3-O-β-D-glucopyranoside; 1,7-bis-(3,4-dihydroxyphenyl)-3-hydroxyheptane; 1,7-bis-(3,4-dihydroxyphenyl)-heptane-3-O-β-D-glucopyranoside; oregonin; hirsutanonol; hirsutenone; platyphylloside [44]; tannins (alnusjaponins A and B); 5-O-galloyl-(−)-shikimic acid, 2,3-(S)-hexahydroxydiphenoyl-D-glucose, 4,6-di-O-galloyl-D-glucose, 1,4-di-O-galloyl-β-D-glucose, 4,6-(S)-valoneoyl-D-glucose, strictinin; gemin D; pedunculagin; praeoxin A; ilosin A; stachyurin; casuarinin [46], lupeol; betulin; betulinic aldehyde, 3-acetoxybetulinic aldehyde, β-sitosterol [45] |
| *Camellia japonica* L. (Theaceae)                        | Cosmetic protectant to keep the skin and hair healthy and as a soothing agent [47] | Fruits               | Antibacterial activity [48], inhibitor of human immunodeficiency virus type 1 protease [49], Epstein-Barr virus inhibitor [50], antitumor activity [51], antioxidant activity [52, 53], inhibitor of human type I procollagen production [54], and antiallergic responses [55], anti-inflammatory [47] | 3β,18β-dihydroxy-28-norolean-12-en-16-one; 18β-hydroxy-28-norolean-12-ene-3,16-dione; camellagenin A, B, and C [56], camellenodiol 3-O-β-D-galactopyranosyl(1→2)[β-D-xylpyranosyl(1→3)]-β-D-glucuronopyranoside; camellenodiol 3-O-4′-O-acetyl-β-D-galactopyranosyl(1→2)[β-D-xylpyranosyl(1→3)]-β-D-glucuronopyranoside; camellenodiol 3-O(β-D-galactopyranosyl(1→2)[β-D-xylpyranosyl(1→3)]-β-D-glucuronopyranoside; maragenin I 3-O(β-D-galactopyranosyl(1→2)[β-D-xylpyranosyl(1→3)]-β′-methoxy-β-D-glucuronopyranoside; camellioside A; camellioside B [57] |
| *Isodon japonicus* (Burman f.) H. Hara (Labiatae)        | Antibacterial, anti-inflammation, and anthelmintic [58]                        | Whole plant          | Cytotoxicity on K562 human leukemia cells and immunomodulatory activity [16], antibacterial activity for plant constituents [59] | Isodonol; epinodosin; sodonosin; epinodosinol [60, 61]; epinodosin; oridonin; taihangjaponicain A; lushanrubescensin J; bisjaponins A and B [58]; isodonol, trichodonin; nodosin; enmein; oridonin; enmein-3-acetate [59] |
| *Lycoris radiata* (L’Her.) Herbert (Amaryllidaceae)      | Laryngeal trouble, furuncle, carbuncle, suppurative wounds [62]               | Leaves, underground parts | Cytotoxicity against B16F10 melanoma cells [17]                                                      | Different types of alkaloids (crinine-type; galanthamine-type; lycorine-type homolycorine-type; tazettine-type; narcissanine-type; and lycorine-type alkaloids); trisphaeridine; galanthine; bicolarine; 11-hydroxyvittatine; 8-O-demethylmaritidine; 8′-demethylgalanthamine; O-demethyllycorine [63] |
Table 1: Continued.

| Plants (and family) | Traditional uses | Part used | Previously reported activity of the plant | Reported chemical constituents |
|---------------------|------------------|-----------|------------------------------------------|-----------------------------|
| *Meliosma oldhamii* Miq. ex. Maxim. (Sabiaeae) | Liver ailments [64] | Stems-stem bark | Low Cholinesterase inhibition (12–19% at 5 mg/mL) [65], moderate alpha glucosidase activity [64] | — |
| *Myrica rubra* Sieb. and Zucc. (Myricaceae) | Diarrhoea; gastroenteritis in China [66] | Stems-stem bark | Antioxidant activity [67]; anti-influenza virus activity [68] | Taraxerone; taraxerol; myricadiol; sitosterol; 28-hydroxy-β-friedoolean-14-en-3-one (); myricanol 5-O-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside; myricanol 5-O-α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside; isomyricanone [69]; cyanidin-3-O-glucoside; myricetin; quercetin-3-O-rutinoside [67] |
| *Rubus corchorifolius* L. f. (Rosaceae) | Stomachache, diarrhea, and dysentery [18] | Whole plant | Antioxidant activity of essential oil [70] | *Ent*-kauran-3β,16β,17,19-tetrol; *ent*-2-carbonyl-16β-hydroxy-kauran-17β-D-glucoside [18]; rubusin A; quercetin; kaempferol [25] |
| *Sageretia theezans* (L.) Brongn (Rhamnaceae) | Tea materials [71] | Leaves, Stems | Antioxidant activity [72] | 7-O-methylmearsnitrin; myricetin, kaempferol 3-O-α-L-rhamnopyranoside, epuretin 3-O-α-L-rhamnopyranoside, and 7-O-methyl quercetin 3-O-alpha-L-rhamnopyranoside; 7-O-methylmearnsetin 3-O-rhamnoside |
| *Sedum middendorfianum* Maxim. (Crassulaceae) | — | Whole plant | — | kaempferol; quercetin; myricetin; arbutin [24] |
| *Sedum takesimense* Nakai (Crassulaceae) | — | Whole plant | Antioxidant activities [26] | Ferulic acid; caffeic acid; gallic acid; methyl gallate; myricetin; quercetin; luteolin; rhodalin; rhodaldin; luteolin-7-O-β-D-glucoside; arbutin; 1-(4-hydroxyphenyl)-2-(3,5-dihydroxyphenyl)-2-hydroxyethanone; gossypetin-8-O-β-D-xylopyranoside; 2,6-di-O-galloylharbutin [26] |
| *Sorbaria sorbifolia* (L.) A. Br. var. *stellipila* MAX. (Rosaceae) | — | Stems | Antioxidant activities, cytotoxicity [73, 74] | Sutherlandin-5-trans-p-coumarate; cardiosiospermin-5-(4-hydroxy) benzoate [75]; noreugenin; wogonin; 5,7,3′,4′-tetrahydroxy-3-methoxyflavone; protocatechuic acid; benzoic acid; emodin; daucosterol [76]; 5,2′,4′-trihydroxy-6,7,3′-trimethoxyflavone; succinic acid; p-hydroxybenzoic acid [77] |
| *Vitis flexuosa* Thunb. (Vitaceae) | — | Aerial parts | — | Flexuosol A; gnetin A; (+)-episoniferin; vitisin A; hopeaphenol [78] |

