TRENDS IN SCIENCES 2026; 23(12): 13826
https://doi.org/10.48048/tis.2026.13826
RESEARCH ARTICLE

Phytochemical Profiling and Evaluation of Antidiabetic and Antihypercholesterolemic Activities of *Guazuma ulmifolia* Leaves

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Received: 14 March 2026, Revised: 17 May 2026, Accepted: 27 May 2026, Published: 20 July 2026

Abstract

Guazuma ulmifolia leaves have traditionally been used for managing diabetes, weight control, and cholesterol reduction. This study evaluated metabolite profiles of *G. ulmifolia* leaf extracts prepared with different solvents and assessed their antidiabetic and antihypercholesterolemic activities. The leaves were extracted using water, water:ethanol mixtures, and ethanol, followed by ^{1}H NMR-based metabolomics and multivariate analysis. Total flavonoid content was determined, and biological activities were evaluated using alpha-glucosidase inhibitory, DPPH, FRAP, and HMG-CoA reductase inhibitory assays. Approximately 31 compounds were putatively identified, including tiliroside, quercitrin, quercetin, rutin, catechin, kaempferol, luteolin, apigenin, chlorogenic acid, and primary metabolites. PCA score plot successfully classified the samples based on the extraction solvents. The evaluation of the total flavonoid content suggested that the flavonoid content increases with a higher ethanol composition in the extraction solvent. The extracts of *G. ulmifolia* leaves showed low alpha-glucosidase inhibitory activity with IC_{50} values ranging from 125.99 ± 5.25 mg/mL to 176.43 ± 10.43 mg/mL. Meanwhile, the ethanol extract exhibited the highest flavonoid content, strongest antioxidant activity, and greatest HMG-CoA reductase inhibition, with 82.79 ± 3.04% inhibition. These findings suggest that ethanol is the most effective solvent for extracting flavonoid-rich *G. ulmifolia* leaf metabolites associated with antioxidants and HMG-CoA reductase inhibitory activities, supporting its potential for further development as a natural cholesterol-lowering candidate.

Keywords: Alpha-glucosidase inhibitory activity, Antihypercholesterolemic activity, *Guazuma ulmifolia* leaves, HMG-CoA reductase inhibition, Metabolomics, Flavonoids, Solvent extraction

Introduction

Guazuma ulmifolia (Malvaceae) is a semi-deciduous tree indigenous to Latin America. The plant is popularly known as Jati Belanda in Indonesia and as mutamba or guácimo in Latin America. *G. ulmifolia* has been employed in traditional medicine across various countries, including Indonesia, Mexico, Brazil, Bolivia, and Cuba [1]. Previous studies had demonstrated that *G. ulmifolia* exhibited a range of biological activities, including anthelmintic [2], antioxidant [3], anticholinesterase [3], antifungal [3], antibiotic [3], antimicrobial [4], and cytotoxic effects [4,5].

Solvent extraction polarity is crucial during the pre-analysis stage, as it significantly impacts the types of compounds extracted. Previous research indicated that the differences in solvent polarity altered the metabolite content and solubility of chemical constituents within biological samples [6]. Thus, optimizing this processing parameter using appropriate

analytical techniques is necessary to enhance both the quality and the efficiency of the extracts.

^{1}H NMR-based metabolomics is extensively used to identify metabolite profiles within biological systems. This method has multiple benefits, including non-destructive nature, straightforward sample preparation, capability for absolute quantification, ability to offer chemical structure information, and lack of dependence on the polarity of the analyte [7]. Recently, ^{1}H NMR-based metabolomics was applied to elucidate the bioactive compounds from *Mangifera caesia* Jack and *Ficus auriculata* which extracted with different solvents [8]. This method also was employed to discriminate the leaf metabolite profiles of 3 endemic Vanilla species from the Peruvian Amazon [9]. ^{1}H NMR-based metabolite profiling of guava leaf extract was used successfully to develop a prototype for standardization of plant extracts [10].

Although several studies have reported the phytochemical composition and biological activities of *G. ulmifolia* [2-5], its solvent-dependent metabolite profiles and their relationship with antidiabetic and antihypercholesterolemic activities remain insufficiently understood. Limited information is available on how different water-ethanol ratios influence the extraction of metabolites associated with antioxidant capacity, α-glucosidase inhibition, and HMG-CoA reductase inhibition. Therefore, integrating ^{1}H NMR-based metabolomics with *in vitro* bioactivity assays may provide a more comprehensive understanding of the chemical features underlying the potential bioactivities of *G. ulmifolia* leaf extracts.

