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Bioavailability, Distribution, and Antioxidative Effects of Selected Triterpenes in Mice

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ABSTRACT: This study analyzed the content of eight triterpenes (oleanolic acid, ursolic acid, arjunolic acid, asiatic acid, boswellic acid, corosolic acid, madecassic acid, and maslinic acid) in ten vegetables and eight fruits. These compounds at 0.5% were supplied to mice for 4 or 8 weeks. The bioavailability, tissue distribution, and antioxidative protection of these triterpenes were examined. Results showed that triterpenes were detected in eight vegetables and six fruits. Basil and brown mustard contained seven test triterpenes, in the range of 14−102 mg/100 g dry weight. The level of each triterpene in plasma, brain, heart, liver, kidney, colon, and bladder increased as the feeding period was increased from 4 weeks to 8 weeks ($P < 0.05$). Renal homogenates from mice with triterpene intake had greater antioxidative effects against glucose-induced glutathione loss and malondialdehyde and oxidized glutathione production when compared with those from control groups ($P < 0.05$). These data support that these triterpenes were absorbed and deposited in their intact forms, which in turn exerted in vivo antioxidative protection.

KEYWORDS: *pentacyclic triterpenes, bioavailability, vegetable, antioxidative*

INTRODUCTION

Oleanolic acid, ursolic acid, arjunolic acid, asiatic acid, boswellic acid, corosolic acid, madecassic acid, and maslinic acid are pentacyclic triterpenes. It has been documented that oleanolic acid and ursolic acid possess many in vivo bioactivities including antioxidative, antiinflammatory, and antiglycative activities,[1,2] which support their application in disease prevention and alleviation.[3−5] Recently, more attention has been paid to the bioactivities of the other six triterpenes. Those studies suggest that these compounds are potent protective agents with multiple actions such as regulating immune function and having a role in the nuclear factor-κB signaling pathway.[6−9]

The recovery of oleanolic acid and ursolic acid in blood after iv injection in human subjects has been confirmed.[10,11] However, tissue distribution of these compounds in their intact forms after oral intake remains unknown. Although the observed bioactivities of these triterpenes could be ascribed to their metabolites after being absorbed and metabolized, the appearance of their intact forms in circulation and/or organs may further ensure their deposition and ability to exert local or systemic protective effects and actions. Thus, an animal study was designed to investigate the bioavailability and tissue distribution of these triterpenes after dietary intake.

The above triterpenes occur naturally in many plants, especially herbs.[12−14] Less information is available regarding their content in fresh vegetables and fruits, which people are more likely to consume. To increase the sources and intake of these triterpenes, their content in several locally available vegetables and fruits was analyzed.

MATERIALS AND METHODS

Chemicals. Oleanolic acid (OA, 99%), ursolic acid (UA, 98%), arjunolic acid (ARA, 98%), asiatic acid (ASA, 98.5%), boswellic acid (BOA, 98%), corosolic acid (CA, 99%), madecassic acid (MCA, 99%), and maslinic acid (MA, 98.5%) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Their structures are shown in Figure 1. All chemicals used in these measurements were of the highest purity commercially available.

Analysis of Triterpene Content in Vegetables and Fruits. Ten fresh vegetables and eight fresh fruits analyzed for the content of eight triterpenes. Test vegetables included gynura (*Gynura bicolor* DC), basil (*Ocimum basilicum*), brown mustard (*Brassica juncea*), mahogany (*Toona sinensis*), madeira vine (*Anredera cordifilia* Moq.), daylily (*Hemerocallis fulva* L.), wild lettuce (*Lactuca indica*), balsam pear (*Momordica charantia*), water convoevueus (*Ipomoea aquatic*), and spinach (*Spinacia oleracea*). Test fruits were mulberry (*Mours alba* L.), carambola (*Averrhoa carambola*), waxapple (*Syzygium samarangensem*), mango (*Mangifera indica* L.), juiube (*Zizyphus mauritiana*), calamondin (*Citrus microcarpa* Bonge), guava (*Psidium guajava*), and loquat (*Eriobotrya japonica*). These vegetables and fruits, harvested in Spring 2012 were purchased from four farms in Taichung City, Taiwan. A 50 g edible portion was chopped, homogenized in a Waring blender, and freeze-dried to a fine powder. A 5 g dry sample was extracted with ethyl ether and methanol in a Soxhlet apparatus. After solvents were evaporated, an equal volume of distilled water was added, followed by extraction with *n*-butanol. After fractionation, the sample was separated by thin layer chromatography on 20 cm ×20 cm glass plates coated with a 0.25 mm layer of Merck silica gel 60H in the solvent system $CHCl_3$/MeOH (97:3, v/v). The individual fractions were localized on plates by comparison with standards and visualized