(—): not reported; the complete list of the tested plants is available in supplementary material.
Figure 3: Cytotoxic activity of hopeaphenol (a) and deoxynarciclasine (b) towards cell lines of different tumor types (mean ± SD).

the NCI cell line panel has been described [12, 13] and the date has been deposited at the NCI website (http://dtp.nci.nih.gov/). For hierarchical cluster analysis, objects were classified by calculation of distances according to the closeness of between-individual distances by means of hierarchical cluster analysis. All objects were assembled into a cluster tree (dendrogram). The merging of objects with similar features leads to the formation of a cluster, where the length of the branch indicates the degree of relation. The distance of a subordinate cluster to a superior cluster represents a criterion for the closeness of clusters as well as for the affiliation of single objects to clusters. Thus, objects with tightly related features appear together, while the separation in the cluster tree increases with progressive dissimilarity. Previously, cluster models have been validated for gene expression profiling and for approaching molecular pharmacology of cancer [12–14]. Hierarchical cluster analyses applying the WARD method were done with the WinSTAT program (Kalmia, Cambridge, MA, USA). Missing values were automatically omitted by the program, and the closeness of two joined objects was calculated by the number of data points they contained. In order to calculate distances between all variables included in the analysis, the program automatically standardizes the variables by transforming the data with a mean = 0 and a variance = 1. For COMPARE analysis, the mRNA expression values of genes of interest and IC50 values for selected phytochemicals of the NCI cell lines were selected from the NCI database (http://dtp.nci.nih.gov/). The mRNA expression has
Table 2: Cross-resistance profile of a panel of cell lines towards hopeaphenol and deoxynarciclasine determined by correlating the IC$_{50}$ values by Pearson's correlation test.

|                | Hopeaphenol | Deoxynarciclasine |
|----------------|-------------|-------------------|
| Doxorubicin    | R-value <0.30 | 0.340             |
|                | P-value >0.05 | 0.010             |
| Daunorubicin   | R-value <0.30 | 0.430             |
|                | P-value >0.05 | 0.001             |
| Vincristine    | R-value <0.30 | 0.331             |
|                | P-value >0.05 | 0.012             |
| Paclitaxel     | R-value <0.30 | 0.330             |
|                | P-value >0.05 | 0.012             |
| Cisplatin      | R-value <0.30 | 0.300             |
|                | P-value >0.05 | 0.020             |
| Melphalan      | R-value <0.30 | 0.388             |
|                | P-value >0.05 | 0.004             |
| Carmustin      | R-value <0.30 | 0.394             |
|                | P-value >0.05 | 0.003             |
| Erlotinib      | R-value −0.353 | >−0.30             |
|                | P-value 0.004  | >0.05             |

Pearson's rank correlation test.

been determined by microarray analyses as reported [12]. COMPARE analyses were performed by a web-based algorithm (http://dtp.nci.nih.gov/) to produce rank-ordered lists of genes expressed in the NCI cell lines. The methodology has been described previously in detail [15]. Briefly, every gene of the NCI microarray database was ranked for similarity of its mRNA expression to the IC$_{50}$ values for the corresponding compound. Scale indices of correlations coefficients (R-values) were created. Pearson’s correlation coefficients with positive algebraic signs indicate that greater mRNA expression in cell lines correlated with enhanced drug resistance, whereas coefficients with negative algebraic signs indicate that greater mRNA expression in cell lines was associated with drug sensitivity. Pearson's correlation test and $\chi^2$ test were implemented into the WinSTAT Program (Kalmia). The one-way ANOVA at 95% confidence level was used for statistical analysis.

3. Results

3.1. Cytotoxic Activity. In the present work, we screened 280 plant extracts derived from traditional Korean medicine belonging to 73 plant families and 198 species (see Supplementary Table) for their cytotoxicity against human CCRF-CEM leukemia cells.

The results of the cytotoxicity assay (Figure 1; Supplementary Table) indicated that among the 280 studied plant extracts, 58 promoted the growth (% growth > 100%) of CCRF-CEM cells, while the remaining samples showed various extents of growth inhibition. Out of these extracts, 207 extracts showed no or a weak inhibition of cancer cell proliferation (<50%) at the tested concentration of 10 $\mu$g/mL. However, 13 samples induced the proliferation of less 50% cells (IC$_{50}$ < 10 $\mu$g/mL). These samples included Sedum midendorffianum whole plant (15.33%), Lycoris radiata underground parts (17.61%), Camellia japonica fruits (22.28%), Myrica rubra stem-stem bark (27.61%), Lycoris radiata leaves (27.73%), Vitis flexuosa aerial parts (29.32%), Sedum takesimense whole plant (32.39%), Sorbaria sorbifolia var. stelligila stems (34.98%), Isodon japonicus whole plant (44.15%), Sageretia theezans leaves and stems (46.35%), Rubus corchorifolius aerial part (46.81%), Alnus japonica stem-stem bark (48.43%), and Meliosma oldhamii stem-stem bark (49.94%) (Figure 1). The traditional used and reported bioactivities are compiled in Table 1.

3.2. Cytotoxicity of Phytochemicals Derived from Koran Medicinal Plants. We searched the literature on the chemical constituents of the cytotoxic Korean plants (Table 1). Subsequently, we mined the NCI database for these compounds. Thirteen compounds with average log$_{10}$IC$_{50}$ values over the entire NCI cell line panel below −4.0 M are depicted in Figure 2. Hopeaphenol and deoxynarciclasine were the most cytotoxic compounds with log$_{10}$IC$_{50}$ values of 0.346 × 10$^{-5}$ M (−0.346 $\mu$M) and 0.233 × 10$^{-5}$ M (−2.33 $\mu$M), respectively.