In this study, ^{1}H NMR-based metabolomics was employed to examine the metabolite profiles of *G. ulmifolia* leaves extracted with various solvents. *In vitro* antidiabetic activity of the *G. ulmifolia* leaf extracts was evaluated with alpha-glucosidase inhibitory assay, and antioxidant tests (1,1-diphenyl-2-picrylhydrazyl (DPPH) and Ferric-Reducing Antioxidant Power (FRAP)). Meanwhile, antihypercholesterolemic was evaluated with HMG-CoA reductase inhibitory assay. The total phenolic content and total flavonoid content of the *G. ulmifolia* leaf extracts were determined as well. This study aimed to identify solvent-dependent metabolite patterns and to explore their association with *in vitro* antioxidant, α-glucosidase inhibitory, and HMG-CoA reductase inhibitory activities. The novelty of this work lies in the integrated evaluation of solvent polarity, ^{1}H NMR-based metabolite profiling, and multiple bioactivity assays to support the standardization and further development of *G. ulmifolia* leaf extracts.

Materials and methods

Materials

G. ulmifolia leaves were purchased from PT Refa Rempah Mulia (Karawang, Indonesia) and deposited in Organic Chemistry Laboratory, Bandung Institute of Technology with voucher number JBD202301. These *G. ulmifolia* leaves were collected in January 2023 from Kediri, East Java, and identified by Dede Kusnandar of PT Refa Rempah Mulia. Deuterium oxide (D_2O), deuterated methanol (CD_3OD), ferric chloride ($FeCl_3$), ethanol, sodium acetate trihydrate ($CH_3COONa.3H_2O$), acetic acid, potassium dihydrogen phosphate (KH_2PO_4), dipotassium hydrogen phosphate (K_2HPO_4), and 3-(trimethylsilyl)- 2,2,3,3-tetradeuteropropionic acid sodium salt (TSP) were bought from Merck (Darmstadt, Germany). Acarbose was obtained from TCI (Tokyo, Japan). Ascorbic acid, 1,1-diphenyl-2-picryl hydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), potassium persulfate ($K_2O_8S_2$), 2,4,6 tripyridyl-s-triazine (TPTZ), potassium persulfate, p-nitrophenyl-alpha-D-glucopyranoside (PNPG) and alpha-glucosidase from Saccharomyces cerevisiae were purchased from Sigma-Aldrich (St. Louis, USA).

Extraction

Around 5 g of *G. ulmifolia* leaf powder was mixed with 40 mL of extraction solvent in a tightly closed vessel to minimize solvent evaporation. The mixture was sonicated for 20 min without external heating, followed by an incubation period at 40 °C in a Memmert wnb 22 waterbath (Memmert, Schwabach, Germany) for 30 min. The mixture was then cooled in water for 10 min before centrifugation at 12,000 rpm for 5 min. The solvent was then removed using a Buchi rotary evaporator apparatus. The solvents employed in the extractions were water, water: Ethanol (7:3), water: Ethanol (3:7), and ethanol. These solvent systems were selected to represent different polarity ranges and to evaluate the influence of solvent composition on the metabolite profile and biological activity of *G. ulmifolia* leaf extracts. Water was used to extract highly polar and

water-soluble compounds, while ethanol was selected to extract semi-polar phytochemicals, particularly phenolic compounds and flavonoids. The water: Ethanol mixtures (7:3 and 3:7) were included to provide intermediate polarity conditions and to assess whether mixed solvents could enhance the extraction of compounds with different solubility characteristics. The dried extracts (15 mg) were dissolved in the corresponding deuterated NMR solvents (600 µL), including deuterium oxide, deuterium oxide:methanol-D4 (7:3), deuterium oxide:methanol-D4 (3:7), and methanol-D4.

^{1}H NMR measurements

^{1}H NMR spectra were captured using a Variant Unity INOVA-500 Spectrometer (Agilent Technologies, Santa Clara, United States). The water signal was treated with the presauration method. The parameters for the ^{1}H NMR measurements included 128 scans, 64 K data points, an 8,012.8 Hz spectral width, a 2.720 s acquisition time, and a 2.0 s delay time. The Free Induction Decay (FID) ^{1}H NMR data were subsequently processed using ACD/Labs 12.0 software (ACD/Labs, Toronto, Canada). Corrections were applied to the spectrum baseline, and the chemical shift was calibrated using the TSP signal.