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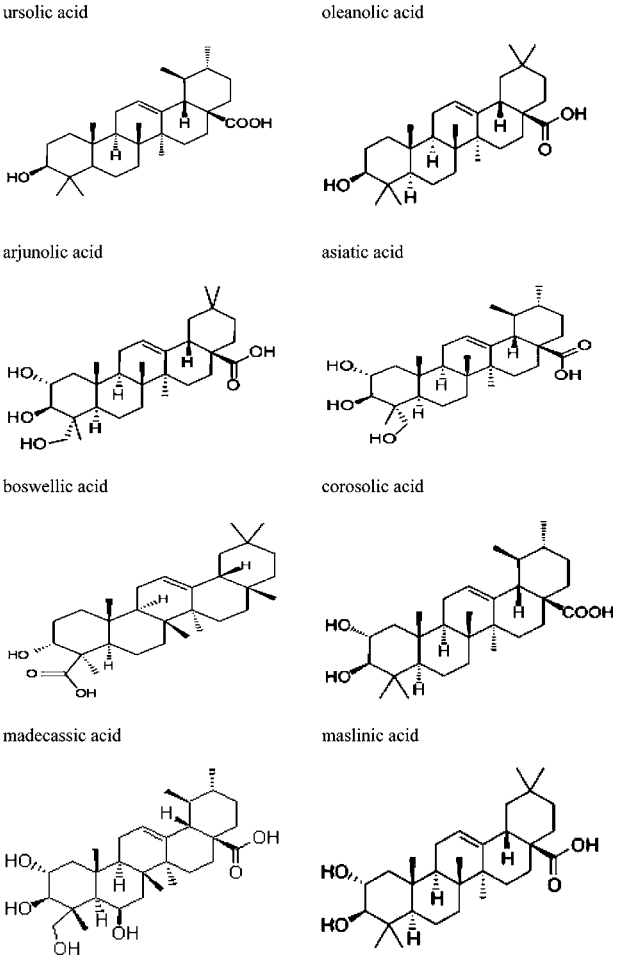

Figure 1. Structure of eight triterpenes.

by spraying with 50% H_2SO_4, followed by heating with a hot-air stream. The method of Lin et al.[15] was used for triterpene identification and quantification by a GC-MS system consisting of an Agilent 6890 Series GC and a TOF mass spectroscope (Micro Mass GCT, time-of-flight, JAS, Eindhoven, Netherlands). Mass spectra were processed in EI mode using 325 °C as interface temperature. The mass spectrometer was operated in positive mode at 70 eV, and the scanning range was m/z 29−650 atomic mass units. GC model 6890 was equipped with an on-column injector and a DB-5 capillary column (30 m × 0.32 mm, i.d., 0.25 μm thickness, Agilent J&W Scientific, Folsom, CA), and helium was used as the carrier gas at a flow rate of 1 mL/min. The individual components were identified and compared with published data on the basis of the mass fragments and e/Z values.

