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Development and Validation of an HPLC-PDA Method for Quantitation of Ten Marker Compounds from *Eclipta prostrata* (L.) and Evaluation of Their Protein Tyrosine Phosphatase 1B, α-Glucosidase, and Acetylcholinesterase Inhibitory Activities

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Abstract – The aerial parts of *Eclipta prostrata* is used as a traditional medicine and vegetable. In traditional folk medicine, it is used for treatment of hemorrhages, hepatic, disease, renal injuries, hair loss, tooth mobility, and viper bites. In this study, ten compounds (**1** – **10**) were isolated from the aerial parts of *E. prostrata*. A reliable high performance liquid chromatography equipped with photometric diode array detector (HPLC-PDA) method was developed to simultaneously quantitate 10 marker compounds [chlorogenic acid (**1**), paratensein 7-*O*-*β*-D-glucoside (**2**), quercetin 7-*O*-*β*-D-glucoside (**3**), luteolin 7-*O*-*β*-D-glucoside (**4**), apigenin 7-*O*-*β*-D-glucoside (**5**), apigenin 4'-*O*-*β*-D-glucoside (**6**), apigenin (**7**), luteolin (**8**), wedelolactone (**9**), and paratensein (**10**)]. In addition, compounds **5** and **6** showed considerable inhibitory effects against protein-tyrosine phosphatase 1B (PTP1B) enzyme. Moreover, compounds **6** – **8**, and **10** exhibited potent α-glucosidase inhibitory effects with IC_{50} values of 24.5 ± 1.9, 33.0 ± 0.5, 45.5 ± 0.1, and 23.8 ± 1.0 μM, respectively. All compounds (**1** – **10**) showed considerable acetylcholinesterase (AChE) inhibitory effects with IC_{50} ranging from 30.1 to 75.2 μM.
Keywords – *Eclipta prostrata,* PTP1B, α-Glucosidase, AChE inhibitory activity

Introduction

Eclipta prostrata (L.) is an annual herb and belongs to Asteraceae family. This plant is widely distributed in tropical, subtropical, and warm temperate areas of the world. *E. prostrata* are used as food and medicinal material. It was traditionally used for treatment of infectious hepatitis, snake venom poisoning, gastritis, and respiratory diseases.[1,2] These studies discovered the constituent of thiophene, polyacetylene, terpenes, terthiophene, triterpene, and phenolic compounds from the aerial parts from this plant using several liquid chromatographic methods Several previous studies[3,4] have found the phenolic and flavonoid components as our study. However, due to different extraction, separation conditions, and samples on different HPLC system, the components of this plant detected were also slightly different. Fang et al reported the phenolic and flavonoid components in this plant using high performance liquid chromatography- diode array detector (HPLC-DAD) coupled to mass spectrometry (MS) throug the electrospray ionization interface (ESI) (HPLC-DAD-ESI-MS/MS), which had a number of differences when comparing to those reported by Han et al using LC/MS.[3,4] Our study focused on extraction and isolation of polar compounds from *n*-BuOH fraction of MeOH extract. Therefore, we have found the components which were isolated for the first time in this plant as apigenin 7-*O*-*β*-D-glucoside (**5**) and apigenin 4'-*O*-*β*-D-glucoside (**6**). Meanwhile, paratensein 7-*O*-*β*-D-glucoside (**2**)[5] and paratensein (**10**)[6] had found from other study. In this study, we describe the isolation and a convenient, complete, and sensitive analytical method to simultaneously quantitate ten marker compounds from the aerial parts of *E. prostrata*. Continuing finding PTP1B, α-glucosidase, and AChE inhibitors from plants, all the isolates were subsequently evaluated for their PTP1B, α-glucosidase, and AChE inhibitory activities.

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Experimental

General experimental procedures – All reagents and solvents used were of analytical grade. Water was purified using a Milli-Q system (Millipore, Bedford, USA). The nuclear magnetic resonance (NMR) spectra were measured in methanol-d_4, pyridine-d_5, or DMSO-d_6 on an Oxford AS 400 MHz instrument (Agilent, Santa Clara, CA, USA). Column chromatography was performed on silica gel (Merck, Darmstadt, Germany; 63 – 200 μm particle size) or RP C-18. Fractions were monitored by thin layer chromatography (TLC), and spots were visualized by spraying with ethanol containing 10% H_2SO_4, followed by heating. The quantitative analyses were carried out on an HPLC chromatography (Waters, Houston, TX, USA) and an Aegispak C18-L column (4.6 × 250 mm, 5 μm particle size; Youngin Biochrom, Korea). Data handling was managed by Empower v.3.0 software.