If the average IC$_{50}$ values over the entire range of cell lines were diversified regarding their tumor types, colon cancer and melanoma cell lines were most sensitive towards hopeaphenol, whereas leukemia and kidney cancer cell lines were most resistant (Figure 3(a)). The cell lines reacted in a different manner towards deoxynarciclasine. Leukemia and colon cancer were most sensitive towards this compound, whereas breast cancer and prostate cancer cell lines were most resistant (Figure 3(b)).

3.3. Cross-Resistance of the NCI Cell Line Panel towards Hopeaphenol and Deoxynarciclasine. The log$_{10}$IC$_{50}$ values of hopeaphenol and deoxynarciclasine were correlated with clinically well-established anticancer drugs. The cell line panel showed statistically significant correlations between deoxynarciclasine and doxorubicin, daunorubicin, vincristine, paclitaxel, cisplatin, melphalan and carmustin. By contrast, cross-resistance was not found between hopeaphenol and these standard drugs, indicating that hopeaphenol might be useful for the treatment of otherwise drug-resistant tumors (Table 2). Interestingly, an inverse correlation was found between the log$_{10}$IC$_{50}$ values for hopeaphenol and the epidermal growth factor receptor tyrosine kinase inhibitor, erlotinib. Such a relationship was not found between deoxynarciclasine and erlotinib (Table 2).
### 3.4. COMPARE and Hierarchical Cluster Analyses of mRNA Microarray Data

We further investigated the microarray-based transcriptomic mRNA expression by COMPARE analyses to test whether the responses of the tumor cells lines to hopeaphenol and deoxynarciclasine were associated with specific gene expression profiles. For this reason, we mined the transcriptome-wide mRNA expression database of the NCI and correlated the expression data with the IC\(_{50}\) values for both compounds. This represents a hypothesis-generating bioinformatical approach, which allows the identification of novel putative molecular determinants of cellular response for hopeaphenol and deoxynarciclasine. The scale rankings of genes obtained by COMPARE computation were subjected to Pearson's rank correlation tests. The thresholds for correlation coefficients were \( R > 0.50 \) for direct correlations and \( R < -0.50 \) for inverse correlations. The genes fulfilling these criteria are shown in Table 3 (for hopeaphenol) and Table 4 (for deoxynarciclasine). These genes were from diverse functional groups for hopeaphenol (Table 3). For deoxynarciclasine, genes were found involved in transcription (ILF2, BCLAF1, MATR3, PSCPI, ZBTB11, CNOT8, IKZF5, SFI, EBP, and MYBL1), RNA metabolism (HNRNP1, LARS, SFRSI, FARSA, NUDT21, DDX39, and SERBP1), translation (GOT1, NGDNR), cellular proliferation (CWF19L1, MKI67, HSPA9, MLF1IP, and DCBLD2), intracellular trafficking (OPTN, SNX6, and RAB1IFIP5), endoplasmic/sarcoplasmic reticulum function (SLN, DNAJC10, LMAN2L, SEC24D, and KDEL2), signal transduction (FRAT2, CHUK, GNA12, and LPP), and others.

As a next step, the genes identified by COMPARE analyses and Pearson's rank correlation tests were subjected to hierarchical cluster analyses. Only the mRNA expression levels but not the IC\(_{50}\) values of the compounds for the cell line panel were used for cluster analyses. Four cluster branches were found in the hopeaphenol-related dendrogram (Figure 4) and three clusters were obtained in the deoxynarciclasine-related cluster analysis (Figure 5). Remarkably, the distribution of cell lines sensitive or resistant to both drugs considerably varied between the different clusters of the dendrograms. Since the IC\(_{50}\) values of the compounds were not included into the cluster analysis, we could address the question whether the gene expression profiles alone are sufficient to predict sensitivity or resistance of cell lines to the compounds.

As shown in Table 5, the distributions of cell lines sensitive or resistant to hopeaphenol and deoxynarciclasine, respectively, were significantly different between the clusters of the dendrograms, indicating that the expression of these genes was not only responsible for the branching of the dendrograms, but also predicted sensitivity or resistance of these cell lines towards these compounds.

### 4. Discussion

#### 4.1. Cytotoxic Activity

The cytotoxicity observed in *Isodon japonicus* in this work is in concordance with previous reports. In effect, the cytotoxic effect of *I. japonicus* extract was reported against five human cancer cell lines (IC\(_{50}\) values below 10 \(\mu\)g/mL on stomach MKN-45, breast MCF-7, leukemia K562, colon HT29, and lung A549 cell lines) with leukemia K562 (IC\(_{50}\) : 2.70 \(\mu\)g/mL) being the most sensitive [16]. Also, the ethanol extract of *Lycoris radiata* exhibited significant antiproliferative effect against B16F10 melanoma cells and induced apoptosis through the activation of p38 and AP-1 pathway [17]. The present report, therefore, provides evidence on the activity of *L. radiata* not only against cell lines derived from solid tumors but also derived from the
Table 4: Genes identified by standard or reverse COMPARE analyses, whose mRNA expression in a panel of 60 cell lines correlated with IC<sub>50</sub> values for deoxynarciclasine.