Data extraction

^{1}H NMR spectra of *G. ulmifolia* leaves underwent further processing through alignment and bucketing techniques using ACD/Labs 12.0 software (ACD/Labs, Toronto, Canada). Bucketing, employing the intelligent option, was performed to generate integrated bins with an equal width of 0.04 ppm within the chemical shifts (δ) range of 0.50 to 12.00 ppm. Buckets containing residual water signals (δ 4.75 - 5.20 ppm) were excluded from the analysis. Subsequently, the processed data were transferred to Microsoft Excel software for normalization using the sum method to mitigate potential biases.

Multivariate data analysis

Following normalization, the data were exported to SIMCA-P version 12.0 (Umetrics, Umeå, Sweden). The data underwent treatment with Pareto scaling to alleviate the masking effect in the subsequent data analysis. Initially, Principal Component Analysis (PCA), an unsupervised approach, was conducted to assess the inherent variation within the data. The 95% confidence interval of the model variation was elucidated using Hotelling's T2 technique, represented by an ellipse in the score plots. Subsequently, in this analysis, Orthogonal Partial Least Square Discriminant Analysis (OPLSDA) was applied for discriminating the metabolite profile of water extract from that of the water: Ethanol (7:3) extracts. The quality of the PCA and OPLSDA model was delineated by R2X, R2Y, and Q2 values. R2X and R2Y characterized the data variation and indicated the goodness of fit, while Q2 elucidated the variation predicted by the models through cross-validation.

Total phenolic content assay

Total phenolics in the samples were measured using a previously reported procedure [11], with a few alterations. Each 0.1 mL of extract-water extract (2,000 mg/L), water: Ethanol (7:3) extract (2,000 mg/L), ethanol extract (2,000 mg/L), and water: Ethanol (3:7) extract (3,000 mg/L)-as well as gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (1 mL, 1:10 diluted with distilled water) and aqueous Na_2CO_3 (0.8 mL, 1 M). The mixture was incubated for 15 min and then measured using colorimetry at 765 nm. A standard curve was created using methanol solutions of 50, 60, 70, 80, 90, 100, 110, and 120 mg/L of gallic acid. Total phenol levels were reported in gallic acid equivalents (mg g-1 dry mass), a standard reference chemical.

Total flavonoid content (TFC) assay

The aluminum chloride colorimetric method was used for the determination of total flavonoids in the samples as reported in previous studies but with slight modifications [11]. Each of 0.25 mL of extracts (water extract (5,000 mg/L), water: Ethanol (7:3) extract (5,000 mg/L), ethanol extract (3,000 mg/L), water: Ethanol (3:7) extract (3,000 mg/L) and quercetin (standard flavonoid compound) were mixed with 0.75 mL of methanol, 0.05 mL of 10% aluminum chloride, 0.05 mL of 1 M sodium acetate and 1.4 mL of distilled water. The reaction mixture was kept at room temperature for 30 min, and its absorbance was measured at 415 nm. The calibration curve was created by diluting quercetin in methanol at concentrations of 50, 60, 70, 80, 90, 100,

110, and 120 mg/L. Total flavonoid contents were expressed as quercetin equivalents (mg/g dry mass), a recognized reference chemical.

Bioactivity assays

In this work, *G. ulmifolia* leaf samples were examined with alpha-glucosidase inhibitory assay and the antioxidant tests (DPPH and FRAP). The procedure of alpha-glucosidase inhibitory assay was based on our previous paper [12]. Meanwhile, DPPH and FRAP test were conducted according to the procedure explained in our recent report [13]. The extracts of *G. ulmifolia* leaves were also evaluated with HMG-CoA reductase inhibitory assay kit (Sigma-Aldrich, St. Louis, USA).

Statistical analysis

The outcomes of the biological assays were expressed as the means of three replicates ± standard deviation (SD). Significant differences among the results were evaluated using Analysis of Variance (ANOVA) and Tukey's Honest Significant Difference (Tukey-HSD) multiple comparison tests, with a 95% confidence interval, through Minitab software version 19.0 (Minitab Inc., State College, PA, USA). The Pearson correlation test was performed to evaluate the relationship between TFC and biological activities using Minitab software version 19.0 (Minitab Inc., State College, PA, USA).

