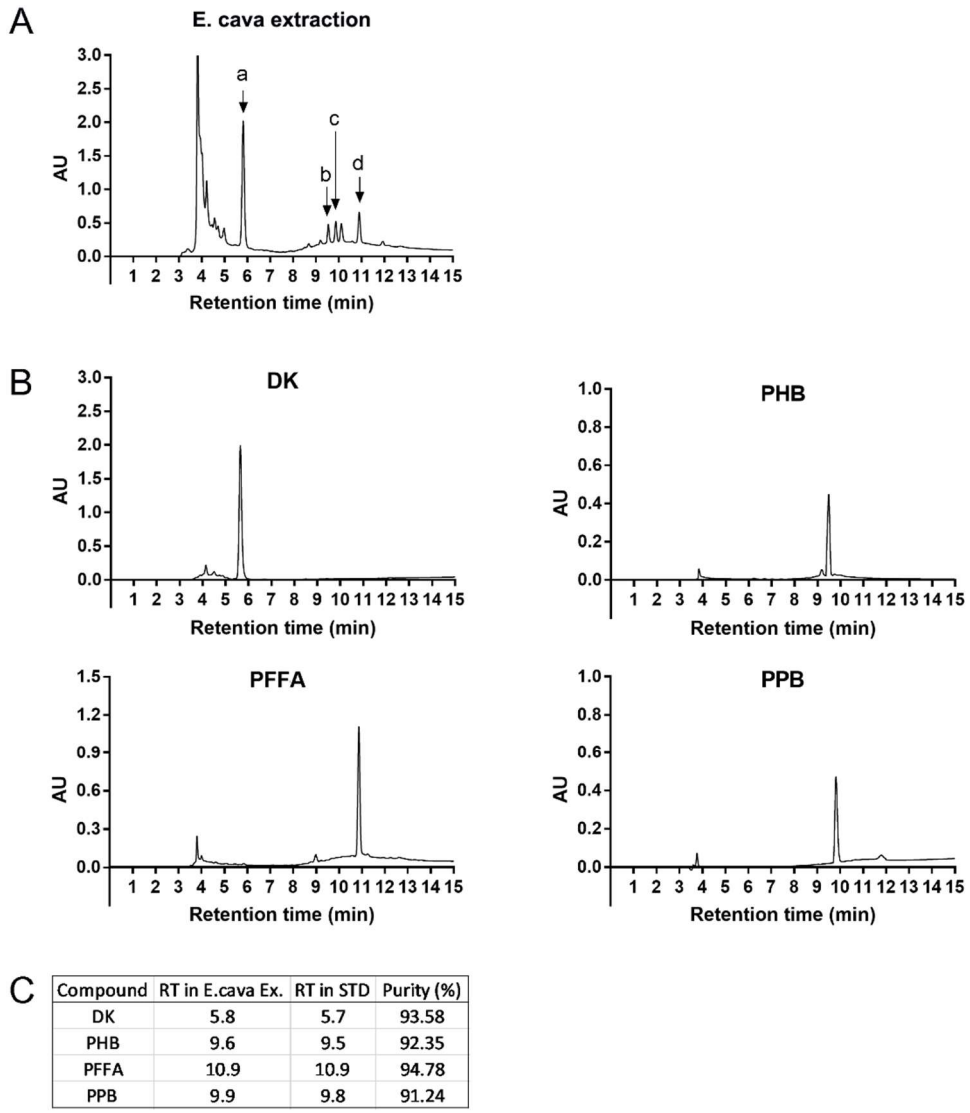
Supplementary Figure

Supplementary Figure 1. HPLC chromatograms and purity of four compounds from *E. cava* extract

