Supplemental Material

Data S1.

Supplemental Materials and Methods

Study design

The study was conducted in accordance with the guidelines of the declaration of Helsinki and was approved by the Ethic Committee of CE Campania Sud, Italy, which registered the study protocol (n° 390-01587470-30081). Written informed consent was obtained from each subject. Systolic and diastolic BP were measured by standard sphygmomanometer after 5 min in the supine position, according to the guidelines of the European Society of Hypertension/European Society of Cardiology. Subjects of both sexes with diagnosis of essential hypertension were screened for the following exclusion criteria: previous cardiac or cerebrovascular event; heart failure; diabetes mellitus; history of atrial fibrillation or other severe arrhythmias; chronic kidney disease (defined as serum creatinine levels >1.4 mg/dL); pre-existing psychiatric disorders; neurodegenerative diseases such as multiple sclerosis, amyotrophic lateral sclerosis, Parkinson's disease, early onset/genetic Alzheimer's disease, neuromuscular pathologies, epilepsy; dementia. In addition, patients requiring any pharmacological treatment beyond anti-hypertensive drugs, pregnant women, and women planning to conceive were also excluded from the study.

We performed two different studies in humans.

The first was a randomized double blind placebo controlled study with a 4 week follow-up during which forty-five treated hypertensive patients with stable, unsatisfactory control of blood pressure were randomized to receive placebo or one pill of a nutraceutical preparation (Akp05, 1gr., Izzek®, Damor Farmaceutici, Italy) containing dry plant extract of 300 mg *Bacopa Monnieri* + 50 mg dry extract of *Ginkgo biloba* leaves + 25mg phosphatidylserine + 40 mg dry extract of green tea leaves. The company also produced the placebo, similar in appearance and organoleptic properties to the nutraceutical preparation.

Since there is no gold standard measurement system that fulfils the criteria for optimal medication-adherence monitoring, in this study, for monitoring of the medication compliance, we relied on patient self-reporting. For study drug monitoring, we delivered to each patient a container containing the number of exact capsules to be used for the entire duration of the treatment. Furthermore, they were required to return the container at the end of the treatment period.

Blood pressure, non-invasive endothelial function, as assessed reactive hyperemia index (RHI), and serum nitric oxide concentration were measured in control conditions and at the end of the follow-up period.

The second outcome was a 1:1 randomized since it represents a novel outcome of the study. For this outcome twenty-four patients who were not taking diuretics participated in a second study conducted according to a single blind active controlled protocol. They were randomized to take, in addition to their medication, either one pill of AkP05 or one pill of diuretic (Chlorothiazide, 1gr/day) and underwent 24-hour ambulatory blood pressure monitoring and a cardiopulmonary exercise test (CPET) at baseline and after 4 weeks of treatment.

Cardiopulmonary exercise test

All subjects were instructed to refrain from alcohol consumption and strenuous exercise for 12 h prior to exercise testing. Before starting the tests, the gas analyzer was calibrated using air and a gas mixture of known composition (16% O_2 and 5% CO_2) (Cosmed). Each patient was then acclimated to the

Ergo-Select 100 cycloergometer (Ergoline, Italy). ECG was performed with a QUARK CEPT ECG (Cosmed). Respiratory gas exchange data was measured continuously, and the hardware was set to "facemask" use. After recording baseline activity at 0 W for 30 s, patients underwent a pre-exercise phase for 1 min at 6 W. Subsequently, subjects started the exercise phase, using a ramp protocol with an increment of 1 W every 6 s. The maximal exercise test lasted until inability to maintain the cycling cadency or cessation due to substantial fatigue. The highest systolic BP (SBP), one metabolic equivalent (MET), and VO_2 peak reached during the exercise phase of the incremental test were considered as the maximal values. At the end of the active-exercise phase, patients continued the activity at for 1 min at 50 W, and starting from the 2^{nd} min, power was reduced to 20 W until complete recovery of physiological parameters.

