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Ethanolic Extracts and Isolated Compounds from Small-Leaf Grape (*Vitis thunbergii* var. *taiwaniana*) with Antihypertensive Activities

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ABSTRACT: This study aimed to investigate the antihypertensive effects of ethanolic extracts (EE) and compounds isolated from the small-leaf grape (*Vitis thunbergii* var. *taiwaniana*, VTT). The highest antiangiotensin-converting enzyme (anti-ACE) was found in stem-EE (IC_{50} was 69.5 μg/mL). In spontaneously hypertensive rats (SHRs), stem-EE effectively reduced blood pressure 24 h after administration of a single oral dose or when administered daily for 4 weeks. The isolated compounds, including (+)-vitisin A, ampelopsin C, and (+)-ε-viniferin, were shown to have anti-ACE and vasodilating effects against phenylephrine-induced tensions in an endothelium-intact aortic ring, with (+)-vitisin A being the most effective compound. Compared to control rats, SHRs showed significantly reduced systolic and diastolic blood pressures 24 h after a single oral dose of (+)-vitisin A (10 mg/kg) or captopril (2 mg/kg). These results suggest that the development of functional foods with VTT extracts may be beneficial for regulating blood pressure.

KEYWORDS: *angiotensin converting enzyme, aortic ring relaxation, blood pressure, spontaneously hypertensive rat (SHR), Vitis thunbergii var. taiwaniana (VTT), (+)-vitisin A*

INTRODUCTION

The number of people with hypertension is increasing worldwide. Together with additional risk factors, hypertension can cause cardiovascular diseases, including all types of stroke, ischemic stroke, intracerebral hemorrhage, and subarachnoid hemorrhage. Therefore, hypertension is considered to be the central causative factor in stroke, with approximately 33% of stroke-related deaths attributed to untreated high blood pressure (BP).[1,2] Several successful classes of therapeutic antihypertensive drugs, such as calcium channel blockers, β-blockers, α-blockers, vasodilators, renin−angiotensin receptor system blockers, and angiotensin-converting enzyme (ACE) inhibitors, have been used for clinical treatment.[3] ACE (EC 3.4.15.1) is a dipeptide-liberating exopeptidase that hydrolyzes angiotensin I to angiotensin II, resulting in vasoconstriction and hypertension.[4] ACE inhibitors, which are recommended in treatment guidelines for hypertension, stable coronary artery disease, myocardial infarction, and heart failure, are reported to have a relatively low rate of adverse side effects in patients.[5,6]

However, in recent years, the development of natural, antihypertensive compounds and food components for preventing and treating hypertension has been considered, particularly for prehypertensive patients for whom the use of drugs is not recommended. In the past decades, natural compounds and small peptides of ACE inhibitors have been discovered from herbs, traditional Chinese medicine, fruits, and food proteins. For example, the tripeptides valyl-prolyl-proline (VPP) and isoleucyl-prolyl-proline (IPP) are well-known ACE inhibitory peptides isolated from fermented milk that were shown to be effective in decreasing BP in spontaneously hypertensive rats (SHRs) and also reduced BP in several clinical trials.[7,8] Additionally, pomegranate juice,[9] myricetin galloylglycosides,[10] geraniin,[11] green tea catechins,[12] and extracts from *Taiwanofungus camphoratus* (Chang-Chih),[13] as well as extracts from tissue cultures of *Vitis thunbergii* var. *taiwaniana* (VTT)[14] were also reported to have ACE inhibitory activity. However, although the variation of ACE inhibitory candidates have been detected *in vitro*, it is extremely important to use relevant models of human hypertensive disease for evaluating antihypertensive treatment effectiveness; SHR is the most widely accepted animal model of hypertensive disease.[15] Moreover, with a steady increase in their systolic blood pressure (SBP) to around 180−200 mmHg, SHRs develop a pathophysiology similar to that of humans with essential hypertension. Hence, SHRs are good models for evaluating the antihypertensive effects of natural products or functional foods.[16]

The small-leaf grape (VTT), also called wild grape, has leaves smaller than those of grape (*Vitis vinifera*) and is a member of

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the *Vitis* genus. It has been classified by The Endemic Species Research Institute, Council of Agriculture, Taiwan, as an endemic, herb in Taiwan, and has long been used in folk medicine for the treatment of hepatitis, jaundice, diarrhea, and arthritis.[17] VTT's small fruit is not as favorable as grape for eating, but the dried branches and leaves of VTT are frequently used as tea substitutes in Taiwan. In the literature, the active components from the *Vitis* genus have been reported to be resveratrol or its derivatives[18,19] and polyphenols.[20] Recently, extracts or purified compounds from VTT were reported to have antimicrobial,[21] anti-inflammatory,[22] and neuroprotective[23] activities. However, an antihypertensive compound from VTT has not been identified or reported. Therefore, in this article, ethanolic extracts (EE) of 4 different plant parts of VTT, including its stem, root, leaf, and branch (VTT-S, VTT-R, VTT-L, and VTT-B) were used to determine their ACE inhibitory activities *in vitro*. The short- and long-term antihypertensive activities of EE-VTT-S were further investigated *in vivo* by oral administration in SHRs. The isolated compounds from EE-VTT-S were tested for ACE inhibitory activity, vaso-relaxing effects against phenylephrine (PE)-induced tensions by using endothelium-intact aortic ring from Sprague–Dawley (SD) rats, and antihypertensive activity in SHRs. Our results suggest that the development of VTT extracts as functional foods may be beneficial for BP regulation.