| Pearson's correlation coefficient | Experimental ID | Gene symbol | Name | Function |
|----------------------------------|-----------------|-------------|------|----------|
| 0.572                            | GC74997         | SNAP25      | Synaptosomal-associated protein, 25 kDa | Regulation of neurotransmitter release |
| 0.56                             | GC32186         | OPTN        | Optineurin | Maintenance of membrane trafficking |
| 0.557                            | GC26586         | FAMIL16B    | Family with sequence similarity 116, member B | Unknown |
| 0.531                            | GC187393        | ZDHHC7      | Zinc finger, DHH type containing 7 | Palmitoyltransferase |
| 0.529                            | GC12575         | CLEC9A      | C-type lectin domain family 9, member A | Endocytic receptor for necrotic cells |
| 0.528                            | GC188718        | SNX6        | Sorting nexin 6 | Involved in intracellular trafficking |
| 0.528                            | GC154565        | RAB11FIP5   | RAB11 family interacting protein 5 (class I) | Involved in protein trafficking |
| 0.527                            | GC18848         | NTAN1       | N-terminal asparagine amidase | Ubiquitin-dependent turnover of intracellular proteins |
| 0.526                            | GC16433         | DNAJC10     | DnaJ (Hsp40) homolog, subfamily C, member 10 | Endoplasmic reticulum co-chaperone |
| 0.524                            | GC100099        | PCOLCE2     | Procollagen C-endopeptidase enhancer 2 | Binds to procollagens |
| 0.524                            | GC45803         | LMAN2L      | Lectin, mannose-binding 2-like | Regulation of export from the endoplasmic reticulum |
| 0.524                            | GC82897         | NGDN        | Neurouquin, EIF4E binding protein | Involved in the translational repression |
| 0.519                            | GC75800         | SEC24D      | SEC24 family, member D (S. cerevisiae) | Transport of ER-derived vesicles |
| 0.517                            | GC90440         | KDELR2      | KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2 | Retention of luminal endoplasmic reticulum proteins |
| 0.514                            | GC173264        | GNA12       | Guanine nucleotide binding protein (G protein) α12 | Transducer in transmembrane signaling |
| 0.512                            | GC170473        | MYBL1       | V-Myb myeloblastosis viral oncogene homolog (avian)-like 1 | Transcriptional activator |
| 0.512                            | GC175225        | LPP         | LIM domain containing preferred translocation partner in lipoma | Signal transduction from cell adhesion sites to the nucleus |
| 0.511                            | GC14006         | ITGB5       | Integrin, β5 | Receptor for fibronectin |
| 0.509                            | GC150035        | DCBLD2      | Discoidin, CUB, and LCCL domain containing 2 | Involved in tumor progression |
| 0.508                            | GC73833         | NCEH1       | Neutral cholesterol ester hydrolase 1 | Promotes tumor cell migration |
| 0.507                            | GC177466        | AHNAK       | AHNAK nucleoprotein | Involved in neuronal cell differentiation |
| 0.502                            | GC40305         | ADAL        | Adenosine deaminase | Putative nucleoside deaminase |
| 0.502                            | GC28174         | MPG         | N-methylpurine-DNA glycosylase | Hydrolysis of alkylated DNA |
| 0.501                            | GC18079         | CRI         | Complement component (3b/4b) receptor 1 (Koops blood group) | Mediates cellular binding of particles and immune complexes |
| -0.658                           | GC18354         | ILF2        | Interleukin enhancer binding factor 2, 45 kDa | Transcription |
| -0.608                           | GC44240         | NDUFA2      | NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 2, 8 kDa | Part of mitochondrial membrane respiratory chain |
| -0.605                           | GC65788         | HNRNPA1     | Heterogeneous nuclear ribonucleoprotein A1 | Involved in RNA replication |
| -0.589                           | GC43237         | CWF9L1      | CWF9-like 1, cell cycle control (S. pombe) | Cell cycle control |
| -0.572                           | GC174320        | BCLAF1      | BCL2-associated transcription factor 1 | Death-promoting transcriptional repressor |
| -0.572                           | GC40779         | LARS        | Leucyl-tRNA synthetase | Editing of tRNA |
| -0.567                           | GC151509        | FRAT2       | Frequently rearranged in advanced T-cell lymphomas 2 | Wnt signaling regulator |
| -0.561                           | GC175610        | IK          | IK cytokine, downregulator of HLA II | May bind to chromatin |
| -0.555                           | GC30213         | SLN         | Sarcolipin | Sarcoplasmic reticulum proteolipid |
| -0.555                           | GC162737        | ANKH2D      | Unknown | Unknown |
| -0.552                           | GC37532         | SFRS28      | Serine/arginine-rich splicing factor 1 | Splicing regulator |
| -0.55                             | GC185046        | SFRS1       | Serine/arginine-rich splicing factor 1 | Splicing regulator |
| -0.55                             | GC37292         | FARSA       | Phenylalanyl-tRNA synthetase, α subunit | Phenylalanine-tRNA ligase |
| -0.548                           | GC32318         | MKI67       | Antigen identified by monoclonal antibody Ki-67 | Cell proliferation |
| -0.548                           | GC149101        | MATR3       | Matrin 3 | Regulator of transcription |
hematopoietic system, that is, CCRF-CEM leukemia cells. Sorbaria sorbifolia was cytotoxic towards HepG-2 cells via induction of apoptosis and cell cycle arrest [18], and evidence of this activity towards CCRF-CEM leukemia cells is provided herein.

In the US NCI plant screening program, a crude extract is generally considered to have in vitro cytotoxic activity, if the IC50 value following incubation between 48 and 72 h is less than 20 μg/mL [19]. In this study, we reduced the cutoff point to 10 μg/mL. All extracts were tested at this concentration and only samples with an inhibitory effect >50% were considered to have highly promising activities against leukemia cells. Under this condition, 13 samples from 12 medicinal plants (Figure 1) were identified as promising anticancer products. Additionally, Kim et al. [21] reported the presence of two well-known cytotoxic compounds, betulin and lupeol [22] in the extract of this plant. Apoptosis induction via Fas-mediated pathway was also reported in human MCF-7 breast adenocarcinoma cells by prod璘helinib B-2 3,3'-di-O-gallate, a constituent of M. rubra [23]. The presence of such cytotoxic compounds could explain the good activity of the crude extract of A. japonica and M. rubra. Similarly, the presence of cytotoxic compounds such as quercetin or ferulic acid [22], in R. corchorifolius and S. middendorfianum extracts [24, 25]

