Antioxidant, Anti-inflammatory, and Antiproliferative Activities of *Taxillus sutchuenensis*

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ABSTRACT.

Inflammation is related to several chronic diseases including cancer and atherosclerosis. *Taxillus sutchuenensis (Lecomte) Danser* is a special folk medicinal plant in Taiwan. The aim of this study was to evaluate the antioxidant, anti-inflammatory, and antiproliferative activities of the aqueous-ethanol extract from the *T. sutchuenensis* (AETS) and fractions. TEAC, DPPH radical, total phenolic compounds, total flavonoid content, inhibition on NO productions by LPS-induced RAW264.7 cell, and inhibition on cancer cell proliferation were employed. Among all fractions, ethyl-acetate (EA) fraction showed the highest TEAC and DPPH radical scavenging activities, respectively. EA fraction also had highest contents of polyphenol and flavonoid contents. EA fractions also decreased the LPS-induced NO production and expressions of iNOS and COX-2 in RAW264.7 cells. The antiproliferative activities of the aqueous/ethanol extract and fractions were studied *in vitro* using A549 cells, and the results were consistent with their antioxidant capacities. EA fractions had the highest antiproliferative activity with an IC_{50} of 454.38 ±1.48 μg/mL. Quercetin also had good pharmacology activity in the antioxidant, anti-inflammation, and antiproliferation. Quercetin might be an important bioactive compound in the *T. sutchuenensis*. The above experimental data indicated that the *T. sutchuenensis* is a potent antioxidant medicinal plant, and such efficacy may be mainly attributed to its polyphenolic compound.

Keywords: Chinese medicine; *Taxillus sutchuenensis*; Antioxidant Activity; Anti-inflammatory activity; Antiproliferation;

INTRODUCTION

It is commonly accepted that in a situation of oxidative stress, reactive oxygen species (ROS) such as superoxide ($O2^{\cdot -}$, OOH), hydroxyl ($OH^{\cdot}$) and peroxyl ($ROO^{\cdot}$) radicals are generated. The reactive oxygen molecules play an important role related to the degenerative or pathological processes of various serious diseases such as aging, cancer, coronary heart disease, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, and inflammation (Halliwell, 2007). The use of traditional medicine is widespread and plants still present a large source of natural antioxidants that might serve as leads for the development of novel drugs. Several anti-inflammatory, digestive, antinecrotic, neuroprotective, and hepatoprotective drugs have recently been shown to have an antioxidant and/or antiradical scavenging mechanism as part of their activity (Raj et al., 1999; Lai et al., 2009; Lai et al., 2010a).

Inflammation has been n shown to be associated with a number of chronic diseases, including asthma, rheumatoid arthritis, inflammatory bowel disease, atherosclerosis, and Alzheimer's disease, and also has a role in various human cancers (Lin & Tang, 2008). One of the major factors involved in the inflammation response is nitric oxide synthase (iNOS), which is induced by lipopolysaccharide (LPS) and various inflammatory mediator cytokines such as interferons, interleukins and tumor necrosis factor (TNF)-α (Wang, Zhou & Lin, 2011).

Taxillus sutchuenensis (Lecomte) Danser is a parasitic plant that attacks the plant including species of Aceraceae, Anacardiaceae, Euphorbiaceae, Fabaceae, Fagaceae, Juglandaceae, Moraceae, Rosaceae, and Rutaceae, which is called "Sang Ji Sheng" in Taiwan, and the whole plant (stems and leaves) has been traditionally used for the treatment of rheumatoid arthralgia, threatened abortion and hypertension and also been applied as an anti-obesity herbal medicine (Wang, et al., 2006). Chemical and biological

studies of Loranthaceous plants of several species in the family are well known as traditional medicines (Gong et al., 1996). In this study, we further investigated on the antioxidant, inflammatory, and antiproliferative activities of the AETS and fractions by characterizing their antioxidative potencies, polyphenol contents, anti-inflammatory and cancer growth inhibition activities. Additionally, an HPLC fingerprint of the extract was also established in this study.