MATERIALS AND METHODS

Meterials. ACE (1 unit, rabbit lung) was purchased from Fluka Chemie GmbH (Switzerland). Acetylcholine, *N*-(3-[2-furyl]acryloyl)-Phe-Gly-Gly (FAPGG), *N*-nitro-L-arginine (L-NNA), and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

EE-VTT Preparations. VTT plants were purchased from Hualien (Taiwan) and identified by Dr. Hsu, Tsai-Wen (Endemic Species Research Institute, Nantou, Taiwan). Each part of the VTT plant, including the stem, root, leaf, and branch, was air-dried at 40 °C in an oven and cut into pieces for extraction. The EEs from the different VTT plant parts were obtained by individual reflux for 1 h and filtered, and the residues were re-extracted once more; the filtrates were collected, concentrated in a rotating evaporator under reduced pressure, and then lyophilized for further use.

ACE Inhibitory Assay. The ACE inhibitory activity was measured by making some modifications to a previously reported method.[24] Different parts of EE-VTT (200 μg) were screened for anti-ACE activities, and 2 extracts (EE-VTT-S, 41, 82, and 164 μg/mL; EE-VTT-R, 123, 164, 246, and 328 μg/mL) were further investigated for their dose effects. Commercial ACE (20 μL, 20 mU, 1 U/mL from rabbit lung) was mixed with different concentrations of the EEs or purified compounds (200 μL), including resveratrol (100 μM), (+)-vitisin A (4.1, 8.2, 12.3, and 16.4 μM), ampelopsin C (8.2, 16.4, 24.6, 32.8, and 41 μM), and (+)-ε-viniferin (24.6, 32.8, 41, and 50 μM), and then 1 mL of 0.5 mM FAPGG (dissolved in 50 mM Tris-HCl buffer [pH 7.5] containing 0.3 M NaCl) was added. The decreased absorbance at 345 nm (ΔA_{sample}) was recorded within 1.5 min at room temperature and expressed as ΔA_{sample}/min. The extracted solvent, ethanol, or DMSO was used in blank experiments and expressed as ΔA_{blank}/min. ACE inhibition (%) was calculated as follows: $[1 - (\Delta A_{sample}/\text{min} \div \Delta A_{blank}/\text{min})] \times 100$. Means of triplicates were determined. The 50% inhibition (IC_{50}) value was calculated as the concentration of samples that inhibited 50% of ACE activity under tested conditions.

Chromatograms of High-Performance Liquid Chromatography and Compounds Isolated from EE-VTT-S. EE-VTT-S was analyzed by HPLC as follows. The reversed-phase LiChrospher 100 RP-18e column (4 mm × 250 mm, 5 μm, Merck); column temperature, 40 °C; mobile phase, 0.05% trifluoroacetic acid–acetonitrile (0 min, 95:5; 50 min, 55:45; 60 min, 55:45; 61 min, 95:5; 70 min, 95:5) (v/v); and flow rate, 1.0 mL/min, monitored at 280 nm. Ten microliter portions were injected into the column. Four peaks that were characteristic of resveratrol (30 min), ampelopsin C (35.8 min), (+)-ε-viniferin (38.6 min), and (+)-vitisin A (41.1 min) were identified by comparison with each pure compound and electrospray ionization mass spectra (ESI/MS).[19,22] The detailed procedures and identification methods used for the purification and isolation of the active compounds from EE-VTT have been reported previously.[19,22] Briefly, the EE-VTT-S was dissolved into methanol and then subjected to chromatography by using a Sephadex LH-20 gel column, in which methanol to 70% acetone was used as the mobile phase. The fractions were further separated by using a LiChroprep RP-18 (2.5 cm i.d. × 57 cm) column and 0.05%TFA/CH_3CN (70:30) as the mobile phase to obtain ampelopsin C and (+)-vitisin A; and 0.05%TFA/CH_3CN (75:25) as the mobile phase to obtain resveratrol; and 0.05%TFA/CH_3CN (75:25→75:30) as the mobile phase to obtain resveratrol and (+)-ε-viniferin. Structural determinations of active oligostilbenes were estimated by ^{1}H- and ^{13}C NMR, including the use of 2D-NMR techniques and by comparison with data describing the authentic compounds.[19,22] The purities of resveratrol, (+)-ε-viniferin, ampelopsin C, and (+)-vitisin A were determined by HPLC and were 98.4%,95.6%, 94.8%, and 97.0%, respectively.[22]