Animal Experiments. Three-week-old male C57BL/6 mice were obtained from National Laboratory Animal Center (National Science Council, Taipei City, Taiwan). Use of the mice was reviewed and approved by a China Medical University animal care committee. Mice were housed on a 12-h light−12-h dark schedule and fed with mouse standard diet for one week acclimation. Mice were then divided into nine groups: one consumed normal diet, and the others consumed a normal diet plus 0.5% OA, UA, ARA, ASA, BOA, CA, MCA, or MA prepared by mixing 0.5 g of target compound with 99.5 g of powder diet. All mice had free access to food and water at all times. Consumed water volume, feed, and body weight were recorded. After a 4- or 8-week supplementation, mice were killed with carbon dioxide after an overnight fast. Blood was collected directly after the head was severed, and plasma was separated from erythrocytes immediately. Brain, heart, lung, liver, kidney, spleen, colon, and bladder were collected and weighed. Each organ (0.1 g sample) was homogenized in 2 mL of ice cold phosphate-buffered saline (PBS, pH 7.2). The homogenate was further passed through Whatman No. 1 filter paper, and the filtrate was collected. The protein concentration of the organ filtrate was determined by the method of Lowry et al.[16] using bovine serum albumin as a standard. In all experiments, the sample was diluted to a final concentration of 1 g protein/L using PBS, pH 7.2.

Determination of Triterpene Content in Plasma and Tissues. The content of target compound in plasma or organ was analyzed by the method described in Song et al.[17] and Gerbeth et al.[18] The plasma or tissue homogenate, 100 μL, was mixed with glycyrrhetinic acid as an internal standard (10 μL of 2.0 μg/mL methanol solution) and followed by extracting with 1 mL of ethyl acetate and centrifuging at 3500g for 10 min at 4 °C. After evaporation by nitrogen gas, the residue was reconstituted in 100 μL of methanol and water, the HPLC mobile phase. Identification and quantification was processed by an HPLC-MS system (Agilent Corp, Waldbronn, Germany), in which an Agilent 1100 series HPLC system equipped with a BDS RP-C18 column (100 mm × 4 mm, 3 μm, Thermo Electron, Bellafonte, PA) and a diode array and fluorescence detector was used. An ion-trap mass spectrometer equipped with an electrospray ionization source was coupled with HPLC, and a negative single ion mode was used for analysis. The limits of detections were 0.1 μg/mL plasma or 0.1 μg/g tissue. The relative standard deviations of precision and accuracy for test compounds were less than 5%.

Antioxidative Effects of Triterpenes in the Renal Homogenate. Glucose at 50 mmol/L was used to initiate lipid oxidation in the

Table 1. Content (mg/100 g dry weight) of Eight Triterpenes in Eight Vegetables and Six Fruits Harvested in Spring 2012 (data are mean ± SD (n = 10))

	OA	UA	ARA	ASA	MCA	BOA	CA	MA
gynura	–[a]	–	–	55 ± 4	72 ± 9	–	–	39 ± 7
basil	25 ± 8	17 ± 6	61 ± 2	60 ± 6	56 ± 3	–	41 ± 3	32 ± 5
brown mustard	–	14 ± 5	74 ± 8	102 ± 7	32 ± 5	18 ± 3	15 ± 4	33 ± 8
daylily	28 ± 9	19 ± 5	–	64 ± 6	73 ± 3	90 ± 9	26 ± 3	–
balsam pear	18 ± 4	42 ± 5	10 ± 3	–	–	33 ± 4	16 ± 2	–
water convoevueus	–	–	–	–	45 ± 8	26 ± 5	14 ± 4	–
spinach	–	–	17 ± 8	39 ± 8	30 ± 5	–	–	11 ± 4
mahogan	30 ± 7	57 ± 8	8 ± 3	15 ± 4	–	–	73 ± 6	17 ± 3
mulberry	–	–	17 ± 5	–	15 ± 5	–	26 ± 4	–
carambola	9 ± 2	13 ± 4	–	17 ± 2	29 ± 6	–	14 ± 3	–
waxapple	–	–	–	–	–	9 ± 3	11 ± 4	–
mango	–	–	16 ± 4	9 ± 4	–	–	–	–
calamondin	–	–	32 ± 6	25 ± 4	11 ± 4	–	7 ± 3	13 ± 5
guava	–	12 ± 2	15 ± 3	–	–	8 ± 2	–	–

[a]Too low to be detected.