Results and discussion

Metabolite annotation

In this research, the metabolites were putatively annotated in the ^{1}H NMR spectra of *G. ulmifolia* leaves (**Figure 1**) by detecting their fingerprint signals and comparing with the reference spectrum obtained from Human Metabolome Database (https://hmdb.ca/) and the Natural Product Magnetic Resonance Database (https://www.npmrd-project.org/). The detected peaks were confirmed with COSY spectrum analysis and further validated with the data from the correlated literature [14-19]. In total, 31 metabolites were successfully putatively annotated in the ^{1}H NMR spectra. **Figure 2** describes molecular structures of some identified metabolites in *G. ulmifolia* leaves.

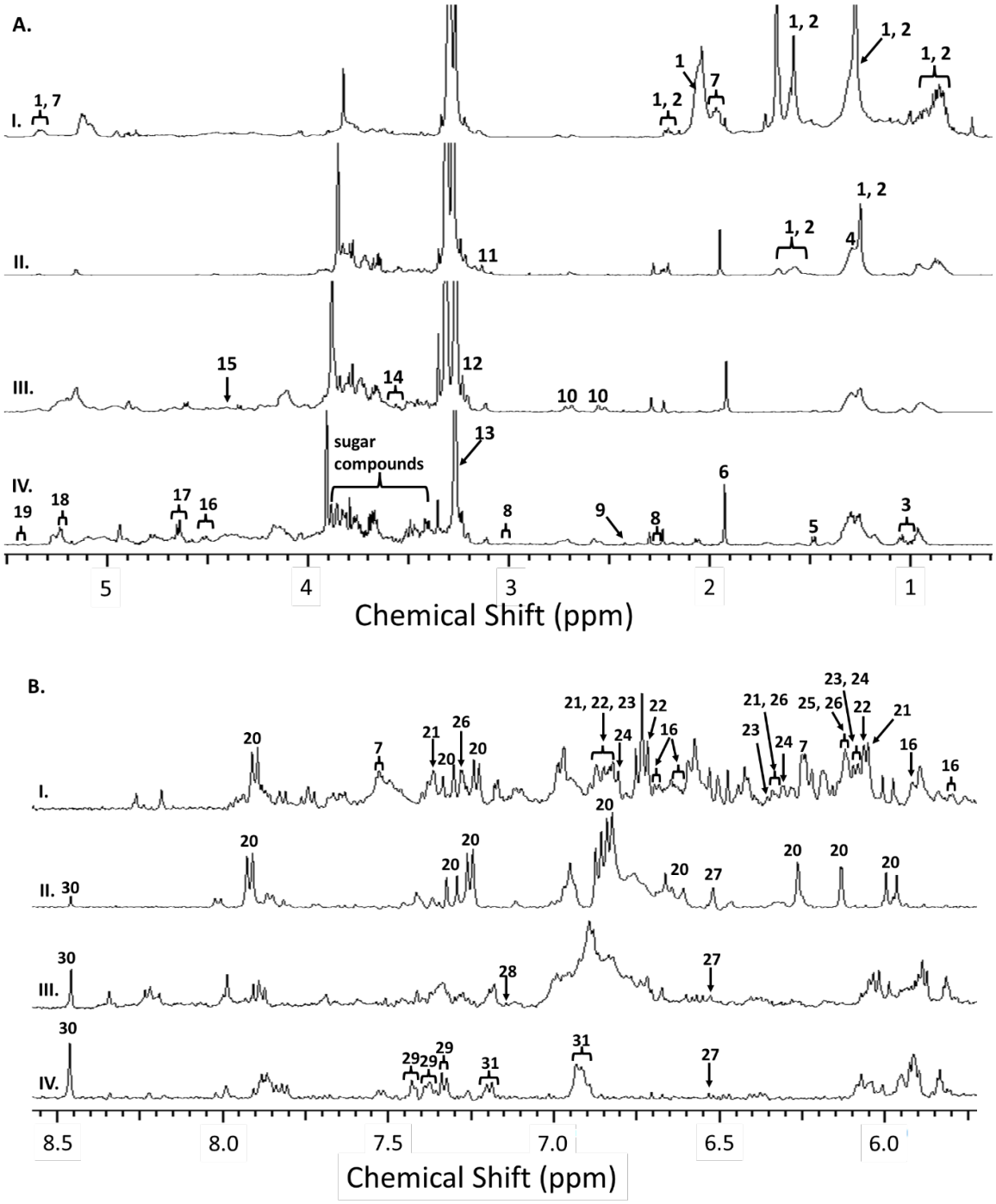