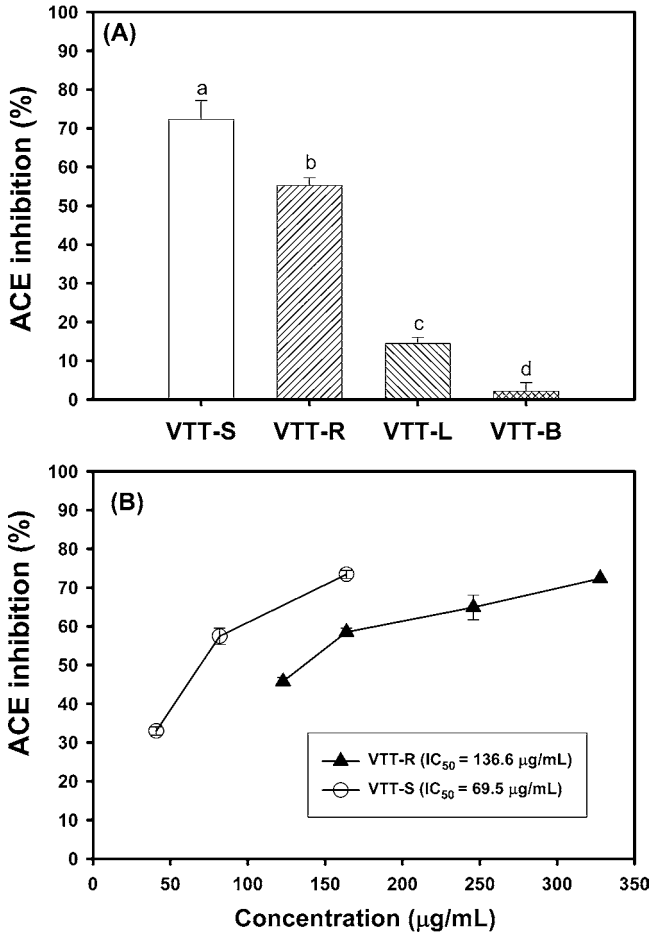

Figure 1. Effect of (A) 200 μg/mL of ethanolic extracts of different parts of VTT (VTT-S, stem; VTT-R, root; VTT-L, leaf; VTT-B, branch) on 20 mU ACE inhibitory screening assay; (B) different concentrations of VTT-R and VTT-S on 20 mU ACE inhibition. The 50% inhibition (IC_{50}) of ACE activity was calculated as the concentrations of samples that inhibited 50% of ACE activity under these conditions. Data were expressed as the mean ± SE (n = 3), and the difference between each group was analyzed using one-way ANOVA, followed by the post-hoc Tukey's test for multiple mean comparisons. Values not sharing the same letter were significantly different ($P < 0.05$).

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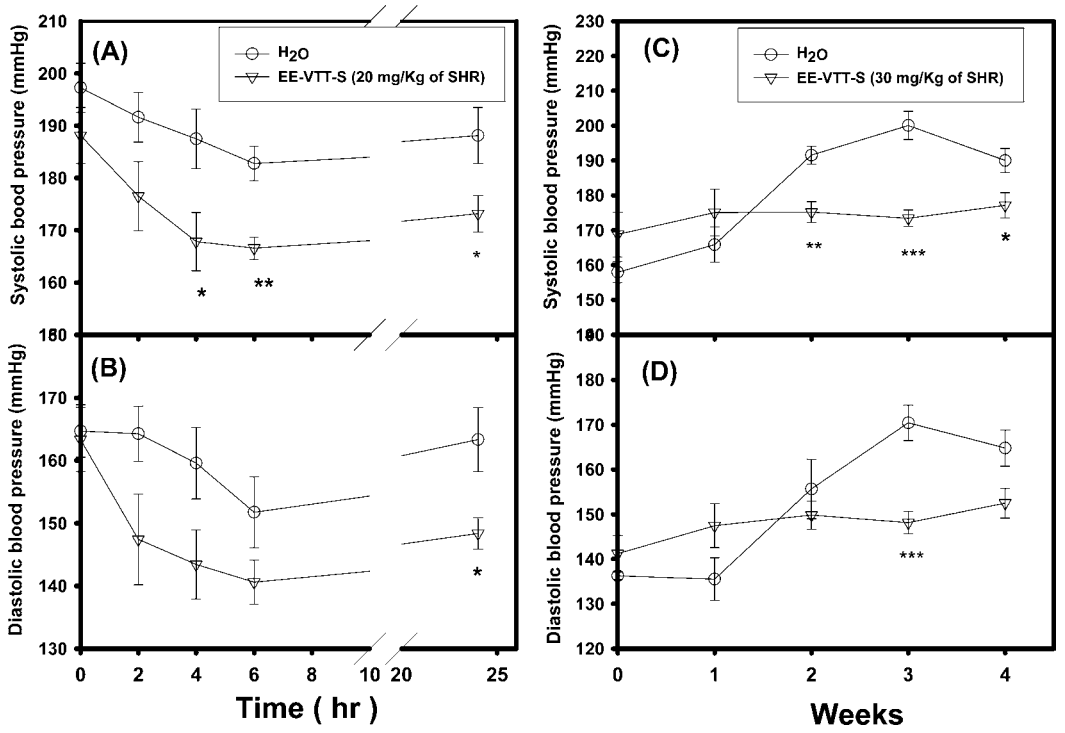

Figure 2. Effects of short-term (A,B) and long-term (C,D) antihypertensive activities of EE-VTT-S on SHR. For the short-term experiment, a single oral administration (20 mg/kg) was performed for 24 h; for the long-term experiment, daily oral administration (30 mg/kg) was performed for 4-weeks. The systolic blood pressure (A,C) and diastolic blood pressure (B,D) were recorded at the fixed time. The BP value was expressed as the mean ± SE (n = 6), and the differences between the blank and the experimental group at the same time was analyzed using Student's t-test. P-values of less than 0.05 (*), 0.01 (**), and 0.001 (***) were recognized as significantly different.