| Pearson’s correlation coefficient | Experimental ID | Gene symbol | Name | Function |
|-----------------------------------|----------------|-------------|------|----------|
| -0.547                            | GC150688       | PSPC1       | Paraspeckle component 1 | Regulator of androgen receptor-mediated gene transcription |
| -0.546                            | GC80581        | ZBTB11      | Zinc finger and BTB domain containing 11 | Regulator of transcription |
| -0.546                            | GC52470        | MLF1IP      | MLF1 interacting protein | Involved in mitotic progression |
| -0.545                            | GC81490        | NUDT21      | Nudix (nucleoside diphosphate linked moiety X)-type motif 21 | Involved in pre-mRNA 3’-processing |
| -0.543                            | GC33504        | DDX39       | DEAD (Asp-Glu-Ala-Asp) box polypeptide 39A | Involved in pre-mRNA splicing |
| -0.543                            | GC33560        | NDUGB8      | | |
| -0.539                            | GC36766        | CHUK        | Conserved helix-loop-helix ubiquitous kinase | Involved in NF-kappa-B signaling |
| -0.538                            | GC33463        | POLD1       | Polymerase (DNA directed), δ1, catalytic subunit | DNA synthesis |
| -0.537                            | GC38208        | SERBP1      | SERPINE1 mRNA binding protein 1 | Regulation of mRNA stability |
| -0.536                            | GC13558        | CNOT8       | CCR4-NOT transcription complex, subunit 8 | Transcription factor |
| -0.535                            | GC17771        | GOT1        | Glutamic-oxaloacetic transaminase 1, soluble (aspartate aminotransferase 1) | Involved in amino acid metabolism |
| -0.535                            | GC43091        | NUDCD2      | NudC domain containing 2 | Unknown |
| -0.534                            | GC39149        | HSPA9       | Heat shock 70 kDa protein 9 (mortalin) | Control of cell proliferation and cellular aging |
| -0.533                            | GC91403        | IKZF5       | IKAROS family zinc finger 5 (Pegasus) | Transcriptional repressor |
| -0.533                            | GC31641        | SF1         | Splicing factor 1 | Transcriptional repressor |
| -0.532                            | GC161453       | KDM2B       | Lysine (K)-specific demethylase 2B | Histone demethylase |
| -0.53                             | GC35520        | EBP         | CCAAT/enhancer binding protein (C/EBP), gamma | Transcription factor |
| -0.53                             | GC10226        | ABCB7       | ATP-binding cassette, sub-family B (MDR/TAP), member 7 | Heme transport |

Information on gene functions was taken from the OMIM database, NCI, USA, (http://www.ncbi.nlm.nih.gov/Omim/) and from the GeneCard database of the Weizmann Institute of Science, Rehovot, Israel (http://bioinfo.weizmann.ac.il/cards/index.html).
and Sedum takesimense [26] could also provide some explanation on their anti proliferative potentials. Nonetheless, it is interesting to know that the activity does not only depend on the presence of a cytotoxic substance in a plant, but also on their quantities and possible interaction with other plant constituents. For example, cytotoxic compounds such as in canone [known to be active on HL60 leukemia cells (IC_{50} value of 6 μM) [25]) or verbascoside [27] (active on human HEP-2 larynx epidermoid carcinoma, human RD rhabdomyosarcoma and human MCF-7 breast adenocarcinoma cell lines [28]) were reported in Caryopteris incana. But extract from Caryopteris incana did not show significant activities against CCRF-CEM leukemia cells in the present study.

We screened the NCI database of the Developmental Therapeutics Program of the NCI for the constituents of our panel of Korean medicinal plants. The two most cytotoxic compounds were hopeaphenol and deoxynarciclasine. Both compounds have been previously reported to be cytotoxic [29–32]. While hopeaphenol’s activity has been demonstrated in mouse tumors in vitro and in vivo [31, 32], the present investigation shows that this compounds also active against human tumor cells. To the best of our knowledge, the mechanisms of action of these two compounds have not been investigated in the past and are addressed in our analysis for the first time.

4.2. COMPARE and Hierarchical Cluster Analyses of mRNA Microarray Data. To gain insight into possible modes of action of both phytochemicals, we investigated gene expression profiles of the NCI cell line panel. By microarray-based gene expression and COMPARE analyses, we correlated the IC_{50} values for both compounds of 60 tumor cell lines with transcriptomic mRNA expression levels of this cell line panel [12]. This approach has been successfully used to unravel the mode of action of novel compounds [33]. Cluster and COMPARE analyses are also useful for comparing gene expression profiles with IC_{50} values for investigational drugs to identify candidate genes for drug resistance [34, 35] and to identify prognostic expression profiles in clinical oncology [36, 37].

Eleven genes passing the correlation thresholds of \( R > 0.5 \) and \( R < -0.5 \) were identified to significantly correlate with sensitivity or resistance to hopeaphenol. Except for fibroblast growth factor 9 [38], none of them have been associated with response of tumor cells to cytostatic drugs. This point of view is supported by the fact that the NCI cell lines did not exert cross-resistance between hopeaphenol and anticancer drugs, such as doxorubicin, vincristine, cisplatin, and others.

The genes correlating with the response of tumor cells to deoxynarciclasine were also not known to confer drug resistance as of yet, but many of them belong to functional
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Cell line: Tumor type:

- U251: Brain cancer
- SK-MEL-2: Melanoma
- SNB-19: Melanoma
- SK-MEL-5: Melanoma
- MCF7: Breast cancer
- SW-620: Colon cancer
- HT29: Colon cancer
- LOX IMVI: Lung cancer
- HCT-116: Lung cancer
- OVCAR-3: Lung cancer
- HCT-15: Lung cancer
- NCI-H522: Lung cancer
- SN12C: Lung cancer
- NCI-H460: Lung cancer
- RPMI-8226: Leukemia

- RXF 393: Renal cancer
- HOP-92: Renal cancer
- UO-31: Renal cancer
- SK-OV-3: Ovarian cancer
- UACC-62: Melanoma
- SK-MEL-28: Melanoma
- UACC-257: Melanoma
- MALME-3M: Colon cancer
- NCI/ADR-RES: Lung cancer
- SW-620: Lung cancer
- HT29: Lung cancer
- OVCAR-8: Lung cancer
- CAKI-1: Renal cancer
- SF-295: Renal cancer
- NCI-H266: Lung cancer
- SF-539: Lung cancer
- NCI-H23: Lung cancer
- GEP: Lung cancer
- A549/ATCC: Lung cancer

**Figure 5:** Dendrograms obtained by hierarchical cluster analysis of microarray-based gene expressions for deoxynarciclasine of the panel of NCI cell lines. The dendrograms were obtained by clustering using the WARD method.