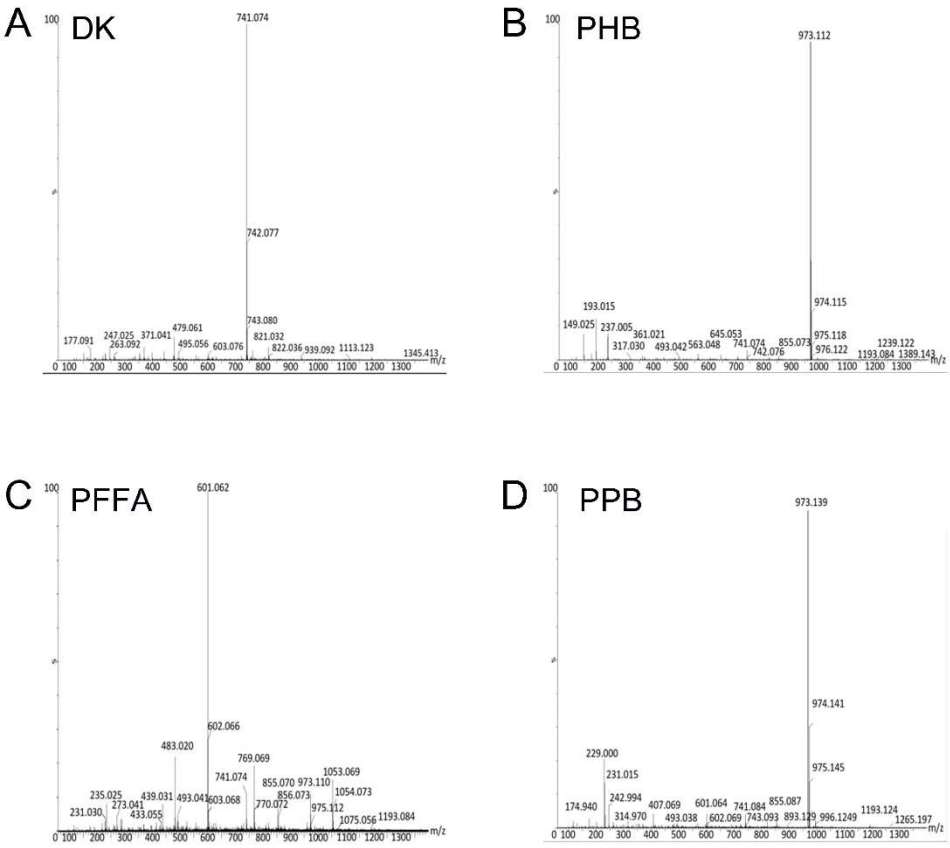


C

Compound	RT in E.cava Ex.	RT in STD	Purity (%)
DK	5.8	5.7	93.58
PHB	9.6	9.5	92.35
PFFA	10.9	10.9	94.78
PPB	9.9	9.8	91.24

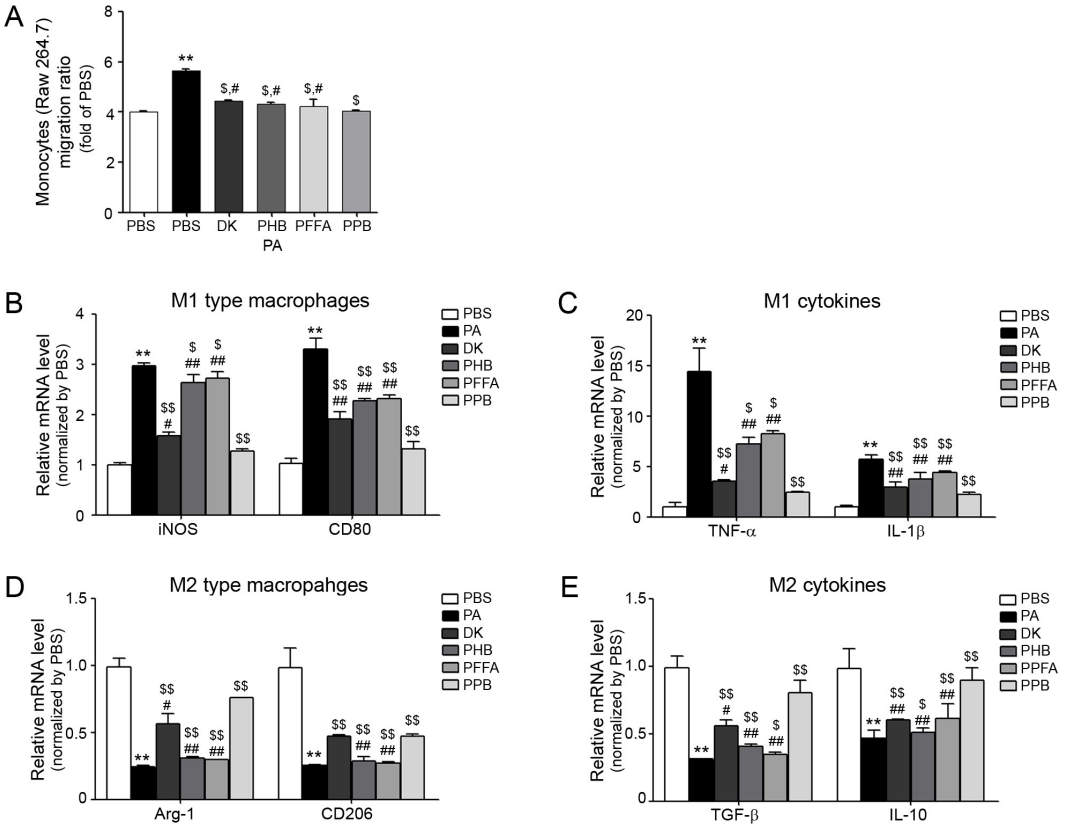
(A) HPLC chromatograms of *E. cava*. The peaks labeled a-d correspond to dieckol, PHB, PPB and PFFA, respectively and checked purity of them. a: dieckol, b: 2,7-phloroglucinol-6,6-bieckol (PHB), c: pyrogallol-phloroglucinol-6,6-bieckol (PPB), d: phlorofucofuroeckol-A (PFFA). (B) Separately isolated single compound was validated HPLC chromatograms. (C) Purity of isolated four compounds from E. cava extract