Preparation of extracts

The AkP05 powder or its single components (namely, *Bacopa monnieri* (BM), *Ginkgo biloba* leaves (GBE), extract of green tea leaves (GTE), and phosphatidylserine (PS)) was first ground with a top ball mill (Galena, Italy) to reduce particle size. Powder of each compound and of AkP05 were lyophilized to a particle size of 45 µm with an LS 13320 Tornado Dry Powder System with an Aqueous Liquid Module (Beckman Coulter). After, the powder (30 g) was extracted with 240 mL of 0.1 M sodium phosphate buffer (ratio w/v of 1:16), with 3 freeze-thaw cycles of 4 h (from -20°C to 37°C); after every cycle, the suspension was sonicated for 30 min at 550 W power and then centrifuged (× 6400 rpm for 25 min at 4°C). The supernatant was removed and the pellet retreated with a fresh solution. Finally, all supernatants were pooled and lyophilized. Approximately 15 g for each compound was obtained and diluted in Krebs buffer solution prior to use.

For analytic evaluation of the compounds contained within AkP05, 903.9 mg of the nutraceutical combination was extracted with 5 mL of a methanol:water mixture (80:20, v/v). First, the sample was vigorously mixed for 1 min on a vortex, then phytochemical extraction was accomplished by maceration in an ultrasonic bath for 1 h at 25 °C. Subsequently, samples were centrifuged for 5 min at 5000 rpm, at 25 °C. The operation was repeated twice. The supernatants were pooled, dried under reduced pressure, and dissolved in a methanol:water mixture (80:20, v/v) to obtain a concentration of 15 mg/mL, which was filtered with 0.45 µm filters and then injected.

UHPLC-PDA-MS/MS parameters

UHPLC-PDA-MS/MS analyses were performed on a Nexera UHPLC (Shimadzu, Milan, Italy) coupled to a hybrid Ion Trap-Time of Flight mass spectrometer LCMS-IT-TOF (Shimadzu, Kyoto, Japan). The separation was performed on a KinetexTM C18 150 mm × 2.1 mm, 2.6 µm column protected with a security Guard UltraTM C18 cartridge. The mobile phases were: A, 0.1 % acetic acid in water; B, acetonitrile plus 0.1 % acetic acid. The gradient was 0-15 min, 5-25 B%, 14-22 min, 25-70%B, 22-24 min, 70-95%B, hold for 0.50 min, return to the initial condition in 0.01 min. Column oven: 40°C. Injection volume: 2 µL. Flow rate: 0.5mL/min. PDA parameters were: lamp D2, sampling rate 12.5 Hz, time constant 0.160s. Chromatogram extracted at 254, 280, and 330 nm. The MS detection was operated in negative mode by an ESI source. Flow rate from the LC was split 50:50 prior the source. ESI source parameters were: interface and CDL temperatures 250°C, nebulizing and drying gas 1.5 and 9.5 L/min. Probe voltage: -3.5 kV. MS1 range: 150-1500 m/z. MS/MS was performed in data-dependent mode. Identification of compounds was performed by accurate MS and MS/MS spectra by comparison with literature and molecular formulas provided by the software Formula Predictor (Shimadzu). Quantitation of main compounds was performed by external

calibration employing 5 standards with 5 concentration levels (1, 5, 10, 15, 25 µg/mL). Three replicates of each LC run were carried out.

Experimental models

All experiments involving animals conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 2011) and were approved by the IRCCS INM Neuromed review board (n° 1070/2015 PR). Male C57BL/6 mice (25±0.9 g body weight) were bred in our animal facility. Male eNOS knockout mice (24±0.9 g body weight) were obtained from Charles River Laboratories. All animals were randomly divided into control and treated groups. All efforts were made to minimize the number of animals used and their suffering. Mice were fed standard chow and water ad libitum. C57BL/6 and eNOS deficient mice were 8 weeks old when used in this study. In vivo administration of AkP05 or single components was performed by gavage. Nobody animal showed adverse events or death during treatments.

Ex vivo vascular reactivity studies and staining for superoxide

Vasorelaxation was expressed as percent reduction of phenylephrine-induced contraction. To evaluate the vascular function of AkP05 or its single components, we exposed precontracted vessels to increasing doses (0.1 mg/mL to 0.8 mg/mL) of each test substance.