MATERIAS AND METHODS

Chemicals

All solvents used were purchased from Merck (Darmstadt, Germany). Folin and Ciocalteu’s phenol reagent, sodium carbonate, catechin, 1,1 dipheny l-2-picrylhydrazyl radical (DPPH・), ABTS, 2,2’-azinobis (3-ethylbenzothiazoline-6-sulfonic acid), quercetin, Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid), MTT [3-(4,5-dimethylthiazol-2-yl) -2, 5-diphenyltetrazolium bromide] and LPS (lipopolysaccharide) were purchased from Sigma Aldrich Ltd (Steinheim, Germany). Anti-iNOS, anti-COX-2, and anti-β-actin antibodies (Abcam, Cambridge, UK) were obtained as indicated.

Plant materials

Plant materials were collected from Nantou country in Taiwan. They were identified and authenticated by Dr. Yuan-Shiun Chang, Professor, School of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, College of Pharmacy, China Medical University. A plant specimen was deposited in the Institute.

Extraction and Fractionation

The coarse powder of *T. sutchuenensis* (5 kg) was extracted with 50% aqueous/ethanol three times. The extract was evaporated under reduced pressure using a rotavapor, and then stored under light protection. A yield equivalent to 15.51 % of the original weight was obtained. Next, AETS (775.51 g) was dissolved and suspended in 100 ml of water in a separatory funnel prior to being partitioned in sequence with *n*-hexane, chloroform, ethyl acetate and *n*-butanol (800 mL each for three times). Under reduced pressure, fractions were yielded and collected: *n*-hexane fraction（105.92 g, 13.66%), chloroform fraction (189.38 g, 24.42%), ethyl acetate fraction (278.79 g, 35.95%), *n*-butanol fraction (102.44 g, 13.21%) and aqueous fraction (98.95 g, 12.76%). All extracts were stored in the refrigerator before the use.

Fingerprint Analysis by HPLC

The HPLC system consisted of a Shimadzu (Kyoto, Japan) LC-10ATvp liquid chromatography equipped with a DGU-14A degasser, a SIL-10ADvp auto injector, an SPD-M10Avp diode array detector, and an SCL-10Avp system controller. Peak areas were calculated using Shimadzu Class-LC10 software (Version 6.12 sp5). The column was a GL Science inertsil ODS-2 (5 µm，4.6 × 250 mm) column. The samples were analyzed by HPLC on a inertsil ODS-2 column and detected at 254 nm with methanol / 0.2% phosphate solution (54: 46, *v/v*) as the mobile phase at a flow rate of 0.8 mL/min.

Determination of antioxidant activity by ABTS$^{\cdot+}$ *scavenging ability*

The ABTS$^{\cdot+}$ scavenging ability was determined according to the method of Huang *et al*., (2008). Aqueous solution of ABTS (7 mM) was oxidized with potassium peroxodisulfate (2.45 mM) for 16 hrs in the dark at room temperature. The ABTS$^{\cdot+}$

solution was diluted with 95% ethanol to an absorbance of 0.75 ± 0.05 at 734 nm (Beckman UV-Vis spectrophotometer, Model DU640B). An aliquot (20 μL) of each sample (125 μg/mL) was mixed with 180 μL $ABTS^{\cdot+}$ solution and the absorbance was read at 734 nm after 1 min. Trolox was used as a reference standard.

Determination of antioxidant activity by DPPH radical scavenging ability

The effects of crude extracts and positive controls (BHT) on DPPH radicals were estimated according to the method of Huang *et al*., (2008). Aliquot (20 μL) of crude extracts at various concentrations were each mixed with 100 mM Tris-HCl buffer (80 μL, pH 7.4) and then with 100 μL of DPPH in ethanol to a final concentration of 250 μM. The mixture was shaken vigorously and left to stand at room temperature for 20 min in the dark. The absorbance of the reaction solution was measured spectrophotometrically at 517 nm. The percentages of DPPH decolorization of the samples were calculated according to the equation: % decolorization = $[1- (ABS_{sample} /ABS_{control})] \times 100$. EC_{50} value was the effective concentration at which DPPH radicals were scavenged by 50% and was obtained by interpolation from linear regression analysis.