Antihypertensive Effects of EE-VVT-S and (+)-Vitisin A in SHRs. Experimental procedures were performed according to published guidelines[25] and were reviewed and approved by the Institutional Animal Care and Use Committee of Taipei Medical University (LAC-95-0056 and LAC-96-0044). Male SHRs (age, 10 weeks; obtained from National Laboratory Animal Center, Taipei, Taiwan) were housed individually in steel cages kept at 24 °C under a 12-h light−dark cycle, with free access to water and a standard mouse/rat chow (Prolab RMH2500, 5P14 Diet, PMI Nutrition International Brentwood, MO, USA). For 24-h (short-term) experiments, SHRs were randomly divided into 4 groups (6 rats/group); a single dose of EE-VTT-S (20 mg/kg) and (+)-vitisin A (10 mg/kg) was orally administered to the SHRs, and SBP and diastolic blood pressure (DBP) were recorded after 0, 2, 4, 6, and 24 h. Distilled water (0.5 mL) was administered to the SHRs in the control group. The captopril (2 mg/kg) was used as the positive control to compare the antihypertensive effects of (+)-vitisin A. For 4-week (long-term) experiments, SHRs were orally administered EE-VTT-S (30 mg/kg) once a day for 4 weeks, with 0.5 mL of distilled water administered to control SHRs. SBP and DBP were measured on a weekly basis by using an indirect tail-cuff BP meter (BP98-A, Softron, Tokyo, Japan).

Vascular Study on the Effects of Resveratrol, (+)-Vitisin A, Ampelopsin C, and (+)-ε-Viniferin on PE-Induced Tension. The effects of the compounds purified from EE-VTT on vascular tension were examined according to the method of Wang et al.[27] Male Sprague−Dawley rats (SD rats) were killed by decapitation, and sections of the thoracic aorta between the aortic arch and the diaphragm were excised carefully and placed in a Petri dish with oxygenated Krebs' buffer (120 mM NaCl, 4.5 mM KCl, 2.5 mM $CaCl_2$, 1 mM $MgSO_4$, 27 mM $NaHCO_3$, 1 mM KH_2PO_4, and 10 mM glucose, pH 7.4 with 95% O_2 and 5% CO_2) and gently dissected free of fat and connective tissue. The isolated aortic rings, 3 to 4 mm in length, were fixed isometrically in organ chambers under passive tension (1.8 g) for 60 min. Changes in vascular tension were recorded with a polygraph (Gould, model 2400, Valley View, OH, USA) via a force displacement transducer (Grass FT03, Quincy, MA, USA). After equilibration, the near maximal concentration was induced by PE (0.3 μM). When the rings achieved a stable tension, acetylcholine (1 μM) was added to the bath to assess endothelial integrity. The relaxing effects of resveratrol, (+)-vitisin A, ampelopsin C, and (+)-ε-viniferin on PE (0.7 μM) precontracted endothelium-intact aortic rings were examined. When PE treatment reached a steady state after about 10 min (considered as 100%), resveratrol, (+)-vitisin A, ampelopsin C, (+)-ε-viniferin (0.1−100 μM), or vehicle were then tested for their ability to induce aortic ring relaxations. To assess the participation of nitric oxide (NO) in aortic ring relaxations, L-NNA (100 μM) was also preincubated for 10 min before PE treatments.

Statistical Analysis. Data were expressed as the mean ± SE. For screening ACE inhibitory activities from EE-VTT, the difference between each group was analyzed using one-way ANOVA, followed by the post-hoc Tukey's test for multiple mean comparisons. Values not sharing the same letter were significantly different (P < 0.05). For animal experiments, the differences between the blank and the experimental group at the same time were analyzed using Student's t-test, and P-values of less than 0.05 (*), 0.01 (**), and 0.001 (***) were recognized as significantly different. The statistical analysis was performed using GraphPad Prism Software 5.0.