Table 2. Content of Eight Triterpenes in Plasma (μg/mL) or Tissues (μg/g) of Mice That Consumed Target Compounds for 4 or 8 Weeks (data are mean ± SD (n = 10))[a]

	plasma	brain	heart	liver	kidney	colon	bladder
OA, 4 week	–[b,a]	0.5 ± 0.1[a]	2.7 ± 0.6[a]	5.9 ± 0.8[a]	3.0 ± 0.3[a]	3.4 ± 0.7[a]	1.2 ± 0.5[a]
OA, 8 week	0.55 ± 0.08[b]	1.7 ± 0.2[b]	4.2 ± 0.8[b]	10.3 ± 1.4[b]	5.5 ± 0.5[b]	6.0 ± 1.0[b]	3.7 ± 0.4[b]
UA, 4 week	–[a]	0.7 ± 0.1[a]	2.2 ± 0.4[a]	5.2 ± 0.5[a]	3.3 ± 0.4[a]	3.5 ± 0.6[a]	1.1 ± 0.2[a]
UA, 8 week	0.58 ± 0.09[b]	1.6 ± 0.2[b]	3.9 ± 0.7[b]	9.7 ± 0.7[b]	5.9 ± 0.7[b]	6.4 ± 0.9[b]	2.9 ± 0.6[b]
ARA, 4 week	–[a]	0.6 ± 0.2[a]	2.4 ± 0.5[a]	6.4 ± 0.4[a]	2.9 ± 0.2[a]	3.1 ± 0.3[a]	1.4 ± 0.3[a]
ARA, 8 week	0.51 ± 0.07[b]	1.8 ± 0.4[b]	4.8 ± 0.9[b]	13.2 ± 1.0[c]	5.7 ± 0.9[b]	6.3 ± 0.8[b]	3.1 ± 0.5[b]
ASA, 4 week	–[a]	0.4 ± 0.1[a]	2.8 ± 0.6[a]	6.2 ± 0.8[a]	3.4 ± 0.6[a]	3.0 ± 0.6[a]	1.3 ± 0.2[a]
ASA, 8 week	0.46 ± 0.09[b]	1.2 ± 0.3[b]	4.5 ± 0.8[b]	10.4 ± 0.9[b]	6.6 ± 0.8[b]	5.9 ± 0.5[b]	2.9 ± 0.6[b]
MCA, 4 week	–[a]	0.6 ± 0.2[a]	2.6 ± 0.4[a]	5.0 ± 0.3[a]	3.2 ± 0.3[a]	3.6 ± 0.4[a]	1.5 ± 0.3[a]
MCA, 8 week	0.48 ± 0.06[b]	1.7 ± 0.4[b]	3.4 ± 0.5[b]	9.1 ± 0.9[b]	6.3 ± 0.7[b]	6.2 ± 0.7[b]	3.0 ± 0.4[b]
BOA, 4 week	–[a]	0.7 ± 0.2[a]	3.1 ± 0.6[a]	5.2 ± 0.6[a]	3.6 ± 0.5[a]	3.1 ± 0.4[a]	1.3 ± 0.3[a]
BOA, 8 week	0.61 ± 0.08[b]	1.5 ± 0.3[b]	5.0 ± 0.9[b]	11.0 ± 1.2[b]	7.5 ± 0.8[b]	6.8 ± 1.1[b]	2.8 ± 0.7[b]
CA, 4 week	–[a]	1.0 ± 0.3[a]	2.5 ± 0.3[a]	5.8 ± 0.9[a]	3.1 ± 0.5[a]	3.7 ± 0.7[a]	1.0 ± 0.3[a]
CA, 8 week	0.51 ± 0.07[b]	1.9 ± 0.4[b]	4.1 ± 0.6[b]	12.9 ± 1.2[c]	5.6 ± 0.7[b]	6.3 ± 0.6[b]	2.6 ± 0.8[b]
MA, 4 week	–[a]	0.8 ± 0.3[a]	2.7 ± 0.5[a]	5.4 ± 0.6[a]	3.6 ± 0.6[a]	3.5 ± 0.7[a]	1.0 ± 0.2[a]
MA, 8 week	0.47 ± 0.07[b]	1.6 ± 0.5[a]	3.7 ± 0.7[b]	9.6 ± 0.9[b]	6.5 ± 0.8[b]	7.0 ± 0.8[b]	1.9 ± 0.5[a]

[a]Means in a row without a common letter (a or b) differ, $P < 0.05$. [b]Too low to be detected.

renal homogenate. After a 3-day incubation at 37 °C, lipid oxidation was determined by measuring the level of malondialdehyde (MDA), glutathione (GSH), and oxidized glutathione (GSSG) via commercial colorimetric assay kits (OxisResearch, Portland, OR).