Figure 1 ^{1}H NMR spectra of *G. ulmifolia* leaves extracted with different solvents. The aliphatic region (A) and the aromatic moiety (B) of the ^{1}H NMR spectra. i: ethanol extract, ii: water: ethanol (3:7) extract, iii: water: ethanol (7:3) extract, iv: water extract. 1: oleic acid, 2: palmitic acid, 3: valine, 4: lactic acid, 5: alanine, 6: acetic acid, 7: chlorogenic acids, 8: GABA, 9: succinic acid, 10: citric acid, 11: malonic acid, 12: choline, 13: betaine, 14: *myo*-inositol, 15: trigonelline, 16: cathecin, 17: beta-glucose, 18: alpha-glucose, 19:sucrose, 20: tiliroside, 21: luteolin, 22: apigenin, 23: kaempferol, 24: quercetin, 25: rutin, 26: quercitrin, 27: fumaric acid, 28: gallic acid, 29: phenylalanine, 30: formic acid, 31: tyrosine.

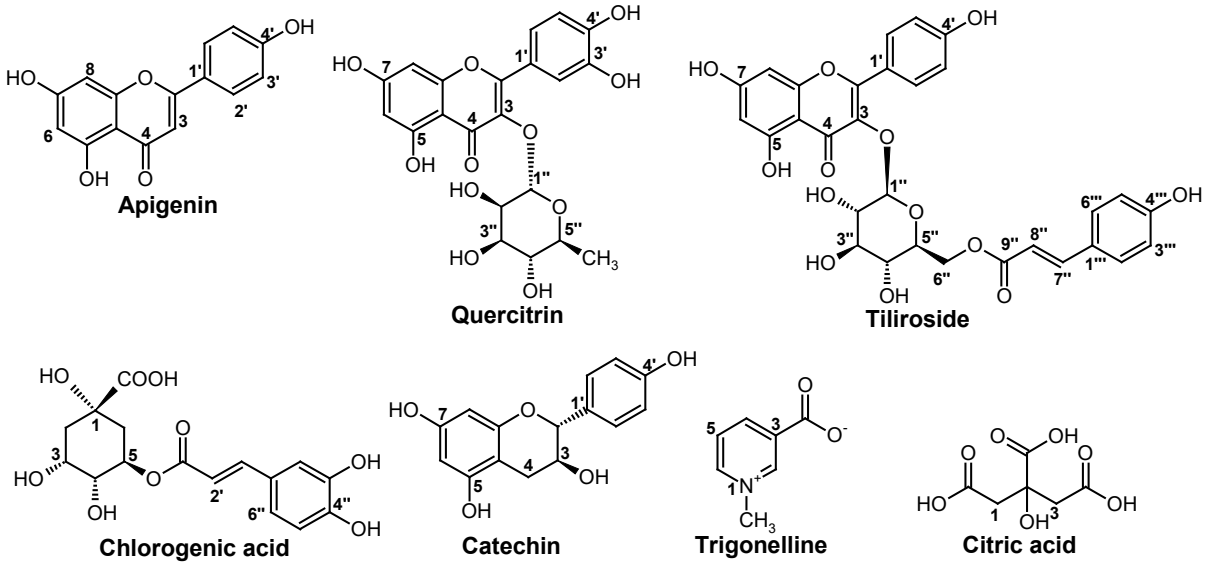


Figure 2 Some molecular structures of annotated metabolites in the ^{1}H NMR spectra of *G. ulmifolia* leaf extracts.