RESULTS AND DISCUSSION

ACE Inhibitory Activity of EE-VTT. The screening of ACE inhibitory activities is straightforward, and potential candidates can be further tested in animal experiments and mechanistic investigations. The different portions of EE-VTT were assayed for anti-ACE activities at the same dose (200 μg/mL) (Figure 1A). The order of ACE inhibition among the different portions of EE-VTT was found to be VTT-S (72.27%) > VTT-R (55.35%) > VTT-L (14.48%) > VTT-B (2.17%). Therefore, the dose effects of EE-VTT-S and EE-VTT-R against 20 mU ACE were further investigated (Figure 1B). Both EE-VTT-S and EE-VTT-R exhibited

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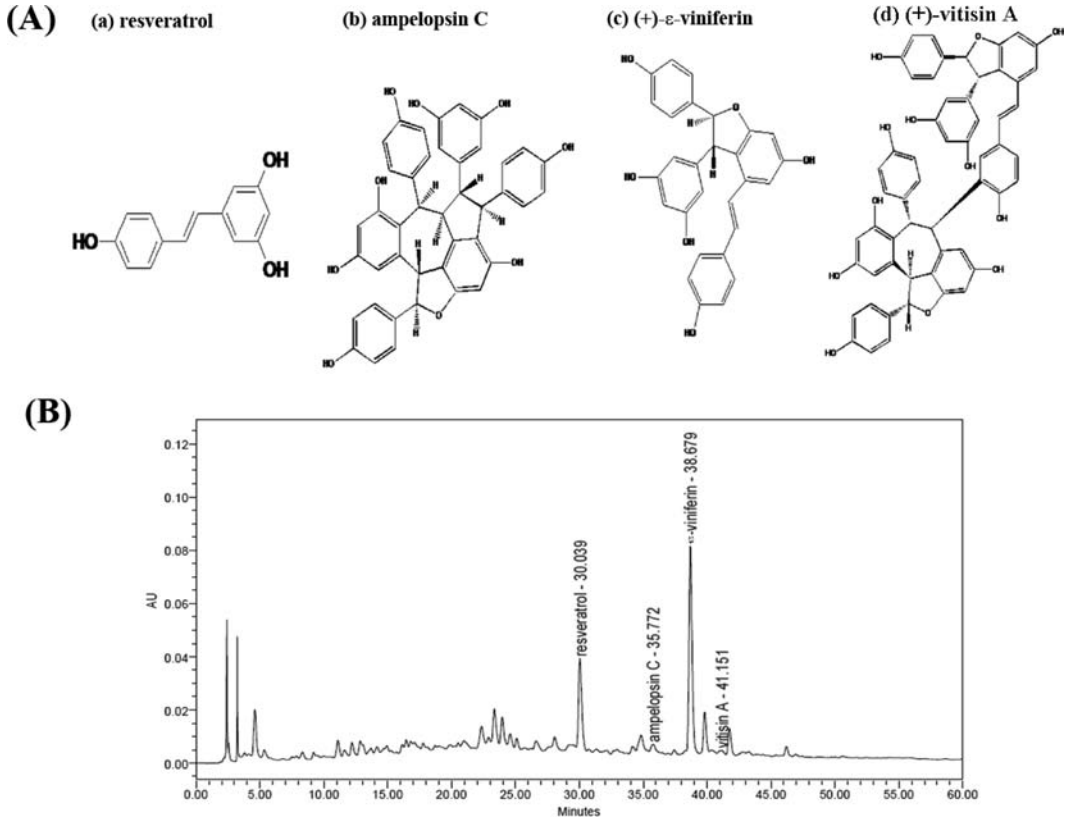

Figure 3. (A) Chemical structures of isolated compounds and (B) the HPLC chromatogram of EE-VTT-S. The resveratrol (30 min), ampelopsin C (35.8 min), (+)-ε-viniferin (38.6 min), and (+)-vitisin A (41.1 min) were identified in EE-VTT-S by comparison with each pure compound and electrospray ionization mass spectra. The reversed-phase LiChrospher 100 RP-18e column (4 mm × 250 mm, 5 μm, Merck); column temperature, 40 °C; mobile phase, 0.05% trifluoroacetic acid-acetonitrile (0 min, 95:5; 50 min, 55:45; 60 min, 55:45; 61 min, 95:5; 70 min, 95:5) (v/v); flow rate, 1.0 mL/min, monitored at 280 nm. Ten microliter portions were injected into the column.

dose-dependent ACE inhibitory activity, with IC_{50} values of 69.5 and 136.6 μg/mL, respectively.

Antihypertensive Effects of EE-VTT-S in SHRs. To evaluate its antihypertensive effects *in vivo*, EE-VTT-S was orally administered to SHRs and their BP was recorded at fixed times (Figure 2). For the short-term experiment, SHRs received a single oral dose of EE-VTT-S (20 mg/kg), and SBP (Figure 2A) and DBP (Figure 2B) were recorded before (0 h) and at 2, 4, 6, and 24 h after administration. EE-VTT-S was found to lower both the SBP and DBP of SHRs within 24 h, and the two values showed significant differences compared with the corresponding values in time-matched controls (P < 0.05 [*] or 0.01 [**]). The average lowering effects at the 2nd, 4th, 6th, and 24th after oral administration were 5.9, 10.5, 7.1, and 5.8 mmHg for SBP, and 15.5, 14.8, 9.8, and 13.6 mmHg for DBP for the 2nd, 4th, 6th, and 24th, respectively. Notably, the 24-h lasting effect was also observed after the administration of a single dose of 20 mg/kg (Figures 2A and B). For the long-term experiment, EE-VTT-S (30 mg/kg) was orally administered daily to SHRs for 4 weeks, and SBP (Figure 2C) and DBP (Figure 2D) were recorded each week. EE-VTT-S (30 mg/kg) was found to lower the BP of SHRs during the 4-week period of oral administration, and the values showed significant differences, particularly the SBP value, compared with time-matched control values (P < 0.05 [*], 0.01 [**], or 0.001 [***]). The average lowering effects for SBP were 27.2, 37.5, and 23.7 mmHg at the second, third, and fourth weeks, respectively. These results are consistent with SBP reductions reported in tissue culture experiments[14] with VTT in SHRs.