To characterize intracellular signaling, some myograph-mounted mesenteric arteries were pre-treated with the following before data for dose-response curves were obtained: 10 µM of phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) inhibitor LY294002 (Selleckchem, Cat No.S1105) for 1 h; 2 µM of the AKT inhibitor Akt Inhibitor X (Sant Cruz Biotechnology; sc-203811) for 1 h; 300 µM of the eNOS inhibitor Nω-nitro-L-arginine methyl ester (L-NAME) (Sigma-Aldrich, N5751) for 30 min; 2.5 µM of the PLC inhibitor U73122 (Selleckchem, S8011) for 1 hour; 2 µM of the PKC inhibitor Ro31-8220 (abcam, ab120374); or with 200 µM of the selective connexin-43 and connexin-37 inhibitor Gap27 (apexbt, A1045). Vasomotor response was quantified by a second individual who was blind to the genotype of the animal and/or the hypothesis that was being tested for each group. In all vascular experiments, precontraction was obtained by exposure to increasing doses of phenylephrine (10^{-9} to 10^{-6} M) in order to obtain a similar level of pre-contraction equal to 80% of initial KCl-induced constriction.

Dihydroethidium (DHE, Life Technologies) was used to evaluate production of reactive oxygen species (ROS) in mouse mesenteric arteries, as previously described.[1] Briefly, vessels were incubated with 5 µM of DHE for 20 min and subsequently observed under a fluorescence microscope (Zeiss). Images were acquired by a digital camera system (Olympus Soft Imaging Solutions). A second, estimation of total ROS production in mouse vessels was performed with the membrane-permeable fluorescent probe 5-(6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (CM-H_2DCF/DA) (Invitrogen). After treatment, vessels were incubated with Krebs solution containing 5 µM CM-$_2$DCFDA for 30 min at 37°C, and then washed two times with PBS prior to fluorescence measurement using a fluorescence microplate reader (TECAN infinite 200 Pro) with 495 nm excitation and 520 nm emission.

Ex vivo vascular reactivity studies and staining for superoxide

Vascular reactivity studies were performed on second-order branches of the mesenteric artery, as previously described.[2] Quantification of vasomotor response and blood pressure, and molecular analyses were performed by a second individual who was blind to the genotype of the animal and/or

the hypothesis that was being tested for each group. Briefly, vessels were isolated and dissected from fat and connective tissue in ice-cold Krebs solution and gassed with 95% O_2 and 5% CO_2. Subsequently, arteries were mounted on a wire myograph in organ chambers with Krebs solution and treated with increasing concentrations of phenylephrine (10^{-9} to 10^{-6} M) in order to obtain a similar level of pre-contraction in each ring (80% of initial KCl-induced contraction). Caution was taken to avoid endothelium damage, and functional integrity of the endothelium was confirmed by the vasodilation response to acetylcholine (10^{-9} to 10^{-6} M).

Cell culture and NO measurement

Commercially available human umbilical vein endothelial cells (HUVECs) or coronary artery smooth muscle cells (CASMCs) were purchased from Lonza (Walkersville, MD, USA) and grown in EGM-2 and SmBM basal medium, respectively. Cells were used within passage five and at 70% confluence for the following sets of experiments. Total nitrite content in the supernatants of HUVEC cultures was measured in an additional experiment. Cells were grown in 100 mm dishes and supernatants collected after stimulation with acetylcholine as control (100 µM for 15 min) or with compounds as reported in figure legends. In some experiments, HUVECs were pretreated for 30 min with 300 µM L-NAME, a well-characterized eNOS inhibitor, to conform the detection of NO. The NO concentration in the supernatant was assayed as nitrite, the stable breakdown product of NO, by a chemiluminescence detector (Sievers 280i NO Analyzer), as previously described.[3]