Statistical Analysis. The effect of each treatment was analyzed from ten different preparations (n = 10). Data were reported as mean ± standard deviation (SD) and subjected to analysis of variance (ANOVA). Differences among means were determined by the Least Significance Difference Test with significance defined at $P < 0.05$.

RESULTS

As shown in Table 1, seven triterpenes were detected in basil and brown mustard, in the range of 14–102 mg/100 g dry weight. Among test fruits, five triterpenes were measured in calamondin and carambola, in the range of 7–32 mg/100 g dry weight. The content of test triterpenes in wild lettuce, madeira vine, juiube, and loquat was too low to be detected. After an 8 week intake, the plasma level of triterpene was in the range of 0.46–0.61 μg/mL (Table 2). The level of each triterpene in brain, heart, liver, kidney, colon, and bladder increased as the feeding period was increased from 4 weeks to 8 weeks ($P < 0.05$). After an 8 week supplementation, liver had the highest content of each triterpene, followed by kidney and colon. The hepatic level of triterpenes was in the range of 5.0–13.2 μg/g tissue.

As shown in Table 3, glucose treatment led to GSH loss, GSSG formation, and MDA production in the renal homogenate of mice from control groups. Compared with control groups, renal homogenates from mice with target compound intake had a higher GSH level and lower MDA and GSSG production ($P < 0.05$). There was a significant difference in GSH, GSSG, and MDA levels between renal homogenates from the 4 and 8 week feeding period for each triterpene ($P < 0.05$).

Table 3. Effect of Test Triterpenes in Kidney Homogenate from Mice without (control) or with Target Compound Intake for 4 or 8 Weeks against Glucose-Induced Oxidative Stress (data are mean ± SD (n = 10))[a]

	GSH nmol/mg protein	GSSG nmol/mg protein	MDA μmol/mg protein
control, 4 week	2.02 ± 0.13[a]	1.68 ± 0.30[d]	2.72 ± 0.42[d]
control, 8 week	2.19 ± 0.21[a]	1.57 ± 0.26[d]	2.61 ± 0.34[d]
OA, 4 week	4.60 ± 0.34[b]	1.02 ± 0.11[c]	1.63 ± 0.26[c]
OA, 8 week	5.45 ± 0.40[c]	0.54 ± 0.09[b]	1.19 ± 0.31[b]
UA, 4 week	4.78 ± 0.28[b]	1.07 ± 0.12[c]	1.54 ± 0.23[c]
UA, 8 week	5.80 ± 0.31[c]	0.61 ± 0.07[b]	1.09 ± 0.26[b]
ARA, 4 week	4.26 ± 0.20[b]	0.89 ± 0.05[c]	1.15 ± 0.17[b]
ARA, 8 week	5.35 ± 0.37[c]	0.48 ± 0.06[b]	0.71 ± 0.10[a]
ASA, 4 week	4.48 ± 0.19[b]	0.94 ± 0.08[c]	1.50 ± 0.15[c]
ASA, 8 week	5.60 ± 0.24[c]	0.48 ± 0.04[b]	1.15 ± 0.21[b]
MCA, 4 week	4.31 ± 0.26[b]	0.63 ± 0.06[b]	1.23 ± 0.18[b]
MCA, 8 week	5.52 ± 0.33[c]	0.29 ± 0.09[a]	0.79 ± 0.09[a]
BOA, 4 week	4.66 ± 0.32[b]	0.97 ± 0.10[c]	1.18 ± 0.11[b]
BOA, 8 week	5.59 ± 0.18[c]	0.53 ± 0.09[b]	0.66 ± 0.08[a]
CA, 4 week	4.87 ± 0.44[b]	1.03 ± 0.11[c]	1.57 ± 0.23[c]
CA, 8 week	6.05 ± 0.39[c]	0.55 ± 0.10[b]	1.01 ± 0.19[b]
MA, 4 week	4.76 ± 0.29[b]	1.08 ± 0.07[c]	1.48 ± 0.20[c]
MA, 8 week	5.68 ± 0.36[c]	0.59 ± 0.09[b]	1.12 ± 0.16[b]

[a]Means in a row without a common letter (a or b) differ, $P < 0.05$.