4.3. Conclusion. The present work provides evidence that some plants derived from Korean medicine could be useful in the treatment of leukemia and supports the advanced investigation of the most active plants extracts. It also provides first pharmacological data on the cytotoxicity of some plants, such as *Adenophora racemosa*, *Cinnamomum japonicum*, *Eurya japonica*, *Sedum middendorffianum*, and *Vitis flexuosa*. The identification of cytotoxic phytochemicals from these plants, for example, hopeaphenol, and deoxynarciclasine, and the investigation of their possible molecular modes of action may foster the development of novel treatment strategies against otherwise drug-resistant and refractory tumors.

**Conflict of Interests**

No potential conflict of interests was disclosed.

**Table 5:** Separation of clusters of 60 cancer cell lines obtained by hierarchical cluster analysis for hopeaphenol and deoxynarciclasine. The log$_{10}$ IC$_{50}$ median values (M) of each compound were used as cut-off values to define cell lines as being sensitive or resistant. $P > 0.05$ was considered as not significant ($\chi^2$ test).

|                  | Sensitive | Resistant | $\chi^2$ test |
|------------------|-----------|-----------|---------------|
| **Hopeaphenol**  |           |           |               |
| Partition*       | $<-5.80$  | $>5.80$   |               |
| Cluster 1        | 0         | 7         |               |
| Cluster 2        | 0         | 4         |               |
| Cluster 3        | 15        | 0         |               |
| Cluster 4        | 12        | 16        | $P = 4.49 \times 10^{-6}$ |

| **Deoxynarciclasine** |                 |            |               |
|-----------------------|-----------------|------------|---------------|
| Partition*            | $<-6.40$        | $>6.40$    |               |
| Cluster 1             | 3                | 22         |               |
| Cluster 2             | 13               | 2          |               |
| Cluster 3             | 7                | 0          | $P = 1.76 \times 10^{-6}$ |

* log$_{10}$ IC$_{50}$ (M).

**Authors’ Contribution**

Victor Kuete and Ean-Jeong Seo contributed equally to this paper.

**Acknowledgments**

The authors are thankful to Mrs. Christine Köppel for her technical assistance performing the cytotoxicity assays and Mrs. Ilona Zirbs for her secretarial support.
References

[1] S. Lee, H. S. Lillehoj, H. Chun et al., “In vitro treatment of chicken peripheral blood lymphocytes, macrophages, and tumor cells with extracts of Korean medicinal plants,” Nutrition Research, vol. 27, no. 6, pp. 362–366, 2007.

[2] D. S. Bhakuni, M. Bittner, C. Marticorena et al., “Screening of Chilean plants for antimicrobial activity,” Lloydia, vol. 37, no. 4, pp. 621–632, 1974.

[3] N. R. Farnsworth, O. Akerele, and A. S. Bingel, “Medicinal plants in therapy,” Bulletin of the World Health Organization, vol. 63, no. 6, pp. 965–981, 1985.

[4] C. Stévigny, C. Bailly, and J. Quetin-Leclercq, “Cytotoxic and antitumor potentialities of aporphinoid alkaloids,” Current Medicinal Chemistry, vol. 5, no. 2, pp. 173–182, 2005.

[5] D. J. Newman and G. M. Cragg, “Natural products as sources of new drugs over the last 25 years,” Journal of Natural Products, vol. 70, no. 3, pp. 461–477, 2007.

[6] Y. S. Cheong, E. W. Park, S. M. Yoo et al., “Use of traditional medicine and folk remedies in hypertensive patients: based on Cheonan practice-based research network,” Journal of the Korean Academy of Family Medicine, vol. 19, no. 2, pp. 141–149, 1998.

[7] C. D. Hong, “Complementary and alternative medicine in Korea: current status and future prospects,” Journal of Alternative and Complementary Medicine, vol. 7, supplement 1, pp. S33–S40, 2001.

[8] M. S. Hong, K. H. Chun, H. J. Song, and I. W. Park, “Attitudes towards complementary and alternative medicine in Suwon city,” Korean Journal of Preventive Medicine, vol. 32, no. 2, pp. 162–169, 1999.

[9] G. A. Cordell, C. W. W. Beecher, and J. M. Pezzuto, “Can ethnopharmacology contribute to the development of new anticancer drugs?” Journal of Ethnopharmacology, vol. 32, no. 1–3, pp. 117–133, 1991.

[10] J. Popoca, A. Aguilar, D. Alonso, and M. L. Villarreal, “Cytotoxic activity of selected plants used as antitumors in Mexican traditional medicine,” Journal of Ethnopharmacology, vol. 59, no. 3, pp. 173–177, 1998.

[11] J. O’Brien, I. Wilson, T. Orton, and F. Pognan, “Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity,” European Journal of Biochemistry, vol. 267, no. 17, pp. 5421–5426, 2000.

[12] U. Scherf, D. T. Ross, M. Waltham et al., “A gene expression database for the molecular pharmacology of cancer,” Nature Genetics, vol. 24, no. 3, pp. 236–244, 2000.

[13] S. A. Amundson, K. T. Do, L. C. Vinikoor et al., “Integrating global gene expression and radiation survival parameters across the 60 cell lines of the National Cancer Institute Anticancer Drug Screen,” Cancer Research, vol. 68, no. 2, pp. 415–424, 2008.

[14] T. Effertth, U. Fabry, and R. Osiak, “Apoptosis and resistance to daunorubicin in human leukemic cells,” Leukemia, vol. 11, no. 7, pp. 1180–1186, 1997.

[15] K. Wosikowski, D. Schuurhuis, K. Johnson et al., “Identification of epidermal growth factor receptor and c-erbB2 pathway inhibitors by correlation with gene expression patterns,” Journal of the National Cancer Institute, vol. 89, no. 20, pp. 1505–1515, 1997.

[16] Y. J. Hwang, J. Kim, D. S. Park, and K. A. Hwang, “Study on the immunomodulation effect of Isodon japonicus extract via splenocyte function and NK anti-tumor activity,” International Journal of Molecular Sciences, vol. 13, no. 4, pp. 4880–4888, 2012.