Determination of total polyphenol content

The total polyphenol contents of crude extracts were determined according to the method of Huang *et al* (2008). 20 μL of each extract was added to 200 μL distilled water and 40 μL of Folin-Ciocalteu reagent. The mixture was allowed to stand at room temperature for 5 min and then 40 μL of 20 % sodium carbonate was added to the mixture. The resulting blue complex was then measured at 680 nm. Catechin was used

as a standard for the calibration curve. The polyphenol content was calibrated using the linear equation based on the calibration curve. The total polyphenol content was expressed as mg catechin equivalence (CE)/g dry weight.

Determination of Total Flavonoid Content

The flavonoid content was determined according to the method of Lamaison and Carnet (1990). 100 μL aliquots of the extract and fractions were added to equal volumes of 2% $AlCl_3 \cdot 6H_2O$ (2 g in 100 mL methanol) solutions. The mixtures were shaken vigorously and left incubating for 10 minutes before the absorbance was read at 430 nm. Rutin was used as standard for the calibration curve, by which a linear equation was derived to determine total flavonoid contents of the samples. Total flavonoid data were expressed in mg of rutin equivalents per gram of dry weight.

Cell culture

A murine macrophage cell line RAW264.7 (BCRC No. 60001) and A549 were purchased from the Bioresources Collection and Research Center (BCRC) of the Food Industry Research and Development Institute (Hsinchu, Taiwan). Cells were cultured in plastic dishes containing Dulbecco's Modified Eagle Medium (DMEM, Sigma, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS, Sigma, USA) in a CO_2 incubator (5% CO_2 in air) at 37°C and subcultured every 3 days at a dilution of 1:5 using 0.05% trypsin–0.02% EDTA in Ca^{2+}-, Mg^{2+}- free phosphate-buffered saline (DPBS).

Cell viability

Raw 264.7 cells (2 x 10^5) were cultured in 96-well plate containing DMEM supplemented with 10% FBS for 1 day to become nearly confluent. Then cells were cultured with the AETS extract and fractions in the presence of 100 ng/mL LPS for 24 h. After that, the cells were washed twice with DPBS and incubated with 100 μL of 0.5 mg/mL MTT for 2 h at 37℃ testing for cell viability. A549 were cultured in DMEM medium supplemented with 10% FBS, 100 U/mL of penicillin, 100 mg/mL streptomycin, and 1 mM sodium pyruvate. The cell cultures were maintained at 37℃ in a humidified atmosphere of 5% CO_2 and 95% air. The cells were then treated with the AETS extract and fractions for 24 hr. Each concentration was repeated three times. After a period of incubation, the medium was removed, and then the cells were washed with PBS. The medium was then discarded and 100 μL dimethyl sulfoxide (DMSO) was added. After 30-min incubation, absorbance at 570 nm was read using a microplate reader.

Measurement of Nitric oxide/Nitrite

NO production was indirectly assessed by measuring the nitrite levels in the cultured media determined by a colorimetric method based on the Griess reaction (Chang et al., 2009). The cells were incubated with the AETS extract and fractions in the presence of LPS (100 ng/mL) at 37^0C for 24 h. Then, cells were dispensed into 96-well plates, and 100 μL of each supernatant was mixed with the same volume of Griess reagent (1% sulfanilamide, 0.1% naphthyl ethylenediamine dihydrochloride and 5% phosphoric acid) and incubated at room temperature for 10 min, the absorbance was measured at 540 nm with a Micro-Reader (*Molecular Devices*, *Orleans Drive*, *Sunnyvale*, *CA*). By using sodium nitrite to generate a standard curve, the concentration of nitrite was measured by absorbance at 540 nm.