Protein extraction and immunoblot analysis

For total protein extraction, mesenteric arteries were lysed in a buffer containing 150 mmol/L NaCl, 50 mmol/L Tris-HCl (pH 8.5), 2 mmol/L EDTA, 1% v/v NP-40, 0.5 % w/v deoxycholate, 10 mmol/L NaF, 10 mM sodium pyrophosphate, 2 mmol/L PMSF, 2 g/ml leupeptin, and 2 g/ml aprotinin (pH 7.4). Lysates were incubated on ice for 15 min and then centrifuged at 38000 x g for 30 min at 4 °C to collect the supernatant. Protein concentration was measured using a dye-binding protein assay kit (Bio-Rad) and read at the spectrophotometer at a wavelength of 595 nm. Immunoblotting was performed as previously described, using the following antibodies: mouse anti-AKT (Santa Cruz, sc-55523; 1:1000); rabbit polyclonal anti-phospho-AKT serine 473 (Cell Signaling, 9271; 1:1000); mouse monoclonal anti-β-actin (Santa Cruz, sc-47778 (C4); 1:2000); mouse monoclonal anti-phospho-eNOS serine 1177 (Enzo Life Sciences, ALX-804-396-C100; 1:600); rabbit monoclonal anti-eNOS (Cell Signaling, 9570; 1:800); mouse monoclonal anti-PKC antibody [M110] (abcam, 23511); and Anti-PKC alpha (phospho T497) antibody (ab76016). Secondary antibodies (1:3000) were purchased from Amersham Life Sciences (GE Healthcare). Bands were visualized with enhanced chemiluminescence (ECL, Amersham Life Sciences), according to the manufacturer's instructions. Immunoblotting data were analyzed using ImageJ software (developed by Wayne Rasband, NIH, USA) to determine density of the bands.

Rac1-GTP pull-down assay

Pooled mouse mesenteric arteries treated with different compounds (AkP05 or single components) at the concentration of 0.4 mg/mL and stimulated with angiotensin II (10^{-5} M) for 1 hour, were lysed in a buffer containing NP-40 equipped with the kit. P21-binding domain (PBD) of p21-activated protein kinase (PAK) bound to agarose beads was added, and active Rac1, binding PAK1, was separated by repetitive centrifugation and washing. After, the specimens were boiled in Laemmli buffer, subjected

to SDS-PAGE, and Rac was quantified by Western blot analysis. In detail, Rac1-GTP was detected with the monoclonal antibodies anti-Rac1-GTPγ (1:800; STA-401-1, Cells Biolab Inc.), and total Rac1 with monoclonal anti-Rac1 (1:1000; Abcam). Immunoblotting data was analyzed using ImageJ software to determine density of the bands. The amount of Rac1-GTP was normalized to the total amount of Rac1.

Protein extraction and immunoblot analysis

For total protein extraction, mesenteric arteries were lysed in a buffer containing 150 mmol/L NaCl, 50 mmol/L Tris-HCl (pH 8.5), 2 mmol/L EDTA, 1% v/v NP-40, 0.5 % w/v deoxycholate, 10 mmol/L NaF, 10 mM sodium pyrophosphate, 2 mmol/L PMSF, 2 g/ml leupeptin, and 2 g/ml aprotinin (pH 7.4). Lysates were incubated on ice for 15 min and then centrifuged at 38000 x g for 30 min at 4 °C to collect the supernatant. Protein concentration was measured using a dye-binding protein assay kit (Bio-Rad) and read at the spectrophotometer at a wavelength of 595 nm.

NADPH oxidase activity

NADPH oxidase activity in mouse mesenteric arteries was measured in control (untreated) vessels and in vessels treated with angiotensin II (10^{-6} M), or angiotensin II plus different compounds pre-incubated for 1 h at the dose of 0.4 mg/mL. Vessels were placed in chilled, modified Krebs/HEPES buffer containing (in mmol/L) NaCl, 99.01; KCl, 4.69; $CaCl_2$, 1.87; $MgSO_4$, 1.20; K_2HPO_4, 1.03; $NaHCO_3$, 25.0; Na HEPES, 20.0; and glucose, 11.1, pH 7.4. Periadventitial tissue was carefully removed, and the vessels were repeatedly washed to remove adherent blood cells. Vessel homogenate was prepared in 50 mmol/L of phosphate buffer containing 0.01 mmol/L EDTA. The homogenate was then subjected to low-speed centrifugation (1000 g) for 10 min to remove unbroken cells and debris. Supernatants (40 µL) were added to glass scintillation vials containing 5 µmol/L lucigenin in 1 mL of phosphate buffer. The chemiluminescence that occurred over the ensuing 5 min in response to addition of 100 µmol/L NADPH was recorded (LS6500 Multipurpose Scintillation Counter; Beckman Coulter, Fullerton, CA, USA). In preliminary experiments, homogenates alone, without addition of NADPH, gave only minimal signals, and NADPH did not evoke lucigenin chemiluminescence in the absence of homogenate.