Plant materials – The plant materials of *E. prostrata* were identified and authenticated by Prof. Byung Sun Min. The voucher specimens (EP.2016006) of the plants were deposited in Herbarium at College of Pharmacy, Daegu Catholic University, Korea.

Isolation of the chemical constituents – The dried aerial parts of *E. prostrata* (2.0 kg) were extracted with 80% methanol (15 L × 3 times) at 80 ºC for 5 h. The methanol extract was filtered and concentrated in *vacuo* to afford methanol extract (400 g). The concentrated extract was then suspended in water (6 L) and successfully partitioned with *n*-hexane (6 L × 5, 31 g), ethyl acetate (6 L × 5, 80 g), and *n*-BuOH (6 L × 5, 180 g). The *n*-BuOH fraction was chromatographed by using a silica gel column (63 – 200 μm particle size, 10 × 120 cm), eluting with a gradient solvent system of CH_2Cl_2/MeOH/H_2O (10/1/0.1 to 1/1/0.1, each 5 L), to yield nine fractions (EP1 – EP9). Sub-fraction EP3 (1.2 g) was purified using Sephadex LH-20 chromatography and eluted with a solvent system of MeOH/H_2O (1/1, v/v) to give compounds **3** (12.0 mg), **5** (20.1 mg), and **8** (8.2 mg), respectively. Sub-fraction EP4 (0.9 g) was subjected to RP-C18 column chromatography and eluted with a gradient solvent system of acetone/H_2O (1/5, 2/5, 3/5, and 5/5, v/v) to give six sub-fractions (EP4A to EP4F). Sub-fraction EP4A was subjected to chromatography over Sephadex LH-20 eluted with MeOH/H_2O (1/1, v/v) to give compounds **1** (11.2 mg), **2** (6.8 mg), and **4** (9.6 mg), separately. Sub-fraction EP4C was purified using Sephadex LH-20 chromatography eluted with a solvent system of MeOH/H_2O (1/1, v/v) to yield compounds **6** (21 mg) and **7** (12 mg). Sub-fraction EP4E (201 mg) was chromatographed on Sephadex LH-20 column, eluted with a solvent system of MeOH/H_2O (1/1, v/v) to give compounds **9** (15 mg) and **10** (6.2 mg).

Standards solutions – The isolated compounds (**1** – **10**) were above 96% pure in HPLC chromatogram. The isolates (**1** – **10**) were used as standard stock solutions for current analytical method. The standard solutions were prepared separately for each analytical standard and internal standard (**IS**) in 2 mL MeOH at 1000 μg/mL. The appropriate concentrations for content determination were obtained by diluting with MeOH. The solutions were transferred to 10 mL glass brown vials, sealed using elastic plastic film (Parafilm, Chicago, IL, USA) and stored in a refrigerator (4 ºC) for analysis.

HPLC instrument and chromatographic conditions – HPLC-PDA analyses were performed on a Waters (Houston, TX, USA) equipped with an autosampler, degasser, quaternary solvent pump, and PDA detector (Waters 2998) scanning in the wavelength range of 190 – 400 nm. The using solvents consisted of high purity water, acetonitrile and methanol (Grade solvent, Honeywell, Korea). Separation was carried out on an Aegispak C18–L column (4.6 × 250 mm, 5 μm particle size; Youngjin Biochromass, Korea). UV detection was recorded at the wavelength of 349 nm.

Sample preparation – Extraction process is an important step affecting the recovery of bioactive natural products. In general, methanol or methanol-water and ethanol or ethanol-water is considered to be the most efficient extracts. In this work, the dried aerial parts of *E. prostrata* were grinded for 5 min and were then sieved through a 250 μm^2 sieve to ensure required sample homogeneity. The composition of ethanol-water mixtures (95%, 75%, 50%, 25%) and methanol-water (100%, 75%, 50%, 25%) were used as extraction solvents for analytical samples. Then, the *E. prostrata* samples (1.0 g) were extracted with 25 mL of above solvent mixtures for 30 min at room temperature in an ultrasonic bath. In comparison between ultra-sonication and the conventional heating reflux at 80 ºC methods were experimented using extraction solvent. The different extraction times (30, 45, 60, and 75 min) were experimented in extraction solvent *via* sonication at room temperature to optimize the extraction time.

Chromatographic condition – The best elution conditions were used as the linear gradient described. The mobile phase was investigated using both methanol-water containing 0.1% formic acid and acetonitrile-water containing 0.1% formic acid systems. Subsequently, the buffer concentration in water was adjusted to obtain the best resolution of chromatogram. The chromatographic

peaks in the sample solutions were identified by comparing their retention times with those of the individual standards as well as UV shapes, and then were confirmed by spiking the samples with the individual compounds.