Western Blotting Analysis

Whole-cell lysates proteins (30 μg of protein) were mixed with an equal volume of electrophoresis sample buffer, and the mixture was then boiled for 10 min. Then, an equal protein content of total cell lysate from control, and different fractions were resolved on 10~12% SDS-PAGE gels. Proteins were then transferred onto nitrocellulose membranes (Millipore, Bedford, MA) by electroblotting using an electroblotting apparatus (Bio-Rad). Nonspecific binding of the membranes was blocked with Tris-buffered saline (TBS) containing 1% (*w/v*) nonfat dry milk and 0.1% (*v/v*) Tween-20 (TBST) for more than 2 h. Membranes were washed with TBST three times for 10 min and then incubated with an appropriate dilution of specific primary antibodies in TBST overnight at 4 °C. The membranes were washed with TBST and then incubated with an appropriate secondary antibody (horseradish peroxidase-conjugated, goat anti-mouse, or anti-rabbit IgG) for 1 h. After washing the membrane three times for 10 min in TBST, the bands were visualized using ECL reagents (Millipore, Billerica, MA). Band intensity on scanned films was quantified using Kodak Molecular imaging (MI) software and expressed as relative intensity compared with control.

Statistical analysis

Data are expressed as mean ± S.D. Statistical evaluation was carried out by one-way analysis of variance (ANOVA followed by Scheffe's multiple range test). Statistical significance is expressed as $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

RESULTS AND DISCUSSION

Fingerprint Analysis by HPLC

To establish the fingerprint chromatogram for the quality control of HF and EA fraction in the AETS. Quercetin was used as marker. The separation of quercetin was carried out by solvent partition and high performance liquid chromatography (HPLC). Quercetin was identified by HPLC and photodiode-array detector as shown in Fig. 1. According to the plot of peak-area ratio (y) vs. concentration (x, μg/mL), the regression equations of the constituents and their correlation coefficients (r) was determined $y = 368857x - 44871$ ($r^2 = 0.9998$). The content of quercetin in EA fraction was consistently high (1.458 mg/ g dry weight), respectively (Fig. 1C). In addition, we can't find quercetin in HF extract fraction. These results evaluated that HF fraction of *Taxillus sutchuensis* might contain other unknown compounds (Fig. 1B).

Trolox Equivalent Antioxidant Capacity

Table 1 shows TEAC values of the AETS extract and fractions. TEAC value of the AETS extract was 57.04 ±0.58 mM /mg extract. As for the fractions, the EA fraction exhibited the strongest antioxidant activity (130.23 ±1.07 mM /mg extract), followed by chloroform fraction (96.77 ±1.38 mM /mg extract), water fraction (69.73 ±0.26 mM /mg extract), *n*-butanol fraction (37.02 ±0.19 mM /mg extract), and *n*-hexane fraction (11.74 ±0.43 mM /mg extract).

Scavenging Activity Against 1, 1-Diphenyl-2-Picrylhydrazyl Radical

A freshly prepared DPPH solution is dark purple in color with a maximum

absorption at 517 nm. This color generally fades and disappears when an antioxidant is present in the solution. The reason behind this is that antioxidant molecules can scavenge DPPH free radicals by supplying protons or by donating electrons, converting them to colorless products. The EA fractions exhibited the strongest antioxidant activities in scavenging DPPH radicals, with IC_{50} values of 93.32 ± 0.94 mg/mL respectively (Table 1).

Determination of Total Phenolic and Total Flavonoid Contents in the Plant Extract and Fractions

Plants containing polyphenols have been reported to possess strong antioxidant activities (Raj et al., 1999). Total Phenolic Content was expressed as mg of catechin equivalent per gram of dry weight. The results showed that the EA and chloroform fractions had the highest phenolic contents of 663.67± 3.47 and 450.45 ± 2.41 mg CE/g, respectively. However, phenolic contents in the hydrophilic fractions were low. The total phenolic content of AETS was 254.06 ± 2.39 mg CE/g (Table 1). Total flavonoid content was expressed as mg of rutin equivalent per gram of dry weight. The results revealed that the total flavonoid contents of the extract and fractions varied from 0.37 ± 0.25 to 94.11 ± 1.14 mg RE/g. Among all the fractions, the EA fraction had the highest total flavonoid content of 94.11 ± 1.14 mg RE/g. Phenols and flavonoids are common groups of polyphenolic compounds. Polyphenolic compounds have important roles in stabilizing lipid peroxidation due to their antioxidative activities (Raj et al., 1999). Many studies have indicated that antioxidant capacities of flavonoids are due to the number and position of hydroxyl groups in their structures (Heim *et al*., 2002).