[17] M. Son, A. Kim, J. Lee et al., “Ethanol extract of Lycoris radiata induces cell death in Bl6F10 melanoma via p38-mediated AP-1 activation,” Oncology Reports, vol. 24, no. 2, pp. 473–478, 2010.

[18] X. W. Zhang, C. X. Cui, and L. Y. Chen, “Inhibition of Sorbaria sorbifolia on proliferation of hepatoma HepG-2 cell line,” Journal of Chinese Medical Materials, vol. 30, no. 6, pp. 681–684, 2007.

[19] J. Boik, Natural Compounds in Cancer Therapy, Oregon Medical Press, Princeton, Minn, USA, 2001.

[20] S. E. Choi, K. H. Kim, J. H. Kwon, S. B. Kim, H. W. Kim, and M. W. Lee, “Cytotoxic activities of diarylheptanoids from Alnus japonica,” Archives of Pharmacal Research, vol. 31, no. 10, pp. 1287–1289, 2008.

[21] H. J. Kim, K. H. Kim, S. H. Yeom et al., “New diarylheptanoid from the barks of Alnus japonica steudel,” Chinese Chemical Letters, vol. 16, no. 10, pp. 1337–1340, 2005.

[22] V. Kuete and T. Effertth, “Pharmacogenomics of Cameroonian traditional herbal medicine for cancer therapy,” Journal of Ethnopharmacology, vol. 137, no. 1, pp. 752–766, 2011.

[23] P. L. Kuo, Y. L. Hsu, T. C. Lin, T. L. Lin, and C. C. Lin, “Induction of apoptosis in human breast adenocarcinoma MCF-7 cells by prodelphinidin B-2 3,3’-di-O-gallate from Myrica rubra via Fas-mediated pathway,” Journal of Pharmacy and Pharmacology, vol. 56, no. 11, pp. 1399–1406, 2004.

[24] G. P. Shnyakina and G. Z. Zapesochnaya, “Flavonols and phenolic compounds of Sedum middendorffianum,” Chemistry of Natural Compounds, vol. 9, no. 5, pp. 645–648, 1975.

[25] Z. L. Sun, Y. Zhang, A. H. Wan, X. L. Zhang, and J. Feng, “A new active compound against kidney deficiency from the fruits of Rubus coreanofolius,” Journal of Asian Natural Products Research, vol. 13, no. 1, pp. 68–74, 2011.

[26] P. T. Thuong, J. H. Kang, M. Na et al., “Anti-oxidant constituents from Sedum takesimensem,” Phytochemistry, vol. 68, no. 19, pp. 2432–2438, 2007.

[27] U. S. Harput, Y. Genc, and I. Saracoglu, “Cytotoxic and antioxidative activities of Plantago lagopus L. and characterization of its bioactive compounds,” Food and Chemical Toxicology, vol. 50, no. 5, pp. 1554–1559, 2012.

[28] D. H. Paper, H. Vogl, G. Franz, and R. Hoffmann, “Defined carrageenan derivatives as angiogenesis inhibitors,” Macromolecular Symposia, vol. 99, no. 1, pp. 219–225, 1995.

[29] G. R. Pettit, V. Gaddamidi, D. L. Herald et al., “Antinociceptive agents, 120. Pancratium littorale,” Journal of Natural Products, vol. 49, no. 6, pp. 995–1002, 1986.

[30] G. R. Pettit, G. Pettit III, R. A. Backhaus, M. R. Boyd, and A. W. Meekrow, “Antinociceptive agents, 256. Cell growth inhibitory agents, 120. Pancratium littorale,” Journal of Natural Products, vol. 60, no. 9–10, pp. 1682–1687, 1993.

[31] S. Mishima, K. Matsumoto, Y. Futamura et al., “Antitumor effect of stilbenoids from Vateria indica against allografted sarcoma S-180 in animal model,” Journal of Experimental Therapeutics and Oncology, vol. 3, no. 5, pp. 283–288, 2003.