Method validation – The analytical method for the aerial parts of *E. prostrata* were validated by the determination of linearity, limit of detection (LOD) and limit of quantitation (LOQ), accuracy, precision, stability, and robustness.

Ten marker compounds (**1** – **10**) were accurately weighed and dissolved in methanol at the concentration of 1000 μg/mL as stock solutions. The stock solutions were then diluted to produce different concentrations for each marker. Linearity was determined by plotting the measurements of area peak ratios (analyte/IS) versus concentrations of analytical standards.

The sensitivity was expressed by the LOD and LOQ. The LOD represents the lowest concentration that can be reliably determined at a signal-to-noise (S/N) ratio of 3. The estimate for the LOQ was calculated using S/N ratio of 10.

Intra-day ($n = 5$) and inter-day ($n = 5$) precisions and accuracies were evaluated by analyzing sets of five independent spiked the low, mid, and high concentration samples (each analyte at 1, 50, and 200 μg/mL). The precision was expressed as RSD% and the accuracy was expressed as bias.

The stability of marker compounds was analyzed by the sample solution of aerial parts of *E. prostrata* through storing extract solution in the dark at 4 ºC and room temperature (25 ºC). The two samples were separately analyzed in triplicate at 0, 1, 3, 7, 15, and 30 days.

PTP1B inhibitory assay – PTB1B (human, recombinant) was purchased from Biomol International LP (USA). Its activity was measured by adding 2 mM *p*-NPP and PTP1B in a 50 mM citrate buffer (pH 6.0, 0.1 M NaCl, 1 mM EDTA, and 1 mM dithiothreitol), with or without tested compounds. The plate was pre-incubated at 37 ºC for 10 min, and then 50 μL of *p*-NPP in buffer was added. After incubating at 37 ºC for 30 min, the reaction was then terminated with 1 N NaOH. The amount of produced *p*-nitrophenyl after enzymatic dephosphorylation was obtained by measuring the absorbance at 405 nm using a VersaMax microplate reader (Molecular Devices, Sunnyvale, CA, USA). The non-enzymatic reactions of 2 mM *p*-NPP were determined by measuring the increase in absorbance at 405 nm without PTB1B enzyme. The PTP1B inhibitory activity (%) was calculated as $(Ac - As) / Ac \times 100\%$, where *Ac* and *As* are the absorbance of the control and sample, respectively. Ursolic acid was used as a positive control.

***α*-Glucosidase inhibitory assay** – Glucosidase inhibition assay was assessed according to the chromogenic method described by Wantanabe. Briefly, the mixture solution including 90 μL of 0.1 M phosphate buffer (pH 7.5, 0.02% NaN_3), 10 μL each sample dissolved in DMSO, and 80 μL of enzyme solution (well concentration 0.05 U/mL) were added to each well. After that, they were incubated at 28 ºC for 10 min before adding PNPG to a final volume of 200 μL. Their product was recorded at 405 nm every 30 s for 35 min in a Multiscan FC microplate photometer with built-in incubator (Thermos Scientific, Waltham, MA), controlled by Skanlt version 2.5.1 software. Acarbose was used as a positive control. The *α*-glucosidase inhibitory activity of test samples were expressed as percentage inhibition and was calculated using the following formula: % inhibition = $(Slope_{blank} - Slope_{sample}) / Slope_{blank}$.

Acetylcholinesterase inhibitory assay – AChE inhibitory activity was experimented using Ellman's method. Acetylthiocholine iodide (ATCI) was used as substrates to determine for the inhibition of AChE. Briefly, 140 μL of sodium phosphate buffer (pH 8.0), 20 μL of each tested sample with different concentrations (4, 20, and 100 μM) and 20 μL enzyme solution were mixed and incubated at room temperature for 15 min. The reactions were initiated by the addition of 10 μL of 0.01 M 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) and 10 μL of 0.075 ATCI. The reaction solution was incubated at 37 ºC for 20 min, then the production of thiocholine reacts with DTNB to produce a yellow compound (5-thio-nitrobenzoate), which was detected at 412 nm. All tested samples and the positive control (berberine) were dissolved in 10% analytical grade dimethyl sulfoxide (DMSO). The reaction was independently experimented for three times and recorded in 96-well microplates using a microplate reader (VersaMax). Percent inhibition was calculated using the formula: % AChE inhibition = $(1 - S / E) \times 100$, where *E* and *S* were the enzyme activities with and without the tested sample, respectively. The 50% inhibition concentration of each tested compound was calculated from the log dose-inhibition curve.

Statistical analysis – Tests were conducted in the means triplicate assays ± standard deviation. The statistical significance was determined by using SPSS software (Version 22.0, SPSS Inc. Chicago, IL, USA). Statistical significances were defined at $p \leq 0.05$.