Relationship between Total Antioxidant Power with Respect to Total Phenolic and Total Flavonoid Contents

Correlation coefficients (R^2) of the total antioxidant power with respect to total phenols and total flavonoid contents of the AETS extract and fractions were estimated in this study. The correlation coefficient (R^2) of TEAC and total phenolic content was 0.519. The R^2 value of TEAC and total flavonid content was 0.352. The results revealed high correlations between TEAC and total phenolic contents. Therefore, the higher the TEAC activity, the higher the total phenolic contents in the samples (data not shown).

Effect of the AETS extract and fractions on LPS-induced NO Production in Macrophages

The effect of the AETS extract and fractions on RAW264.7 cell viability was determined by a MTT assay. Cells cultured with the AETS extract and fractions at the concentrations (0, 62.5, 125, and 250 μg/mL) used in the presence of 100 ng/mL LPS for 24 h did not change cell viability, significantly (Fig. 2A). And when RAW264.7 macrophages were incubated for 24 h with 100 ng/mL of LPS in the presence of quercetin at the 5, 10, and **2**0 μg/mL concentrations, quercetin did not change cell viability (Fig. 2A).

In the present study, effects of the AETS extract and fractions on LPS-induced NO production in RAW 264.7 macrophages were investigated. Nitrite accumulated in the culture medium was estimated by the Griess reaction as an index for NO release from the cells. After treatment with LPS (100 ng/mL) for 24 h, the nitrite concentration increased in the medium. When RAW264.7 macrophages were treated with different concentrations of the AETS extract and fractions together with LPS for 24 h, the the AETS extract and fractions inhibited nitrite production significantly (Fig. 2B). When RAW264.7 macrophages were treated with different concentrations of *n*-hexane,

Chloroform, and EA fraction (0, 62.5, 125, and 250 μg/mL) together with LPS (100 ng/mL) for 24 h, a significant concentration-dependent inhibition of nitrite production was detected. There was either a significant decrease in the nitrite production of group treated with 62.5 μg/mL quercetin ($p < 0.05$), or highly significant decrease of groups treated respectively with 10 and 20 μg/mL of quercetin when compared with the LPS-alone group ($p < 0.001$).

Inhibition of LPS-induced iNOS and COX-2 Protein by ethyl acetate fraction and qucrcetin.

The results showed that incubation with EA fraction and quercetin in the presence of LPS for 24 h inhibited iNOS and COX-2 protein expression in mouse macrophage RAW264.7 cells in a dose-dependent manner (Fig. 3A). The intensity of protein bands were analyzed and showed an average of 77.2% and 23.5% down-regulation of iNOS and COX-2 proteins, respectively, after treatment with EA fraction at 250 μg/mL compared with the LPS-alone (Fig. 3B). And the intensity of protein bands were analyzed and showed an average of 68.8% and 19.2% down-regulation of iNOS and COX-2 proteins, respectively, after treatment with quercetin at 20 μg/mL compared with the LPS-alone.

Acute inflammation is part of the defense response, but chronic inflammation has been found to mediate a wide variety of diseases, including cardiovascular diseases, cancer, diabetes, arthritis, Alzheimer's disease, pulmonary diseases, and autoimmune diseases. Inflammation leads to up-regulation of a series of enzymes in affected areas. Inducible nitric oxide synthase (iNOS) catalyzes the formation of nitric oxide (NO) from L-arginine. High concentration of NO are found to play important roles in inflammation and carcinogenesis. INOS can be induced by bacterial endotoxic

lipopolysaccahride (LPS), interferon-γ (IFN-γ), and a variety of pro-inflammatory cytokines (Wang, Zhou & Lin, 2011). As many of these conditions exhibit rapid onset and development, often resulting in the failure of conventional anti-inflammatory therapies and extremely high mortality rates, a simultaneous suppression of NO production pathways, as shown by *n*-hexane fraction, chloroform fraction, EA fraction and quercetin may satisfy the control of the rapid progression of the inflammatory process.