Supplementary Figure 2. Mass spectrometry analysis of four compounds from *E. cava* extract



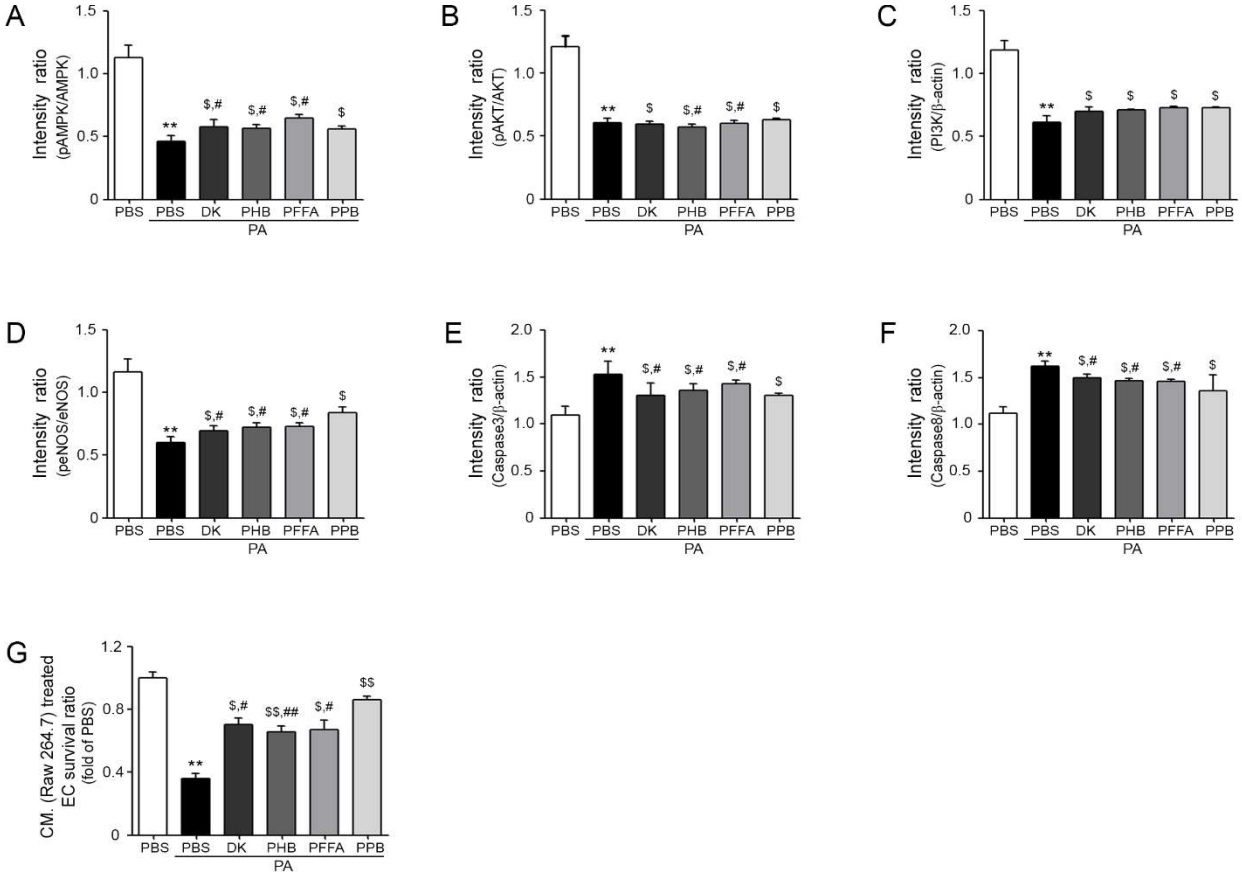
ESI-MS spectra of isolated four phlorotannins. (A) DK (B) PHB (C) PFFA and (D) PPB were validated. DK: dieckol, PHB: 2,7-phloroglucinol-6,6-bieckol, PFFA: phlorofucofuroeckol-A, PPB: pyrogallol-phloroglucinol-6,6-bieckol