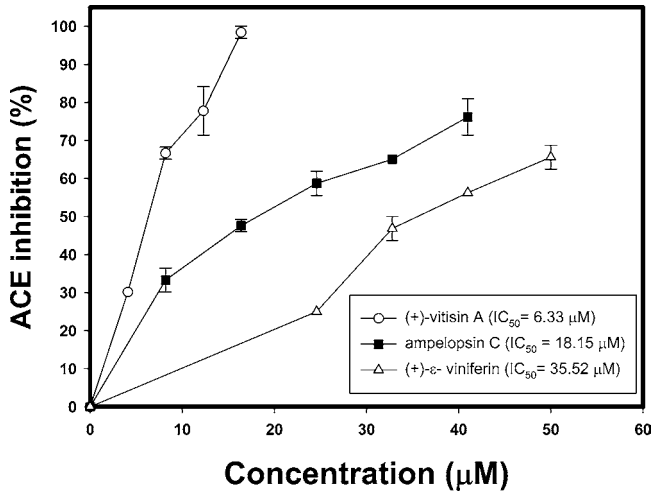

Figure 4. Effect of different concentrations of ampleopsin C, (+)-ε-viniferin, and (+) vitisin A on 20 mU ACE inhibition by continuous spectrophotometric methods. The 50% inhibition (IC_{50}) of ACE activity was calculated as the concentrations of samples that inhibited 50% of ACE activity under these conditions. Data were expressed as the mean ± SE (n = 3).

VTT is an endemic wild grape in Taiwan, and the whole plant or its roots are used as folk medicine; the roots, in particular, are used for the treatment of hepatitis, jaundice, diarrhea, and arthritis.[17] Wang et al.[22] reported that EE-VTT-S inhibited the production of PGE2 in LPS-induced and IL-1β-induced

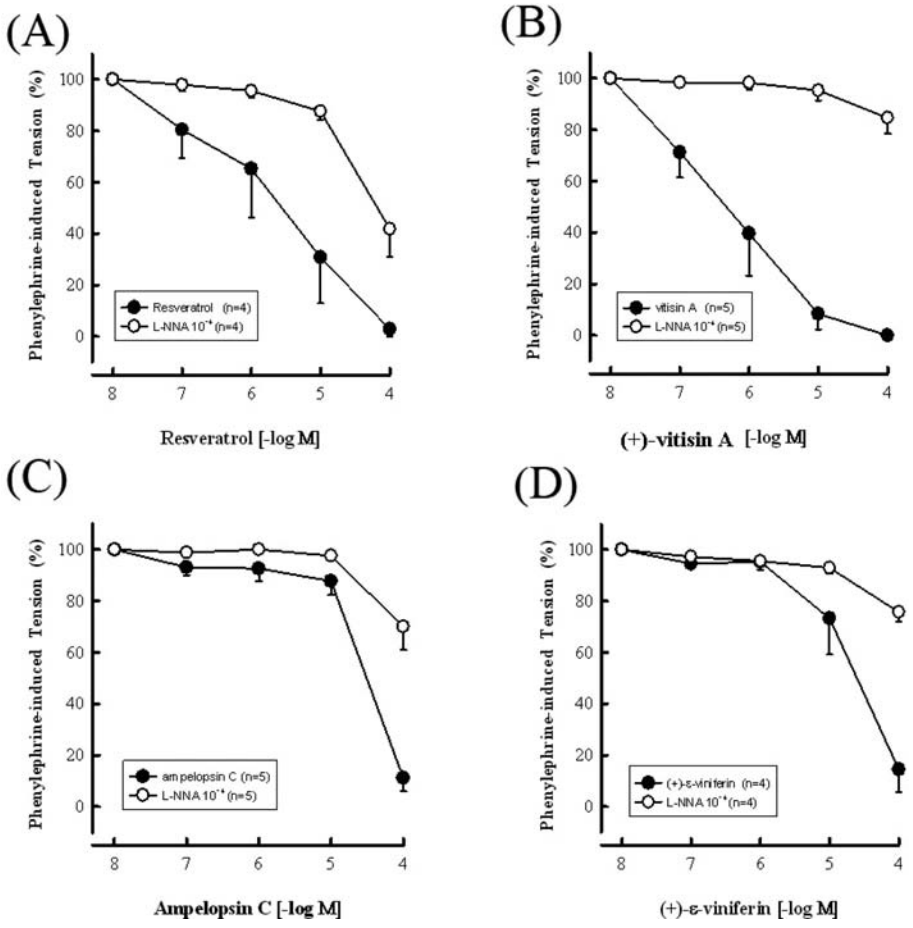

Figure 5. Effects of different concentrations (0.1−100 μM) of (A) resveratrol, (B) (+)-vitisin A, (C) ampleopsin C, and (D) (+)-ε-viniferin on vasodilating activities of phenylephrine-induced tension in endothelium-intact Sprague−Dawley rat thoracic aortic rings in the absence or presence of L-NNA. Data are expressed as the mean ± SE (n = 4−5).