Cell Viability

MTT assay was used to investigate whether the AETS extract and fractions affected the viability of A549 cells. The viabilities of human lung cancer A549 cells treated with 200, 400, 600, 800, and 1000 μg/mL of the extract and fractions were assayed by MTT. After exposing A549 cells to any of the samples with various concentrations, the cell viabilities decreased significantly as compared to the control (100%), indicating cytotoxic effect on A549 cells. Among all the fractions, EA (IC_{50}= 454.38 ± 1.48) fractions showed excellent inhibitory effects on A549 cells, as shown in Table 2. And quercetin also showed excellent inhibitory effects on A549 cells (IC_{50}= 27.48 ± 0.32).

In Taiwan, the whole plant (stems and leaves) are used to treat tumors, arthritis and to relieve pain. In this study, we attempted to use scientific methods to elucidate the antioxidant, anti-inflammatory and anti-cancer properties of *T. sutchuenensis* to justify its use in folk medicine.

Phenolic and flavonoid contents were not only indicated in this experiment to be directly proportional with antioxidant activity (Huang et al. 2008), these phytochemicals may also possess unique or synergic activities on the inhibition of tumor cell proliferation *in vitro*. In previous phytochemical investigations, the parasitic plant

Scurrula atropurpurea, the main biologically active substance for the treatment of cancer as a folk medicine might be octadeca-8,10,12-triynoic acid, catechin, epicatechin, epicatechin-3-*O*-gallate and epigallocatechin-3-*O*-gallate, octadeca-8,10-diynoic acid, and (*Z*)-octadec-12-ene-8,10-diynoic acid may also be active substances used locally in Indonesia (Kazuyoshi, *et al*., 2003). In this studies *T. sutchuenensis*is rich in polyphenolic compounds, it may be developed into an antioxidant, inflammation, and anticancer agent in the future.

CONCLUSION

This study revealed that EA fractions of *T. sutchuenensis* exhibited good antioxidant activities, anti-inflammatory activities and inhibited the growth of A549 cell. These activities may be attributed to the high polyphenolic contents in these fractions. This study also demonstrated that *T. sutchuenensis* had a wide safety dosage range. Furthermore, people are more and more interested in the understanding of antioxidative activities of herbs for the purpose of improving health and preventing of chronic diseases or cancers.

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References

Kazuyoshi O., Hendig W., Mutsuko M., Masahiro I., Made Sri P., Partomuan S., and

Hirotaka S., Indonesian Medicinal Plants. XXV.1) Cancer Cell Invasion Inhibitory Effects of Chemical Constituents in the Parasitic Plant *Scurrula atropurpurea* (Loranthaceae) *Chem. Pharm. Bull.* 51(3): 343—345 , 2003.

Amarowicz, R., R.B. Pegg, P.R. Moghaddam, B. Barl and J.A. Weil. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chem*. 84: 551–562, 2004.

Chang, T.N., G.J. Huang, Y.L. Ho, S.S. Huang, H.Y. Chang and Y.S. Chang. Antioxidant and Antiproliferative Activities of *Crossostephium chinensis* (L.) Makino. *American Journal of Chinese Medicine.* 37: 797-814, 2009.

Gong, Z.N., Z.T. Wang, L.S. Xu, G.J. Xu, and J.R. Wu, Studies on medicinal plants of Loranthaceae in China. *Chinese Wild Plant Resources* 1: 11–15,1996.

Halliwell, B. Oxidative stress and cancer: have we moved forward? *Biochem. J.* 401: 1-11, 2007.

Heim, K. E., A.R. Tagliaferro and D.J. Bobilya. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *J. Nutr. Biochem*. 13(10): 572–584, 2002.

Huang, S.S., G.J. Huang, Y.L. Ho, Y.H. Lin, H.J. Hung, T.N. Chang, M.J. Chang, J.J. Chen and Y.S. Chang. Antioxidant and anti-proliferative activities of the four *Hydrocotyle* species from Taiwan. *Bot. Stud.* 49: 311-322, 2008.