Supplementary Figure 3. Inhibitory effects of PPB in monocyte polarization and related cytokines



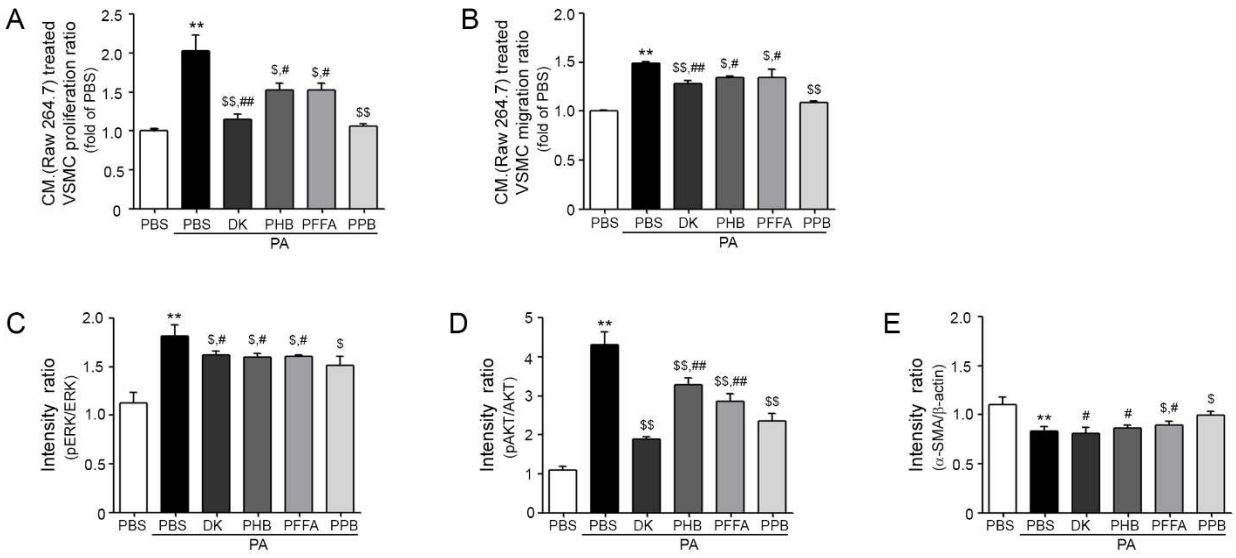
(A) Migrating Raw 264.7 cell levels in 4 compounds with PA-BSA as determined by the trans-well migration assay. (B, C) mRNA expression levels of M1 type macrophages marker (*iNOS* and *Cd80*) and M2 type macrophages marker (*Arg-1* and *Cd206*) as determined by qRT-PCR. (D, E) mRNA expression levels of M1 type macrophages related cytokines (*TNF-α* and *IL-1β*) and M2 type macrophages related cytokines (*TGF-β* and *IL-10*) by qRT-PCR. **, P < 0.01, ***, P < 0.001, vs. PBS; $, P < 0.05, $$, P < 0.01, vs. PA-BSA; #,P < 0.05, ##, P < 0.01, vs. PA-BSA with PPB, DK; dieckol, PHB; 2,7-phloroglucinol-6,6-bieckol, PFFA; phlorofucofuroeckol-A, PPB; pyrogallol-phloroglucinol-6,6-bieckol.