Investigation on aromatic region of the ^{1}H NMR spectra revealed the characteristic signals of flavonoids in the *G. ulmifolia* leaf extracts, including apigenin, catechin, kaempferol, luteolin, quercetin, quercitrin, rutin, and tiliroside. The signals of apigenin, belonging to flavone class, were recorded at δ 6.78 (H-3, *s*), 6.93 (H-3' and H-5', *d*), 7.93 (H-2' and H-6', *d*) ppm. Meanwhile, the other flavone, luteolin, was identified by detecting its characteristic signals at δ 6.20 (H-6, *d*), 6.48 (H-8, *d*), 6.54 (H-3, *s*), 6.92 (H-5', *d*), 7.40 (H-6', *dd*). COSY spectrum analysis revealed the signal correlation between H-2' with H-3' and H-5' with H-6' of apigenin, and between H-5' with H-6' of luteolin as depicted in **Figure S1**.

The signals corresponding to kaempferol, a non-glycoside flavonol, were found at δ 6.15 (H-6, *d*), 6.42 (H-8, *d*), 6.90 (H-3' and H-5', *d*), 8.04 (H-2' and H-6', *d*) ppm. The signal correlation between H-2' with H-3' or H-5' with H-6' of kaempferol was detected in the COSY spectrum (**Figure S1**). The characteristic signals of quercetin, the other non-glycoside flavonol, were discovered at δ 6.18 (H-6, *d*), 6.39 (H-8, *d*), 6.89 (H-5', *d*), 7.65 (H-6', *dd*), and 7.73 (H-2', *d*). COSY spectrum analysis successfully revealed the signal correlation between H-5' and H-6' of quercetin as described in **Figure S1**. Quercitrin, a glycoside quercetin derivative, was found in the ^{1}H NMR spectra of the water, water: Ethanol (3:7), and ethanol extracts. The characteristic peaks of this compound were detected at δ 6.21 (H-6, *d*), 6.40 (H-8, *d*), 6.91 (H-5', *d*), 7.23 (H-6', *dd*), 7.34 (H-2', *d*). Rutin is the other glycoside quercetin derivative and was discovered in the ^{1}H NMR spectra of the water: Ethanol (7:3), water: Ethanol (3:7), and ethanol extracts. Rutin signals were recorded at δ 6.24 (H-6, *d*), 6.42 (H-8, *d*), 6.95 (H-5', *d*), 7.66 (H-6, *dd*), 7.70 (H-2', *d*). Meanwhile, the signals of tiliroside were recorded in the ^{1}H NMR spectra of water: Ethanol (3:7) and ethanol extracts. The peaks at δ 3.48 (H-2", *m*), 4.20 (H-6"a, *d*), 4.30 (H-6"b, *d*), 5.24 (H-1", *d*), 6.14 (H-6, *d*), 6.28 (H-8, *d*), 5.99 (H-8"', *d*), 6.83 (H-3'" and H-5'", *d*), 6.84 (H-3' and H-5', *d*), 7.32 (H-7'", *d*), 7.35 (H-2'" and H-6'", *d*), 8.01 (H-2' and H-6', *d*) were assigned to tiliroside signals. The identification of these glycoside flavonoids (quercitrin, rutin, and tiliroside) was verified by the COSY spectrum analysis as described in **Figure S1**. Catechin, a flavan-3-ol, was the only flavonoid found in the ^{1}H NMR spectra of all samples. Characteristic signals of this compound were detected at δ 2.88 (H-4, *m*), 3.97 (H-3, *m*), 4.56 (H-2, *d*), 5.86 (H-6, *brs*), 5.93 (H-8, *brs*), 6.70 (H-6', *m*), and 6.75 (H-5', *d*). The identification of catechin was supported by detecting the signal correlation between H-3 and H-4 of this compound in the COSY spectrum (**Figure S2**).

Amino acids, including alanine, gamma butyric acid (GABA), phenylalanine, tyrosine, and valine, were found successfully in the ^{1}H NMR spectra of *G. ulmifolia* leaf extracts. Alanine and phenylalanine were detected in the ^{1}H NMR spectra of all sample extracts. The fingerprint proton signals of alanine were clearly found at δ 1.50 (H-3, *d*) and 3.80 (H-2, *m*). Meanwhile,

the proton signals of phenylalanine were identified at δ 7.32 (H-2' and H-6', *d*), 7.36 (H-4', *m*), 7.42 (H-3' and H-5', *m*). Valine signals (0.96 (H-4, *d*), 1.05 (H-5, *d*) ppm) were discovered in almost all ^{1}H NMR spectra, except in the spectrum of ethanol extract. Meanwhile, GABA (1.92 (H-3, *t*), 2.28 (H-2, *t*), 3.04 (H-4, *t*) ppm) and tyrosine (6.76 (H-3' and H-5', d), 7.08 (H-2' and H-6', d) ppm) signals were only recorded in the ^{1}H NMR spectra of the water and water: Ethanol (7:3) extracts. The identification of these amino acids was further verified by COSY spectrum analysis as described in **Figures S1**, **S3**, and **S4**.