Data S2.

Supplemental Results

Chemical characterization of the nutraceutical compound

The resulting MS data (**Table S5 & Figure S1**) highlighted the presence of compounds belonging to the three matrices. In particular, catechins and procyanidin derivatives, typical of green tea, were eluted in the first part of the LC run; glycosylated flavonoids and gynkgolids, typical of *G. Biloba*, in the middle; and saponins characteristic of the *Bacopa* extract were the last compounds to be eluted. Phosphatidyl serine was not detected, probably under the LOQ of the MS method. The most abundant compounds were apigenin and kaempferol aglycones.

Quantitation of main compounds was performed by external calibration employing 5 standards (**Table S6**) with 5 concentration levels (1, 5, 10, 15, 25 μg/mL), three replicates of each LC run was carried out.

Table S1. Baseline characteristics of hypertensive patients of first study.

Variable	Placebo group (n=15)	AkP05 group (n=30)	*p*
Clinical characteristics			
Age (yrs)	57.1±6.4	57.5±9.5	0.8643
Males, n (%)	12 (80)	21 (70)	NS
Height (cm)	169.3±9.7	169±7.6	>0.9999
Weight (kg)	74.8±11.4	79.6±15.6	0.3000
Body mass index (kg/m^2)	26.11±3.3	27.7±4.1	0.2154
SBP (mmHg)	130.5±16.9	133.5±12.5	0.5094
DBP (mmHg)	86.60±9.6	85.1±10.4	0.6429
Heart rate (bpm)	68.87±7.1	67.2±8.1	0.5126
Laboratory characteristics			
Total cholesterol (mg/dl)	201.6±24.2	199.8±23.8	0.8133
HDL cholesterol (mg/dl)	48.0±8.5	49.3±9.4	0.6559
LDL cholesterol (mg/dl)	132.6±26.9	127.1±24.6	0.4978
Triglycerides (mg/dl)	115.9±42.7	122.5±36.9	0.5965
Uric acid (mg/dl)	5.6±1.0	5.4±0.8	0.5452
Serum creatinine (mg/dl)	0.94±0.14	0.98±0.16	0.3112
Glycemia (mg/dl)	96.9±11.8	93.7±9.0	0.3228
Medication, n (%)			
ACE-inhibitors	2 (13.3)	4 (13.3)	
AT II receptor blockers	7 (46.6)	17 (56.6)	
Beta-blockers	3 (20)	7 (23.3)	
Calcium antagonists	4 (26.6)	9 (30)	
Diuretics	5 (33.3)	10 (33.3)	

Data are mean±SD. ACE, angiotensin-converting enzyme; AT II, angiotensin II; SBP, systolic blood pressure; DBP, diastolic blood pressure; NS, not statistically significant. Statistical analysis has been performed using unpaired t-test (two-tails).

Table S2. Pharmacological therapies of patients.

Medications	Placebo group (n=15)	AkP05 group (n=30)
ACE-inhibitors	2	2
Zofenopril		
AT II receptor blockers		
Irbesartan 150mg	1	2
Lortaan 50	0	1
Olpress 20	2	3
Ratacand 16	1	0
Beta-blockers		
Dilatrend 25	3	2
Lobivon 5	0	2
Calcium antagonists		
Amlodipina 10	0	2
Norvasc 5	2	2
Diuretics		
Moduretic	2	1
Esidrex	2	0
Diuretics + ACE-inhibitors		
VASORETIC	0	2
AT II receptor blockers + Calcium antagonists		
GIANT 40/5	1	1
PRITOR 80; Amlodipina 10	0	1
Diuretics + AT II receptor blockers		
Olmegan 40/12.5	0	3
Coaprovel 300/12.5	1	1
Corixil 160/12.5	0	1
AT II receptor blockers + Beta-blockers		
OLPRESS 40; Dilatrend 25 ½ cp	2	0
Beta-blockers + Calcium antagonists		
Norvasc 5 ½ cp; Lobivon 5	0	2
AT II receptor blockers + Beta-blockers + Calcium antagonists		
BIVIS 40/10; Dilatrend 25 ½ cp	1	2
Diuretics + AT II receptor blockers + Beta-blockers		
Olmegan 40/12.5; Lobivon 5	0	1
Diuretics + AT II receptor blockers + Calcium antagonists		
Plaunazide 20/25; Norvasc 5 ½ cp	0	1

Table S3. Statistical analysis of hypertensives that achieve target blood pressure in placebo and active treated groups.