Supplementary Figure 4. Inhibitory effects of PPB in Raw 264.7 cell-associated endothelial cell death



(A-F) Protein level graph shows quantified protein levels of cell-death related molecules, that is, (B) pAMPK/AMPK, (C) pAKT/AKT, (D) PI3K (E) peNOS/eNOS, (F) Caspase 3, and (G) Caspase 8. (G) Conditioned medium (CM) collected from PA-BSA with or without four compound treated Raw 264.7 cell. ECs were validated after CM treatment by a cell survival assay. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, vs. PBS; $, $P < 0.05$, $$, $P < 0.01$, $$$, $P < 0.001$, vs. PA-BSA; #,$P < 0.05$, ##, $P < 0.01$, ###, $P < 0.001$ vs. PA-BSA with PPB, DK; dieckol, PHB; 2,7-phloroglucinol-6,6-bieckol, PFFA; phlorofucofuroeckol-A, PPB; pyrogallol-phloroglucinol-6,6-bieckol.

Supplementary Figure 5. Inhibitory effects of PPB in Raw 264.7-associated VSMC proliferation and migration



(A) Conditioned medium (CM) collected from PA-BSA with or without four compound treated Raw 264.7 cell. VSMC proliferation were measured using a proliferation assay after CM treatment (B) Trans-migrating VSMC numbers were measured using a trans-migration assay. (C-E) Protein level graph shows quantified protein levels of proliferation and migration related molecules, that is, (C) pERK/ERK, (D) pAKT/AKT, (E) α-SMA. **, P < 0.01, ***, P < 0.001, vs. PBS; $, P < 0.05, $$, P < 0.01, vs. PA-BSA; #,P < 0.05, ##, P < 0.01, vs. PA-BSA with PPB, DK; dieckol, PHB; 2,7-phloroglucinol-6,6-bieckol, PFFA; phlorofucofuroeckol-A, PPB; pyrogallol-phloroglucinol-6,6-bieckol.

Supplementary Tables

Supplementary Table 1. List of antibodies for Western blotting

Antibody name	Company	Cat. No.	Dilution rate
β-actin	Cell signaling	4967s	1:1,000
ERK	Cell signaling	9102s	1:1,000
Phospho-ERK	Cell signaling	9101s	1:1,000
AKT	Abcam	81283S	1:1,000
Phospho-AKT	Ab5076	Ab5076	1:5,000
AMPK	Abcam	Ab207442	1:1,000
Phospho-AMPK	Abcam	Ab133448	1:1,000
eNOS	Thermo Fisher	PA3-031A	1:1,000
Phospho-eNOS	Cell signaling	9571	1:1,000
PI3K	FineTest	FNab06422	1:1,000
α-Smooth muscle actin	Abcam	Ab5694	1:1,000
Caspase 3	Cell signaling	9662	1:1,000
Caspase 8	Cell signaling	9746	1:1,000