Results and Discussion

Flavonoids are known as a large group of polyphenolic compounds. Their activities are broad ranging. With high antioxidant capacity both *in vivo* and *in vitro* systems, flavonoids are thought to have health-promoting properties. Flavonoids have human protective effects by against many infectious (bacterial and viral diseases) and degenerative diseases such as cardiovascular, cancer, and other age-related diseases. Therefore, we initially examined the main peaks in the chromatogram of *E. prostrata* MeOH extract using HPLC-PDA system. Based on the manner of UV shape of the main peaks, they were predicted as flavonoids. These effects are related to its use in traditional medicine. Thus, we focused on extraction and isolation of those flavonoids which is partly elucidated in the use of traditional medicine of this plant. In traditional medicine, most medicinal products are extracted as water solvent after heating. The extracted components are sugars and polar constituents. However, a less polar was used in our study as MeOH-H_2O giving high yield efficiency but it is not used clinically due to toxicity.

Chromatographic separation and purification of *n*-BuOH fraction from the aerial parts of *E. prostrata* led to the isolation of ten compounds (**1** – **10**). The structures of these compounds were identified as chlorogenic acid (**1**),[7] paratensein 7-*O*-*β*-D-glucoside (**2**),[5] quercetin 7-*O*-*β*-D-glucoside (**3**), luteolin 7-*O*-*β*-D-glucoside (**4**),[8,9] apigenin 7-*O*-*β*-D-glucoside (**5**),[8,9] apigenin 4'-*O*-*β*-D-glucoside (**6**),[4] apigenin (**7**),[10] luteolin (**8**),[8] wedelolactone (**9**),[11] paratensein (**10**)[6] by comparison of their NMR data with those reported data (Fig. 1).

E. prostrata samples were extracted with composition of ethanol-water mixtures (95%, 75%, 50%, 25%) and methanol-water (100%, 75%, 50%, 25%). The result exhibited that extraction solvent of 50% aqueous methanol showed the highest amount of all the markers in extract. Therefore, extract solvent mixture of methanol-water (50%) was used as extraction solvent for all the samples. Next, the sample assay results showed that the area peaks of all markers in extraction with sonication were similar to those of extraction with reflux. The ultrasonic extraction method was ultimately used due to its flexibility. Finally, the extraction time was consisted in 50% methanol *via* sonication at room temperature. The result indicated similar percent yields between 60 and 75 min. Therefore, the extraction time was selected as 60 min.

All the isolates were well detected at wavelength of 349 nm. Thus, the analytical method was validated at flow rate of 1.0 mL/min with wavelength of 349 nm. The resolution of all marker compounds was affected by the acetonitrile percentage. The mobile phase was initially investigated as methanol and water containing 0.1% formic acid system. However, this elution system could not achieve peak separation requirements. Therefore, the solvent system of ACN and H_2O were then evaluated and showed better separations and peak shapes. When