	PLACEBO N=15 (%)	**AKP05 N=30 (%)**	***P***
Target BP <130/80 mmHg (overall)	1 (6.66)	16 (53.3)	0.002
Target BP <130/80 mmHg (male)	1 (8.33)	10 (47.6)	0.021
Target BP <130/80 mmHg (female)	0	6 (66.6)	0.045

Data are presented as absolute number and percentage (%) of positive patients referred to each sample's population. Statistical analysis was performed using Z Score Calculator for 2 Population Proportions.

Table S4. Baseline characteristics of patients undergoing cardiopulmonary exercise test.

Variable	Supplementation with AkP05 (n=12)	Supplementation with a diuretic (n=12)
Age (yrs)	54.1±7.3	53.7±6.7
Males (%)	6 (50)	6 (50)
Height (cm)	173.8±7.4	170.7±5.6
Weight (kg)	84.3±15.2	84.0±15.3
Body mass index (kg/m^2)	26.8±6.4	27.1±6.0
Medication, n (%)		
ACE inhibitors	2 (16.6)	1 (8.33)
AT II receptor blockers	4 (33.3)	3 (25)
Beta-blockers	3 (25)	4 (33.3)
Calcium antagonists	3 (25)	4 (33.3)

Data are mean±SD. ACE, angiotensin-converting enzyme; AT II, angiotensin II.

Table S5. UHPLC-PDA-MS/MS characterization of AkP05.