Supplementary table 2. List of primer for qRT-PCR

Gene		Primers
β-actin	Forward	5'-ACA AAG CTG TTC AGT GTC TCC A-3′
	Reverse	5'-CTC CGT TTC CAG AAT ACA CAC A-3′
iNOS	Forward	5'- CACAGCAATATAGGCTCATCCA-3′
	Reverse	5'- AGCCTCATGGTAAACACGTTCT-3′
CD80	Forward	5'-GACCGAATCTACTGGCAAAAAC-3′
	Reverse	5'-TTCTTATACTCGGGCCACACTT-3′
CD206	Forward	5'-TGTATTCTTTGCCTTTCCCAGT-3′
	Reverse	5'-GATAAAAGCCAGAAGCAGGAGA-3′
Arg-1	Forward	5'-ACAGAACTAAGCAAACGCCTTC-3′
	Reverse	5'-AGAAAGGAACTGCTGGGATACA-3′
TNF-α	Forward	5'-TTCTGTCTACTGAACTTCGGGGTGATCGGTCC-3′
	Reverse	5'-GTATGAGATAGCAAATCGGCTGACGGTGTGGG-3′
IL-1β	Forward	5'-CTTTTCGTGAATGAGCAGACAG-3′
	Reverse	5'-TCAGCTTCAATGAAAGACCTCA-3′
TGF-β	Forward	5'- CTGGCAGTAGCTCCCCTATTTA-3′
	Reverse	5'- ACCAGGGTAAAAATCGAGATGA-3′
IL-10	Forward	5'-ATGGTGTCCTTTCAATTGCTCT-3′
	Reverse	5'-AGGATCTCCCTGGTTTCTCTTC-3′
E-selectin	Forward	5'-ATGAAATGTCTTCCCAGTGCTT-3′
	Reverse	5'-TGATCCCTTCAGTTCAAATCCT-3′
ICAM-1	Forward	5'-ATAACCGCCAGAGAAAGATCA -3′
	Reverse	5'-GGCTTGTCCCTTGAGTTTTATG-3′
VCAM-1	Forward	5'- GAGACCTGTCACTGTCAACTGC -3′
	Reverse	5'- CATCAGTGTAGTCTCCCCCTTC -3′
vWF	Forward	5'-AAAGCTCCAGCAAGTTGAAGAC-3′
	Reverse	5'-CATCCACACAAACTCCAGAAAA-3′

Supplementary materials and methods

High-performance liquid chromatography

A reverse phase High-performance liquid chromatography (HPLC) system comprising an Poroshell 120 C18 column (4 μm, 4.6 × 150 mm; Agilent) was used to further purify the selected fraction (Alliance 2695; Waters Corp., MA, USA). This was connected to a photodiode array detector (Waters Corp). Gradient elution was performed with 0.1% Formic acid (FA) and water as the eluents. Between 0 min and 2 mins, 43% of the FA and methanol and to 8 mins, 70% of the FA and methanol and to 15 mins 100% of the FA and methanol and to 30 mins 43% of the FA and methanol were used. The column flow rate was maintained at 0.4 mL/min and injection volume was 10 μL. We finally confirmed purity of four phlorotannins is more than 90% was used in the study.

Mass spectrometry analysis

This analysis was performed following previous study [1]. Briefly, Electrospray ionization (ESI)/ spectrometry (MS) analyses were performed using a Hewlett-Packard 1100 series HPLC system equipped with a DAD detector, a degasser, an auto-sampler, a column oven and a binary pump (Hewlett-Packard; Waldbronn, Germany) coupled to a Finnigan MAT LCQ ion-trap mass spectrometer (Finnigan MAT; CA, USA). The MS was equipped with a Finnigan electrospray source and was capable of analyzing ions up to 2000 m/z. Negative ion mass spectra of the column elute was recorded in the range 100-2000 m/z. The source voltage was set to 5 kV and the capillary temperature to 275 ℃. The other conditions were as follows: sheath gas, 80 psi (551.6 kPa); inter-octapole lens voltage, 10 V; capillary voltage, -36.5 V; auxiliary gas, 20 psi (137.9 kPa).

Raw 264.7 cells cultivation

Raw 264.7 cells for monocyte/macrophage cell line were purchased from Korea cell bank (Republic of Korea). Dulbecco's Modified Eagle's medium (DMEM; Hyclone) and 1% penicillin-streptomycin (Gibco) were used as growth medium. To investigate the inhibitory effects of DK, PHB, PFFA and PPB in 0.25 mM PA-BSA treated Raw 264.7 cells, we used the same concentration (2.5 ug/ml) for a treatment time of 48 hrs. To collect conditioned medium (CM), Raw 264.7 cells were treated with PA-BSA with or without DK, PHB, PFFA or PPB for 48 hrs.

Quantification of Western blotting results

The Western blotting band was quantified by image J program. PI3K, Caspase 3, Caspase 8 and α-SMA were normalized b-actin. phospho-form proteins including pAMPK-AMPK, pAKT-AKT, peNOS-eNOS were normalized total-form protein.

Supplementary reference

1. Lee, J.H.; Ko, J.Y.; Oh, J.Y.; Kim, C.Y.; Lee, H.J.; Kim, J.; Jeon, Y.J. Preparative isolation and purification of phlorotannins from Ecklonia cava using centrifugal partition chromatography by one-step. *Food Chem*. **2014**, 158, 433-437.