human chondrocytes. EE-VTT-B and EE-VTT-L showed significant inhibition against inducible NO synthase protein expression and NO production in BV2 microglia.[23] In addition, EE-VTT was reported to reduce serum lipid levels and prevent atherogenesis in a hypercholesterolemic rabbit model.[28] VTT plants have been found to exhibit a variety of biological activities depending on the different portions of the plant being used and thus deserve further investigation.

Anti-ACE Activities of (+)-Vitisin A, Ampelopsin C, and (+)-ε-Viniferin. On the basis of the BP-reducing effects of EE-VTT-S in SHRs, we isolated active compounds for further experiments. The Vitaceae family is known as a rich source of oligostilbenes biosynthesized via isomerization and/or rearrangement, such as (+)-ε-viniferin generating (+)-ampelopsin B, (−)-ampelopsin D, and (+)-ampelopsin F, and oxidative coupling of (+)-ε-viniferin generating resveratrol tetramers, including (+)-hopeaphenol, (+)-vitisin A, (−)-vitisin B, (+)-vitisin C, (−)-isohopeaphenol, and (+)-viniferol A.[29] In this study, 4 stilbenes from EE-VTT-S were isolated and identified as resveratrol, (+)-vitisin A (resveratrol tetramer), ampelopsin C (resveratrol dimer), and (+)-ε-viniferin (resveratrol dimer) (Figures 3A and B), which were then each assayed for ACE inhibitory activities. (+)-Vitisin A, ampelopsin C, and (+)-ε-viniferin showed dose-dependent inhibitory activities against 20 mU ACE (Figure 4), with IC_{50} values of 6.33, 18.15, and 35.52 μM, respectively. However, resveratrol showed no inhibitory ACE activity, even at a concentration of 100 μM. Using aortic tissues from male Wistar−Kyoto rats, it was also reported that ACE activity was not inhibited by resveratrol.[30] Of the stilbenes isolated from EE-VTT-S, (+)-vitisin A displayed 2.9- and 5.6-fold more potent inhibitory effects than ampelopsin C and (+)-ε-viniferin, respectively. This is the first report showing that (+)-vitisin A, ampelopsin C, and (+)-ε-viniferin exhibit ACE inhibitory activities. Several plant phenolic compounds such as anthocyanins, flavanols, isoflavanols, and flavones have been shown to exhibit ACE inhibitory activities.[10,31] Therefore, another model of PE-induced aortic ring tension was used to determine the vasodilating effects of these isolated compounds.

Vasodilating Effect of Resveratrol, (+)-Vitisin A, Ampelopsin C, and (+)-ε-Viniferin in Rat Thoracic Aorta. Several papers have reported the vasorelaxing effects of resveratrol in red wine.[32,33] In particular, resveratrol was found to enhance NO formation by increasing eNOS expression and its activity, as well as mediate calcium concentrations by activating Ca^{2+}-activated and voltage-gated K^+ (K_v) channels.[34,35] Therefore, the vasorelaxing effects of the purified stilbenes were investigated by PE pretreatment in endothelium-intact thoracic aortic rings from SD rats (Figure 5). Four stilbenes, resveratrol (Figure 5A), (+)-vitisin A (Figure 5B), ampelopsin C (Figure 5C), and (+)-ε-viniferin (Figure 5D), were shown to cause dose-dependent relaxation in PE-induced contractions in endothelium-intact aortic rings in the following order: (+)-vitisin A > resveratrol > (+)-ε-viniferin > ampelopsin

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C. Furthermore, the thoracic aortic ring vasodilating activities of the purified compounds were completely abolished by pretreatment with L-NNA (100 μM), the NO synthase inhibitor (Figures 5A–D). NO is an endogenous molecule that is released by endothelial cells via NO synthases and leads to an increase in cyclic guanosine monophosphate levels in smooth muscle cells, which is another contributing factor to blood vessel relaxation and reduction of BP.[36,37] In addition, Seya et al.[38] have reported that vitisin C exhibits vasorelaxation in rabbit aorta by elevating NO production. Given their similar structure, it was suggested that (+)-vitisin A and vitisin C might have BP-lowering activities. Thandapilly et al.[39] reported that resveratrol could prevent the development of concentric hypertrophy and cardiac dysfunction; however, no SBP- and DBP-lowering activities were observed in SHRs. Therefore, (+)-vitisin A was chosen to be orally administered to SHRs for further BP-lowering investigations.