[32] S. Sahidin, E. H. Hakim, L. D. Juliawaty et al., “Cytotoxic properties of oligostilbenoids from the tree barks of Hopea dryobalanoides,” Zeitschrift fur Naturforschung C, vol. 60, no. 10, pp. 1682–1687, 1993.
S. T. Kim, J. D. Kim, S. H. Ahn, G. S. Ahn, Y. I. Lee, and Y. M. W. Lee, T. Tanaka, G. I. Nonaka, and I. Nishioka, “Dimeric T.Efferth, V. B. Konkimalla, Y. Wang et al., “Prediction of broad K. Y. Kim, P. M. Davidson, and H. J. Chung, “Antibacterial ac- J. C. Park, J. M. Hur, J. G. Park et al., “Inhibitory effects of Ko- T. Akihisa, H. Tokuda, M. Ukiya et al., “3-Epicabraldehydeoxy- inhibitory effects on Epstein-Barr virus activation,” Chemical and Pharmaceutical Bulletin, vol. 52, no. 1, pp. 153–156, 2004. D. Miura, Y. Kida, and H. Nojima, “Camellia oil and its dis- tillate fractions effectively inhibit the spontaneous metastasis of mouse melanoma BL6 cells,” FEBS Letters, vol. 581, no. 13, pp. 2541–2548, 2007. K. Onodera, K. Hanashiro, and T. Yasumoto, “Camellianoside, a novel antioxidant glycosome from the leaves of Camellia japonica,” Bioscience, Biotechnology and Biochemistry, vol. 70, no. 8, pp. 1995–1998, 2006. M. J. Piao, E. S. Yoo, Y. S. Koh et al., “Antioxidant effects of the ethanol extract from flower of Camellia japonica via scavenging of reactive oxygen species and induction of antioxidant en- zymes,” International Journal of Molecular Science, vol. 12, no. 4, pp. 2618–2630, 2011. E. Jung, J. Lee, J. Baek et al., “Effect of Camellia japonica oil on human type I procollagen production and skin barrier function,” Journal of Ethnopharmacology, vol. 112, no. 1, pp. 127–131, 2007. J.-H. Lee, J.-W. Kim, N.-Y. Ko et al., “Camellia japonica suppresses immunoglobulin E-mediated allergic response by the inhibition of Syk kinase activation in mast cells,” Clinical and Experimental Allergy, vol. 38, no. 5, pp. 794–804, 2008. H. Itokawa, H. Nakajima, A. Ikuta, and Y. Iitaka, “Two triter- penes from the flowers of Camellia japonica,” Phytochemistry, vol. 20, no. 11, pp. 2539–2542, 1981. N. T. P. Thao, T. M. Hung, T. D. Cuong et al., “28-Nor-oleanane- type triterpene saponins from Camellia japonica and their inhibitory activity on LPS-induced NO production in macro- phage RAW264.7 cells,” Bioorganic and Medicinal Chemistry Letters, vol. 20, no. 24, pp. 7435–7439, 2010. Jiangsu New Medical College, The Chinese Medicine Dictionary, Shanghai People’s Publishing House, Shanghai, China, 1977. I. Kubo, T. Kamikawa, and T. Kubota, “Studies on constituents of Isodon japonicus Hara. The structures and absolute stereochem- istry of isodonol, trichodonin and epinodosin,” Tetrahedron, vol. 30, no. 5, pp. 615–622, 1974. T. Kubota and I. Kubo, “A new bitter principle of Isodon japonicus hara,” Tetrahedron Letters, vol. 8, no. 38, pp. 3781–3784, 1967. E. Fujita, T. Fujita, M. Taoka, H. Katayama, and M. Shibuya, “The structure and absolute configuration of sodoponin and epinodosinol, new minor diterpenoids of Isodon japonicus,” Tetrahedron Letters, vol. 11, no. 6, pp. 421–424, 1970. L. Wang, X. Q. Zhang, Z. Q. Yin, Y. Wang, and W. Ye, “Two new amaryllidaceae alkaloids from the bulbs of Lycoris radiata,” Chemical and Pharmaceutical Bulletin, vol. 57, no. 6, pp. 610–611, 2009. L. Wang, Z. Q. Yin, Y. Cai, X. Zhang, X. Yao, and W. Ye, “Amaryllidaceae alkaloids from the bulbs of Lycoris radiata,” Biochemical Systematics and Ecology, vol. 38, no. 3, pp. 444–446, 2010. S. Sancheti, S. Sancheti, S. Lee, J. Lee, and S. Seo, “Screening of korean medicinal plant extracts for α-glucosidase inhibitory activities,” Iranian Journal of Pharmaceutical Research, vol. 10, no. 2, pp. 261–264, 2011. S. Sancheti, S. Sancheti, B.-H. Um, and S.-Y. Seo, “1,2,3,4,6- penta-O-galloyl-β-d-glucose: a cholinesterase inhibitor from Terminalia chebula,” South African Journal of Botany, vol. 76, no. 2, pp. 285–288, 2010. Z. L. Chen, “The history of bayberries,” Journal of Fruit Science, vol. 13, pp. 59–61, 1996.
[67] J. Bao, Y. Cai, M. Sun, G. Wang, and H. Corke, “Anthocyanins, flavonols, and free radical scavenging activity of Chinese Bayberry (Myrica rubra) extracts and their color properties and stability,” Journal of Agricultural and Food Chemistry, vol. 53, no. 6, pp. 2327–2332, 2005.

[68] K. Mochida, “Anti-influenza virus activity of Myrica rubra leaf ethanol extract evaluated using Madin–Darby canine kidney (MDCK) cells,” Bioscience, Biotechnology and Biochemistry, vol. 72, no. 11, pp. 3018–3020, 2008.

[69] N. Sakurai, Y. Yaguchi, T. Hirakawa, M. Nagai, and T. Inoue, “Two myricanol glycosides from Myrica rubra and revision of the structure of isomyricanone,” Phytochemistry, vol. 30, no. 9, pp. 3077–3079, 1991.

[70] F. Daayf, A. Schmitt, and R. R. Bélanger, “Evidence of phytoalexins in cucumber leaves infected with powdery mildew following treatment with leaf extracts of Reynoutria sachalinensis,” Plant Physiology, vol. 113, no. 3, pp. 719–727, 1997.

[71] S. K. Chung, Y. C. Kim, Y. Takaya, K. Terashima, and M. Niwa, “Novel flavonol glycoside, 7-O-methyl mearnsitrin, from Sageretia theezans and its antioxidant effect,” Journal of Agricultural and Food Chemistry, vol. 52, no. 15, pp. 4664–4668, 2004.

[72] S. K. Chung, C.-Y. O. Chen, and J. B. Blumberg, “Flavonoid-rich fraction from Sageretia theezans leaves scavenges reactive oxygen radical species and increases the resistance of low-density lipoprotein to oxidation,” Journal of Medicinal Food, vol. 12, no. 6, pp. 1310–1315, 2009.

[73] H. Bae, R. Kim, Y. Kim et al., “Effects of Schisandra chinensis Bailon (Schizandraceae) on lipopolysaccharide induced lung inflammation in mice,” Journal of Ethnopharmacology, vol. 142, no. 1, pp. 41–47, 2012.

[74] M. Zhang, Y. Cao, F. L. Du, W. Ou-Yang, and Y. Yuan, “Isolation and identification of two new diterpenoid from Rubus corchorifolius L. f.” Yaoxue Xuebao, vol. 42, no. 11, pp. 1155–1158, 2007.

[75] D. K. Kim and O. P. Zee, “A new cyanogenic glycoside from Sorbaria sorbifolia var. stellipila,” Chemical and Pharmaceutical Bulletin, vol. 48, no. 11, pp. 1766–1767, 2000.

[76] X. Li, L. Wu, X. Zang, and J. Zheng, “Studies on chemical constituents of Sorbaria sorbifolia,” Zhongguo Zhongyao Zazhi, vol. 27, no. 11, pp. 842–843, 2002.

[77] X. Zhang, Y. Zhang, L. Guan, Y. Quan, and Q. Sun, “Study on extraction and isolation of active constituents from Sorbaria sorbifolia and antitumor effect of the constituents in vivo,” Journal of Chinese Medicinal Materials, vol. 27, no. 1, pp. 36–38, 2004.

[78] W. Li, B. Li, and Y. Chen, “Flexuosol A, a new tetrastilbene from Vitis flexuosa,” Journal of Natural Products, vol. 61, no. 5, pp. 646–647, 1998.