DISCUSSION

The presence of test triterpenes in herbs such as *Ligustrum lucidum* Ait. and *Cichorium intybus* L. has been reported.[12−14] Our present study further found that several commonly available vegetables and fruits contained these triterpenes. Thus, these vegetables and fruits are also the sources of triterpenes. Furthermore, we noticed that basil and brown mustard are rich in arjunolic acid, asiatic acid, and madecassic acid. It has been indicated that these three triterpenes possess several protective functions including antioxidative and antiinflammatory activities.[7,19,20] It is possible that these two vegetables, based on their triterpenes levels, might be able to provide healthy benefits.

The recovery or organ distribution of ursolic acid and betulinic acid after iv injection in human and/or animals has been reported.[10,21,22] The results of our present study revealed that the intact form of test triterpene was recovered in blood and tissues after dietary intake. Furthermore, we found that the accumulated level of each triterpene in tissues was increased with an extension of the supplementary period. These findings

indicate that dietary supplements of these compounds were absorbed, metabolized, and deposited in tissues, which in turn exerted their local and/or systemic actions. Many studies reported that dietary intake of these compounds exhibited cardiac, renal, and hepatic protection.[23−25] Because intact forms of triterpenes were available in tissues after oral intake, the observed protective effects from triterpenes in those previous studies are partially ascribed to the recovery of these compounds in their intact forms. In addition, we notified that liver had the highest bioavailability for each triterpene compared with other tissues. It seems that liver is the major organ for triterpene storage and/or metabolism. Although this finding suggests that using these triterpenes for hepatic protection seems possible and feasible, the hepatoxicity of these compounds also needs attention. In addition, the appearance of test triterpenes in brain suggested that these compounds might be able to penetrate the brain−blood barrier after they were absorbed and metabolized. This finding supports the possibility of using these agents for brain protection.

In our present study, high glucose-induced oxidative stress was markedly decreased in the renal homogenate from mice with triterpene intake. The observed antioxidative effects are partially ascribed to the existence of intact triterpenes in the renal homogenate. It is reported that triterpenes possessed nonenzymatic activities such as reducing power and scavenging free radicals. Our data revealed that these compounds could spare GSH. These results once again support that dietary intake of these compounds benefits tissue protection against oxidative injury. It is interesting to find that 8-week ARA and BOA intake led to a renal content of these compounds at 5.5 and 7.0 μg/g tissue. However, their renal homogenates exhibited similar effects in retaining GSH and lowering GSSG and MDA levels. Apparently, the antioxidative effects observed in the renal homogenate could not be solely ascribed to the intact forms of triterpenes. That is, the metabolite(s) of these triterpenes may also contribute to antioxidative protection.

The eight test compounds are pentacyclic triterpenes. Although they have similar structures, they exhibited different bioavailability, tissue distribution, and antioxidative activity. It seems that certain functional groups and/or the position of this group affect the absorption and/or metabolism of a triterpene. So far, more studies are focused on the benefits of ursolic acid and oleanolic acid. Because the other six triterpenes also provided substantial bioavailability, tissue distribution, and antioxidative efficiency, their application should be encouraged.

In conclusion, oleanolic acid, ursolic acid, arjunolic acid, asiatic acid, boswellic acid, corosolic acid, madecassic acid, and maslinic acid were detected in fresh vegetables and fruits locally available in Taiwan. The intake of these triterpenes increased their bioavailability and accumulation in circulation and tissues. Recovery of triterpene from the organ suggests a local or systemic protective effect to benefit the organ's antioxidative defense. These findings link dietary triterpenes and their ability to prevent and/or alleviate diseases.

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Notes

The authors declare no competing financial interest.

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