Huang, Y. S., and Ho, S. C. (2010). Polymethoxy flavones are responsible for the anti-inflammatory activity of citrus fruit peel. *Food Chem.*119: 868-873.

Jeong, C.H., G.N. Choi, J.H. Kim, J.H. Kwak, D.O. Kim, Y.J. Kim and H.J. Heo. Antioxidant activities from the aerial parts of *Platycodon grandiflorum*. *Food Chem.* 118: 278-282, 2010.

Karin C.S.L., Yan S.L., Roberts M.F. and Phillipson J.D. Qucrcetin rhamnosides from *Kalanchoe gracilis*. *J. Nat. Prod.* 52: 970-974, 1989.

Kim, K.S., S. Lee, Y.S. Lee, S.H. Jung, Y. Park, K.H. Shin and B.K. Kim. Antioxidant activities of the extracts from the herbs of *Artemisia apiacea*. *J. Ethnopharmacol.* 85: 69–72, 2003.

Lai, S.C., W.H. Peng, S.C. Huang, Y.L. Ho, T.H. Huang, Z.R. Lai and Y.S. Chang. Analgesic and anti-inflammatory activities of methanol extract from *Desmodium triflorum* DC in mice. *Am. J. Chin. Med.* 37: 573-588, 2009.

Lai, S.C., Y.L. Ho, S.C. Huang, T.H. Huang, Z.R. Lai, C. R. Wu, K. Y. Lian, and Y.S. Chang. Antioxidant and antiproliferative activities of *Desmodium triflorum* DC. *Am. J. Chin. Med.* 38: 329-342, 2010a.

Lai, Z.R., W.H. Peng, Y.L. Ho, S.C. Huang, T.H. Huang, S.C. Lai, Y.R. Ku, J.C. Tsai, C.Y. Wang and Y.S. Chang. Analgesic and anti-inflammatory activities of the methanol extract of *Kalanchoe gracilis (L.)* DC stem in mice. *Am. J. Chin. Med.* 38: 529-546, 2010b.

Lamaison, J.L.C. and A. Carnet. Teneurs en principaux flavonoids des fleurs de *Crataegeus monogyna* Jacq et de *Crataegeus laevigata* (Poiret D. C) en fonction de la vegetation. *Pharm. Acta. Helv.* 65: 315-320, 1990.

Li X., X. Wu, and L. Huang. Correlation between antioxidant activities and phenolic contents of Radix Angelicae Sinensis (Danggui). *Molecules* 14: 5349-5361, 2009.

Lin, J. Y., & Tang, C.Y. (2008). Strawberry, loquat, mulberry, and bitter melon juices exhibit prophylactic effects on LPS-induced inflammation using murine peritoneal macrophages. *Food Chem.* 107: 1587-1596.

Raj, K.J. and K. Shalini. Flavonoids - a review of biological activities. *Indian Drugs* 36: 668-676, 1999.

Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and R.E. Catherine. Antioxidant activity applying an improved ABTS radical cation decolorization assay.

Free Radic. Biol. 26: 1231–1237, 1999.

Wang, Y., S.Y. Zhang, X.F. Ma and W.X. Tian. Potent inhibition of fatty acid synthase byparasitic loranthus (*Taxillus chinensis* (DC.) Danser) and its constituent avicularin. *J. Enzym. Inhib. Med.Chem.* 21: 87–93, 2006.

Wang, A. Y., Zhou, M. Y., & Lin, W.C. (2011). Antioxidative and anti-inflammatory properties of *Citrus sulcata* extracts. *Food Chem.*124: *958-963.*

Wu, P.L., Y.L. Hsu, T.S. Wu, K.F. Bastow and K.H. Lee. Kalanchosides A-C, new cytotoxic bufadienolides from the aerial parts of *Kalanchoe gracilis*. *Org. Lett.* 23: 5207-5210, 2006.

Wu, C.R., M.Y. Huang, Y.T. Lin, H.Y. Ju and H. Ching. Antioxidant properties of Cortex Fraxini and its simple coumarins. *Food Chem.* 104: 1464–1471, 2007.