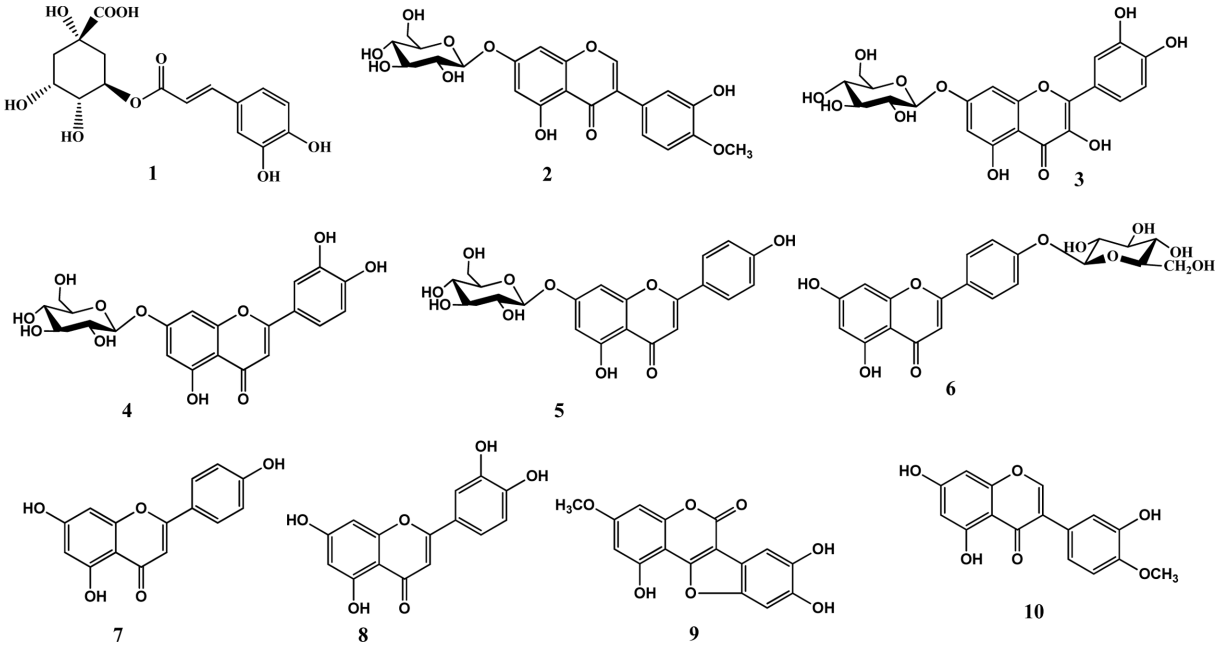

Fig. 1. The structures of isolated compounds from the aerial parts of *E. prostrata*.

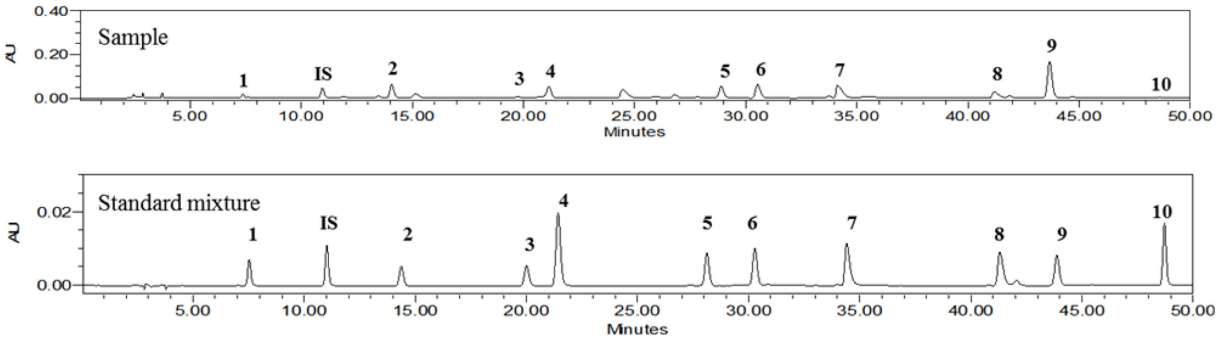

Fig. 2. The HPLC chromatograms of 50% methanol extract of Ecliptae Herba and standard mixture [chlorogenic acid (**1**), paratensein 7-*O*-*β*-D-glucoside (**2**), quercetin 7-*O*-*β*-D-glucoside (**3**), luteolin 7-*O*-*β*-D-glucoside (**4**), apigenin 7-*O*-*β*-D-glucoside (**5**), apigenin 4'-*O*-*β*-D-glucoside (**6**), apigenin (**7**), luteolin (**8**), wedelolactone (**9**), paratensein (**10**), and I.S. (caffeic acid)].

increasing the concentration of formic acid in water solvent, the resolutions of all peaks were improved. Finally, a gradient solvent system elution condition was obtained from acetonitrile (B) and water containing 0.3% formic acid (A) as follows: 0 – 20 min, 14 – 20% B; 20 – 40 min, 20 – 30% B; 40 – 50 min, 30 – 45% B. Caffeic acid with a retention time of 11.1 min was used as an internal standard. Ten marker compounds were well separated without overlapping of adjacent peaks (Fig. 2).

The linearity of the developed method was conducted by analyzing seven concentrations of each analyte (0.625, 6.25, 12.5, 25, 50, 100, and 200 μg/mL) in triplicate analysis. The concentration range is generally chosen as per International Conference on Harmonization guidelines. The calibration curves of each analyte showed excellent linearity over the tested range ($r^2 > 0.9981$) (Table 3). The LOD of each marker was determined to be 0.014 to 0.064 μg/mL and the LOQ was 0.049 to 0.214 μg/mL. This collected results indicated that our developed method is well-established with a good sensitivity.

The accuracy of the developed HPLC method was determined by analyzing the known amounts at the three different concentrations (each analyte: 1, 50, and 200 μg/mL) of analytes spiked into 50% methanol extract solution of the aerial parts of *E. prostrata*. After addition of known amounts of each analyte to the previous 50% methanol extract solution, their recoveries were investigated (Table 4). The method precision was measured by five successive injections, and the precisions were less than 14.50% in intra-day and 14.27% in inter-day. The accuracies of the method were in the range 89.32 – 107.35% in intra-day and 85.12 – 107.51% in inter-day. The method validation indicated that the regression equations of the marker compounds were linear and this method was precise, accurate, and reliable for quantitation of the 10 marker compounds (**1** – **10**).