Effects of (+)-Vitisin A on Short-Term Antihypertensive Activity in SHRs. The present study investigated the antihypertensive effect of a single oral dose of (+)-vitisin A (10 mg/kg) administered to SHRs; SBP and DBP were recorded at 0, 2, 4, 6, 8, and 24 h (Figure 6). Captopril (2 mg/kg) was used as a positive control. (+)-Vitisin A was found to significantly lower SBP (Figure 6A) and DBP (Figure 6B) compared with time-matched control levels ($P < 0.05$, * or $P < 0.01$, **). The greatest reduction in BP was reached 8 h after the single dose was administered, with a reduction of 23.6 and 25.7 mmHg in SBP and DBP, respectively. Under the tested concentration, (+)-vitisin A had higher SBP-lowering activity than captopril (Figure 6A) after a single oral administration during 24 h. Notably, a 24-h long-lasting effect was also observed after the single 10 mg/kg dose of (+)-vitisin A was administered orally (Figures 6A and B). This is the first report showing that (+)-vitisin A exhibits ACE inhibitory activities, vasodilating activities in a PE-induced aorta ring tension model, and antihypertensive activities in SHRs. These results suggest that (+)-vitisin A may be one of the important mediators responsible for the antihypertensive effect of EE-VTT-S.

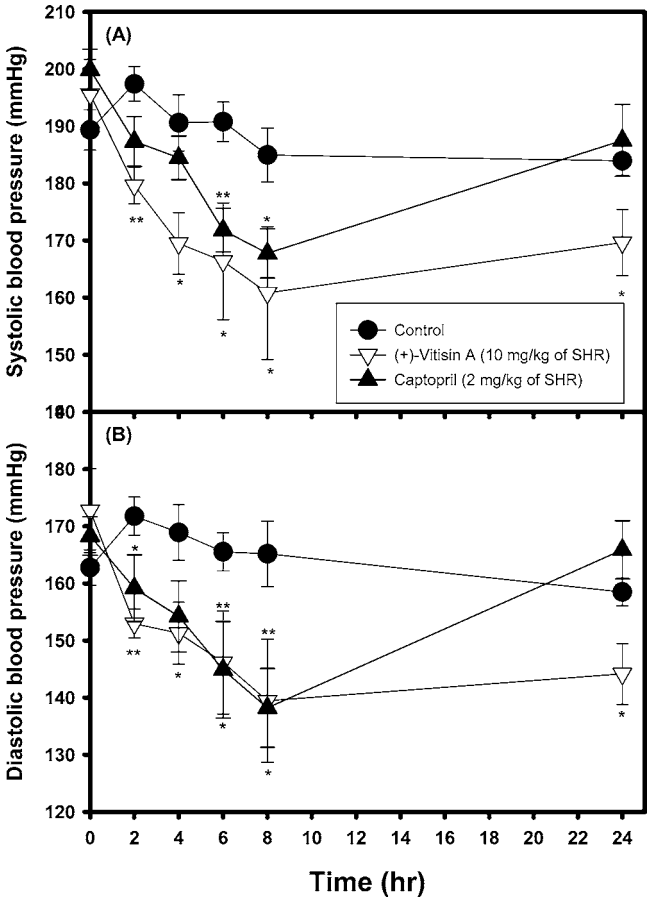

Figure 6. Effects of (+)-vitisin A on (A) systolic blood pressure and (B) diastolic blood pressure of SHR by a single oral administration (10 mg/kg of SHR) during 24 h. Distilled water (0.5 mL) was used for the blank experiment. The captopril (2 mg/kg) was used as the positive control. The BP value was expressed as the mean ± SE ($n = 6$), and the differences between the blank and the experimental group at the same time were analyzed using Student's t-test. P-values of less than 0.05 (*), 0.01 (**), and 0.001 (***) were recognized as significantly different.

In conclusion, VTT, also called wild grape with smaller leaves than grape (*Vitis vinifera*), is a member of the *Vitis* genus that has been classified by The Endemic Species Research Institute, Council of Agriculture, Taiwan as an endemic, herbal plant in Taiwan. VTT has long been used in folk medicine for the treatment of hepatitis, jaundice, diarrhea, and arthritis.[17] Vitaceae family members are known as rich sources of oligostilbenes, which have been shown to have cardiovascular benefits. In this study, (+)-vitisin A was identified and shown to reduce BP in SHRs and exhibit vasodilating effects on PE-induced tension by increasing NO release from endothelial cells. Therefore, on the basis of the observed biological functions of EE-VTT-S, the development of VTT extracts as functional foods may be beneficial for the regulation of BP and need further investigation.

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Author Contributions

▽These authors contributed equally to this study.

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Notes

The authors declare the following competing financial interest(s): Taiwan patent (98102503) pending.

ABBREVIATIONS USED

ACE, angiotensin converting enzyme; DMSO, dimethyl sulfoxide; EE, ethanolic extracts; DBP, diastolic blood pressure; L-NNA, *N*-nitro-L-arginine; NO, nitric oxide; PE, phenylephrine; SBP, systolic blood pressure; SD rat, Sprague–Dawley rat; SHR, spontaneously hypertensive rats; VTT, *Vitis thunbergii* var. *taiwaniana*

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