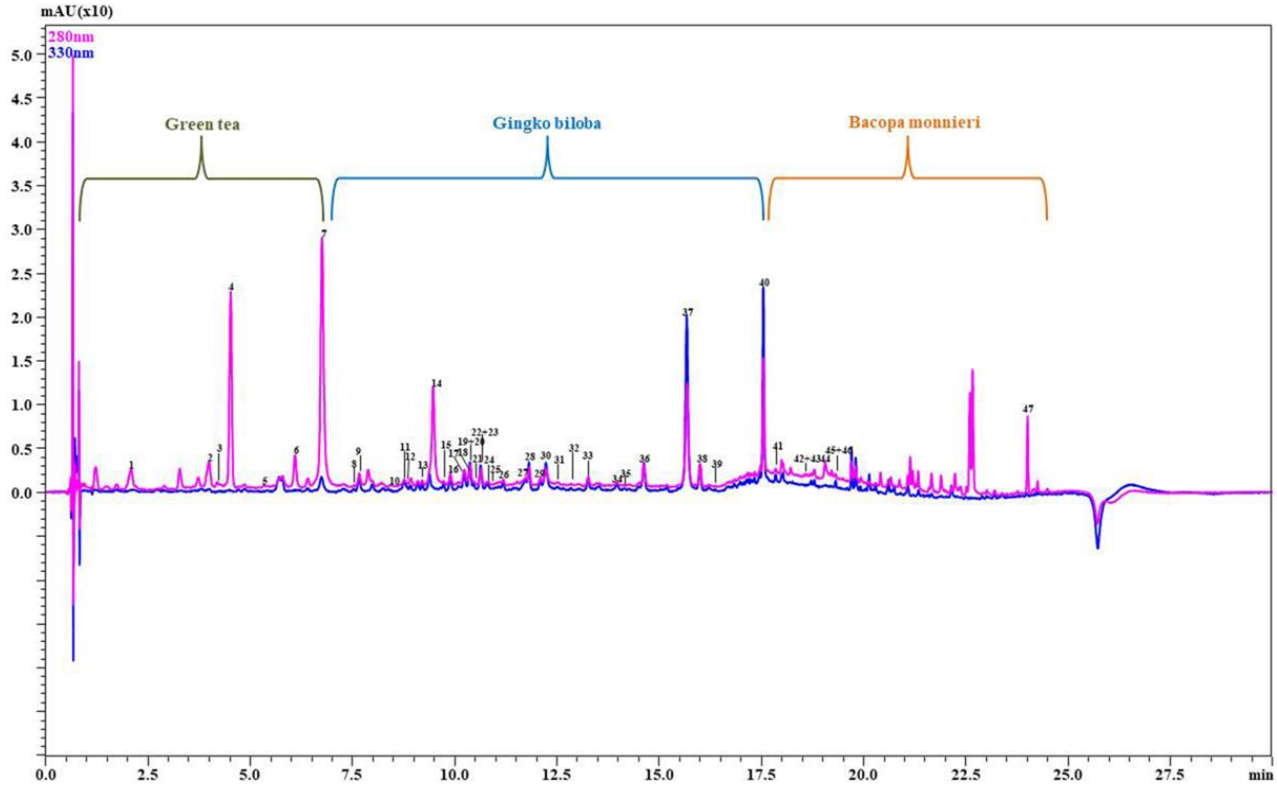
Peak	t_r	Compound	$[M-H]^-$	[MS/MS]	Error (ppm)	Molecular Formula
1	2.11	(-) Epigallocatechin	305.0635	219.0298 125.0421	-10.42	$C_{15}H_{14}O_7$
2	4.00	Gallocatechin	305.0637	219.0695 125.0042 179.0259 2210374	-9.83	$C_{15}H_{14}O_7$
3	4.20	Catechin	289.0688	211.3420 245.0626 161.0527	-6.92	$C_{15}H_{14}O_6$
4	4.54	Unknown	451.1254	281.0680 453.1331 433.1206	1.77	$C_{21}H_{24}O_{11}$
5	5.74	Procyanidin B1	577.1348	289.0643	-2.53	$C_{27}H_{30}O_{15}$
6	6.11	(-) Epicatechin	289.0698	245.0844 161.0427	-6.92	$C_{15}H_{14}O_6$
7	6.75	Epigallocatechin-3-gallate	457.0781	169.0117 193.0212 305.0606	1.09	$C_{22}H_{18}O_{11}$
8	7.50	Kaempferol-3-O-rutinoside	593.1497	285.0377	-2.53	$C_{27}H_{30}O_{15}$
9	7.65	Yinxingestin	491.1224	329.0854	5.90	$C_{23}H_{24}O_{12}$
10	8.49	Ginkgolide C	439.1254	383.1312 259.1273	0.01	$C_{20}H_{24}O_{11}$
11	8.75	Myricetin-3-O-rutinoside	625.1426	316.0179	2.56	$C_{27}H_{30}O_{17}$
12	8.76	Myricetin-3-O-glucoside	479.0829	316.0224		$C_{21}H_{20}O_{13}$
13	9.25	Quercetin-3-O-2’’,6’’-dirhamnosylglucoside	755.2128	300.0226 757.2132	11.65	$C_{33}H_{40}O_{20}$
14	9.49	Unknown	441.0836	289.0677 169.0081	4.99	$C_{22}H_{18}O_{10}$
15	9.75	Apigenin-7-O-glucoside	431.1006	311.0544 283.0611	5.10	$C_{21}H_{20}O_{10}$
16	9.95	Quercetin-3-O-rhamnsosylhexoside-7-O-glucoside	771.2056	301.0306	1.04	$C_{26}H_{44}O_{26}$
17	10.25	Kaempferol-3-O-2’’,6’’-dirhamnosylglucoside	739.2138	284.0349 255.0276	6.36	$C_{33}H_{40}O_{19}$
18	10.28	Kaempferol-3-O-rhamnsosylhexoside-7-O-glucoside	755.2080	285.0292	5.60	$C_{33}H_{40}O_{20}$
19	10.39	Quercetin-3-O-galactoside	463.0848	301.0309		$C_{21}H_{20}O_{12}$
20	10.40	Rutin	609.1461	301.0326	0.01	$C_{27}H_{30}O_{16}$
21	10.41	Quercetin-3-O-glucoside	463.0868	301.0324	-3.02	$C_{21}H_{20}O_{12}$
22	10.49	Isorhamnetin-3-O-2’’,6’’-dirhamnosylglucoside	769.2234	314.0357 299.0338	4.78	$C_{34}H_{42}O_{20}$
23	10.66	Kaempferol-3-O-hexoside	447.0938	285.0365	1.12	$C_{21}H_{20}O_{11}$
24	10.75	Patuletin-3-O-rutinoside	639.1600	331.1402	5.16	$C_{28}H_{32}O_{17}$