The stability of the analytes at room temperature was measured at 0, 1, 3, 7, 15, and 30 days (Table 5). Under lightless temperature (25 ºC) and 4 ºC, all marker compounds (**1** – **10**) displayed stable with recovery ranging from 97.39 to 100.93%.

The contents of isolated constituents from the roots of *E. prostrata* were listed in Table 1. In general, the major components in twelve different samples were compounds **5, 6**, **7**, and **9** at the average concentrations of 0.0258%, 0.0064%, 0.0113%, and 0.0661% on dry weight basis, respectively. Whereas, the amounts of compounds **3** and **10** are minor components in (E01 – E06, E08, E10, and E11) and (E05 – E12) samples, respectively.

Compounds (**1** – **10**) isolated from *E. prostrata* were assessed for their inhibitory potential against PTP1B using *p*-NPP as a substrate. The known PTP1B inhibitor (ursolic acid, $IC_{50} = 9.5 \pm 0.4$ μM) was used as the positive control in the assay. Compounds **5** and **6** displayed considerable inhibitory effects against PTP1B, with IC_{50} values of 81.3 ± 1.7 and 56.9 ± 0.6 μM, respectively. Meanwhile, other compounds (**1** – **4** and **7** – **10**) were inactive ($IC_{50} > 100$ μM) (Table 2).

α-Glucosidase inhibitory effect – In order to evaluate the anti-diabetic potential of all compounds (**1** – **10**) isolated from aerial parts of *E. prostrata*, these compounds were examined *via in vitro* α-glucosidase inhibitory assay. The results were shown in Table 2. Acarbose, a clinical drug was used as the positive control with IC_{50} value of 161.9 ± 1.4 μM. Among isolated compounds, apigenin 4'-*O*-*β*-D-glucoside (**6**), apigenin (**7**), luteolin (**8**), and paratensein (**10**) showed excellent inhibitions on α-glucosidase with IC_{50} values of 24.5 ± 1.9, 33.0 ± 0.5, 45.5 ± 0.1, and 23.8 ± 1.0 μM, respectively. These compounds were much more potent than that of acarbose. Notably, compounds **6** and **7** were high in content of the plant material (Table 2). These compounds might contri-

Table 1. The contents (w/w, %) of **1**–**10** in *E. prostrata* (E01 – E12) samples

Samples	Compounds (% w/w)									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
E01	0.0019	0.0092	ND	0.0098	0.0183	0.0175	0.0148	0.0109	0.069725	0.0002
E02	ND	0.0004	ND	0.0031	0.0069	0.0013	0.0103	0.0068	0.0261	0.0001
E03	0.0084	0.0364	ND	0.0063	0.0695	0.0090	0.0130	0.0048	0.13635	0.0004
E04	0.0028	0.0104	ND	0.0020	0.0198	0.01413	0.0109	0.0005	0.0456	0.0001
E05	0.0005	ND	ND	0.0021	0.0096	0.0022	0.0095	0.0027	0.02904	ND
E06	ND	ND	ND	0.0028	0.0051	0.0018	0.0084	0.0058	0.0242	ND
E07	0.0086	0.0049	0.0010	0.0036	0.0285	0.0101	0.0138	0.0009	0.0831	ND
E08	0.0012	0.0016	ND	0.0035	0.0273	0.0019	0.0061	0.0038	0.0455	ND
E09	0.0083	0.0090	0.0011	0.0092	0.0648	0.0070	0.0177	0.0043	0.1156	ND
E10	0.0002	0.0020	ND	0.0053	0.0089	0.00123	0.0126	0.01197	0.0576	ND
E11	0.0011	0.0006	ND	0.0031	0.0215	0.0022	0.0068	0.0050	0.0541	ND
E12	0.0104	0.0186	0.0002	0.0075	0.0291	0.0080	0.0117	0.0054	0.1061	ND
Average	0.0036	0.0077	0.0002	0.0048	0.0257	0.0064	0.0113	0.0052	0.0661	0

ND: not detect.

Table 2. PTP1B, α-glucosidase, and AChE inhibitory effects of compounds (**1** – **10**) from the aerial parts of *E. prostrata*

Compound	PTP1B assay IC_{50} (μM)	α-Glucosidase assay IC_{50} (μM)	AChE assay IC_{50} (μM)
1	> 100	> 125	70.0 ± 1.0
2	> 100	> 125	70.8 ± 2.4
3	> 100	> 125	56.9 ± 0.6
4	> 100	59.5 ± 2.9	75.2 ± 1.4
5	81.3 ± 1.7	> 125	70.2 ± 1.9
6	56.9 ± 0.6	24.5 ± 1.9	42.2 ± 2.9
7	> 100	33.0 ± 0.5	58.5 ± 1.1
8	> 100	45.5 ± 0.1	58.7 ± 1.7
9	> 100	117.4 ± 0.2	30.1 ± 1.5
10	> 100	23.8 ± 1.0	67.4 ± 0.6
Ursolic acid[b]	9.5 ± 0.4	-	-
Acarbose[c]	-	161.9 ± 1.4	-
Berberine[d]	-	-	0.60 ± 0.07

IC_{50} values (μM) indicate 50% PTP1B inhibitory effects. These data represent the average values of three repeated experiments.
[b]Ursolic acid, [c]acarbose, and [d]berberine were used as the positive control.

bute to their effects in traditional medicine. Compounds **6** – **8** belong to flavone group and compound **10** is isoflavone skeleton. Luteolin 7-*O*-β-D-glucoside (**4**) and wedelolactone (**9**) exhibited considerable inhibitory effects with IC_{50} values of 59.5 ± 2.9 and 117.4 ± 0.2 μM, respectively. Other compounds were very weak or inactive (IC_{50} values > 125 μM). The structure activity relationships of some compounds were deduced. When comparison the structure between compounds **2** and **10** with the same skeleton, the structure of compound **2**, a flavone bearing β-D-glucopyranosyl at C-7, reduced α-glucosidase inhibitory effects. Similarly, compounds **5** and **7** had the same skeleton. Especially, compound **5** with β-D-glucopyranosyl moiety at C-7 also reduced α-glucosidase inhibitory effect.

All isolated compounds were determined for inhibitory effects against AChE. Berberin was used as the positive control with IC_{50} values of 0.60 ± 0.07. As the results, all test compounds showed considerable inhibitory effects with IC_{50} ranging from 30.1 to 75.2 μM. In particular, compounds **6** and **9** exhibited potent inhibitory effects.

In conclusion, ten compounds (**1** – **10**) were isolated from the aerial parts of *E. prostrata*. Particularly, the development of reliable HPLC/PDA method was able to simultaneously quantitate 10 marker compounds [chlorogenic acid (**1**), paratensein 7-*O*-β-D-glucoside (**2**), quercetin

Table 3. The linearity, linear range, LOD, and LOQ

Analytes	Linear range (μg/mL)	Slope	Intercept	Correlation coefficient (r^2)	LOD (μg/mL)	LOQ (μg/mL)
1	0.625 – 200	0.1582	0.0818	0.9999	0.023	0.076
2	0.625 – 200	0.1285	0.0821	0.9992	0.019	0.065
3	0.625 – 200	0.3005	0.0211	0.9998	0.048	0.160
4	0.625 – 200	0.3229	–0.0921	0.9999	0.014	0.049
5	0.625 – 200	0.1926	–0.2303	0.9997	0.039	0.132
6	0.625 – 200	0.2189	–0.0031	0.9981	0.063	0.212
7	0.625 – 200	0.3322	–0.2609	0.9997	0.064	0.214
8	0.625 – 200	0.1821	–0.0078	0.9999	0.034	0.113
9	0.625 – 200	0.1399	0.1127	0.9993	0.039	0.130
10	0.625 – 200	0.3609	0.0110	0.9997	0.021	0.070

Table 4. Intra-day and inter-day precisions and accuracies of the 10 marker compounds in 50% MeOH extract of the Ecliptae Herba