				330.0459 271.0189		
25	10.81	Patuletin-7- O - glucoside	493.1049	330.0375 343.0946	5.15	$C_{22}H_{22}O_{13}$
26	11.20	Kaempferol-3-O-2''-glucosyl-6'-rhamnosylglucoside	755.2079	285.0346 593.1459	5.16	$C_{33}H_{40}O_{20}$
27	11.75	Quercetin-3-O-2''-glucosylrhamnoside	609.1463	300.0239 271.0217 255.0223	6.89	$C_{27}H_{30}O_{16}$
28	11.84	Kaempferol-3-O-neohesperidoside	593.1523	285.0391 255.0296 129.5690	2.02	$C_{27}H_{30}O_{15}$
29	12.13	Kaempferol-3-O-hexoside	447.0924	285.0378	-2.01	$C_{21}H_{20}O_{11}$
30	12.29	Isorhamnetin-3-O-dihexoside	623.1588	315.0467 300.0259	-4.81	$C_{28}H_{32}O_{16}$
31	12.50	Syringetin-3-O-2''-glucosylrhamnoside	653.1710	345.0595	-1.99	$C_{29}H_{34}O_{17}$
32	12.87	Ginkgolide J	423.1291	367.1366 261.1321 395.1071	1.18	$C_{20}H_{24}O_{10}$
33	13.29	Kaempferol-3-O-glucosylrhamnoside	593.1492	284.0250	-1.01	$C_{27}H_{30}O_{15}$
34	13.37	Isorhamentin-7-O-glucoside	477.1034	314.9209	4.92	$C_{22}H_{22}O_{12}$
35	14.00	Unknown	445.0769	269.0405 436.7879 356.9776	11.46	$C_{28}H_{14}O_{6}$
36	14.65	Quercetin-3-O-p-coumaroyldiglucosyde	755.1893	609.1425	0.66	$C_{36}H_{36}O_{19}$
37	15.70	Kaempferol	285.0391	287.0569 229.0408	-4.21	$C_{15}H_{10}O_{6}$
38	16.02	Kaempferol-p-coumaroyldiglycoside	739.1965	593.1451	11.50	$C_{36}H_{36}O_{17}$
39	16.25	Quercetin-p-coumaroyldiglucoside	755.1879	609.1358 300.0234	3.89	$C_{36}H_{36}O_{19}$
40	17.50	Apigenin	269.0432	271.0409 145.0323 225.0450	-8.55	$C_{15}H_{10}O_{5}$
41	17.88	Bacoside A_3	973.5042	913.4650 791.3708	6.21	$C_{47}H_{76}O_{18}$
42	18.76	p-glc-Glu-ara	987.5243	941.4830	7.33	$C_{47}H_{74}O_{19}$
43	18.80	Bacopaside II	927.5036	633.3889 795.4484	8.30	$C_{47}H_{76}O_{18}$
44	19.10	Deoxybacopaside I	959.4397	827.3751	5.79	$C_{46}H_{72}O_{19}S$
45	19.27	Bacopaside A oxy-p-2-glucosyl-malonylpentoside	369.1192	177.0226 371.1151 139.1229	5.20	$C_{14}H_{26}O_{9}S$
46	19.28	Bacopaside N1	795.4608	633.3852	9.05	$C_{42}H_{68}O_{14}$
47	24.00	Bacopaside I	977.4548	845.3959	12.99	$C_{46}H_{74}O_{20}S$

Table S6. Quantification of the most abundant peaks.

Compound	R^2	µg/mg	CV%
Apigenin	0.996	0.14 ± 0.01	2%
Kaempferol	0.999	0.25 ± 0.01	1%
Rutin	0.999	0.09 ± 0.01	3%
Kaempferol-3-*O*-glucoside	0.999	0.05± 0.01	3%
(-) Epicatechin	0.999	0.06 ± 0.03	3%

Figure S1. AKP05 profile with UHPLC-PDA-MS/MS peak assignment.



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