Analyte	Fortified conc. (μg/mL)	Sample conc. (μg/mL)	Intra-day (n=5)				Sample conc. (μg/mL)	Inter-day (n=5)			
			Observed (μg/mL)	SD	Accuracy (%)	Precision (%)		Observed (μg/mL)	SD	Accuracy (%)	Precision (%)
	1	1.94	2.84	0.09	90.15	9.90	1.94	2.89	0.23	85.12	11.68
1	50	1.94	51.610	0.67	99.34	1.35	1.94	51.77	0.32	99.67	0.63
	200	1.94	203.66	1.39	100.86	0.69	1.94	200.70	3.87	101.87	1.93
	1	10.01	11.08	0.13	107.35	13.70	10.01	11.18	0.19	107.51	12.78
2	50	10.01	57.35	0.64	94.68	1.28	10.01	56.08	0.37	91.92	2.31
	200	10.01	202.00	2.14	96.00	1.07	10.01	206.82	4.06	98.40	2.03
	1	0.55	1.44	0.06	89.32	6.18	0.55	1.44	0.07	89.34	6.86
3	50	0.55	50.44	0.31	99.78	0.62	0.55	50.62	0.36	100.10	0.74
	200	0.55	200.77	1.18	100.11	0.59	0.55	200.66	1.97	100.05	0.98
	1	4.25	5.28	0.09	103.48	9.09	4.25	5.24	0.11	103.95	4.51
4	50	4.25	53.11	0.47	97.72	0.94	4.25	53.24	0.51	97.97	1.03
	200	4.25	201.85	4.41	98.80	2.20	4.25	202.50	4.15	99.12	2.07
	1	8.50	9.47	0.10	97.91	10.26	8.50	9.44	0.10	95.10	10.23
5	50	8.50	55.53	1.23	94.06	2.46	8.50	55.44	0.99	93.90	1.99
	200	8.50	201.88	1.21	98.69	0.61	8.50	206.29	1.64	98.89	0.82
	1	8.29	9.26	0.10	96.72	10.13	8.29	9.28	0.04	99.18	3.89
6	50	8.29	58.66	0.53	100.73	1.06	8.29	58.88	0.99	101.17	1.07
	200	8.29	206.33	2.58	99.01	1.29	8.29	205.15	1.28	98.42	0.64
	1	6.72	7.62	0.12	90.35	12.55	6.72	7.59	0.14	86.84	14.33
7	50	6.72	56.42	0.24	99.39	0.47	6.72	56.62	0.64	99.80	0.30
	200	6.72	203.17	2.85	98.23	1.42	6.72	204.24	5.07	98.76	2.54
	1	6.47	7.41	0.25	93.14	13.95	6.47	7.52	0.15	104.36	14.27
8	50	6.47	55.75	0.29	98.55	0.58	6.47	55.61	0.64	98.26	1.29
	200	6.47	202.95	3.26	100.23	1.63	6.47	206.65	5.41	100.09	2.70
	1	58.54	59.46	0.14	92.17	14.50	58.54	59.18	0.42	85.86	4.23
9	50	58.54	109.91	1.27	102.74	2.55	58.54	113.03	1.69	104.08	2.30
	200	58.54	201.29	5.73	101.37	2.86	58.54	203.29	1.07	102.37	3.36
	1	0.04	1.05	0.02	101.57	2.18	0.04	1.05	0.02	100.85	2.00
10	50	0.04	50.94	0.64	101.81	1.28	0.04	50.32	1.51	100.57	3.02
	200	0.04	201.12	2.14	100.54	1.07	0.04	201.28	1.21	100.62	0.60

Table 5. Stability of marker compounds (**1** – **10**)

Compound	Temperature (ºC)	Day (%)						Mean	RSD (%)
		0	1	3	7	15	30		
1	4	100	97.07	98.84	102.96	100.58	101.27	100.14	2.26
	25	100	99.50	98.69	98.17	97.49	100.90	98.95	1.31
2	4	100	98.98	99.29	98.37	99.13	102.31	99.61	1.54
	25	100	98.50	96.21	100.04	99.69	96.74	98.23	1.71
3	4	100	99.21	99.40	99.30	99.61	99.78	99.46	0.23
	25	100	97.86	99.61	98.74	99.15	100.82	99.23	1.72
4	4	100	98.46	98.19	99.23	101.02	100.88	99.46	0.23
	25	100	99.05	100.50	99.57	98.66	98.01	99.23	1.09
5	4	100	99.31	99.82	100.39	99.92	96.97	99.56	1.32
	25	100	100.15	99.92	100.80	97.45	97.06	99.16	0.94
6	4	100	99.07	100.05	101.36	99.84	99.59	99.28	1.34
	25	100	98.88	97.24	99.06	99.08	98.06	99.07	1.69
7	4	100	99.48	100.03	99.23	100.51	101.99	99.98	0.85
	25	100	98.99	102.11	99.82	98.13	97.95	98.46	0.80
8	4	100	98.68	99.73	99.81	99.68	97.36	100.24	1.09
	25	100	97.28	98.13	99.21	98.77	98.70	99.42	0.74
9	4	100	99.39	97.14	98.32	98.76	100.89	98.90	1.38
	25	100	98.53	99.28	98.59	100.49	97.29	98.83	1.13
10	4	100	101.56	105.39	98.42	94.90	96.74	99.40	4.14
	25	100	102.79	107.72	101.63	101.81	99.99	102.78	2.93

7-*O*-*β*-D-glucoside (**3**), luteolin 7-*O*-*β*-D-glucoside (**4**), apigenin 7-*O*-*β*-D-glucoside (**5**), apigenin 4'-*O*-*β*-D-glucoside (**6**), apigenin (**7**), luteolin (**8**), wedelolactone (**9**), and paratensein (**10**)]. The validation results indicated that the method was simple for widespread and routine use. In PTP1B assay, compounds **5** and **6** showed considerable inhibitory effects against PTP1B enzyme. Compounds **6** – **8** and **10** showed potent inhibitory activity against *α*-glucosidase. All test compounds displayed considerable AChE inhibitory effects.

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Conflict of Interest

The authors declare no conflict of interest.

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