Acidic compounds were also detected in the *G. ulmifolia* leaf extracts. The signals assigned to acetic acid (1.93 (H-2, *s*) ppm), fumaric acid (6.53 (H-2 and H-3, *s*) ppm), lactic acid (1.34 (H-3, *d*), 4.27 (H-2, *m*) ppm), succinic acid (2.44 (H-2 and H-3, *s*) ppm), and malonic acid (3.14 (H-2, *s*) ppm) were identified in the ^{1}H NMR spectra of all sample extracts. The characteristic signals of formic acid (8.46 (H-1, *s*) ppm) and gallic acid (7.13 (H-2 and H-6, *s*) ppm) were discovered in the ^{1}H NMR spectra of water, water: Ethanol (7:3), and water: Ethanol (3:7) extracts. Meanwhile, citric acid signals (2.60 (H-1a, H-3a d), 2.72 (H-1b, H-3b, d) ppm) were found exclusively in the ^{1}H NMR spectra of water and water: Ethanol (7:3) extracts. In contrast, the signals corresponding to chlorogenic acids at δ 5.34 (H-1, *m*), 6.28 (H-3', *d*), 6.85 (H-5", *d*), 7.02 (H-6", *d*), 7.53 (H-2', *d*), were only recorded in the ^{1}H NMR spectra of ethanol and water:ethanol (3:7) extracts. COSY spectra analysis revealed the signal correlations of chlorogenic acid (**Figure S1**), lactic acid (**Figure S3**), and citric acid (**Figure S4**).

Sugar compounds were clearly discovered in the *G. ulmifolia* leaf extracts, including alpha-glucose, beta-glucose, myo-inositol, and sucrose. The detailed signals assigned to these compounds in the correlated ^{1}H NMR and COSY spectra were described in **Table 1** and **Figures S3 - S5**. The other compounds identified in the *G. ulmifolia* leaf extracts were betaine, choline, oleic acid, palmitic acid, and trigonelline. The investigation of ^{1}H NMR spectra revealed signals corresponding to betaine (3.27 (-N-$(CH_3)_3$, *s*), 3.91 (H-2, *s*) ppm) and choline (3.24 (N$(CH_3)_3$, *s*), 3.51 (H-1, *t*), 3.91(H-2, *m*) ppm) in all samples. The intense signals belonging to the oleic were detected at δ 0.90 (H-18, *t*), 1.30 (H-4 to H-7 and H-12 to H-17, *m*), 1.62 (H-3, *m*), 2.04 (H-8 and H-11, *m*), 2.25 (H-2, *t*), 5.35 (H-9 and H-10, *m*) in the ^{1}H NMR spectra of ethanol and water:ethanol (3:7) extracts. Meanwhile, palmitic acid signals were recorded at δ 0.94 (H-16, *t*), 1.30 (H-4 to H-15, *m*), 1.66 (H-3, *m*), 2.32 (H-2, *t*) in the ^{1}H NMR spectra of ethanol and water: Ethanol (3:7) extracts, as well. In contrast, the signals of trigonelline (4.46 (N-CH_3, s), 9.13 (H-2, *s*) ppm) were only found in the ^{1}H NMR spectra of water and water: Ethanol (7:3) extracts. The identification of choline, oleic acid, and palmitic acid were confirmed by COSY spectrum analysis as described in **Figures S3** and **S5**.

In this study, 8 flavonoids were putatively identified in the *G. ulmifolia* leaves, including apigenin, catechin, kaempferol, luteolin, quercetin, quercitrin, rutin, and tiliroside. The identification of flavonoids in *G. ulmifolia* leaves is consistent with findings from previous reports [2,3,20]. Interestingly, all these compounds were successfully detected in the ^{1}H NMR spectra of the ethanol extract, indicating an abundance of flavonoids in this extract. Furthermore, apigenin, kaempferol, luteolin, and quercetin were exclusively discovered in the ethanol extract of *G. ulmifolia*. Meanwhile, the glycoside kaempferol derivative, tiliroside, was exclusively detected in the ^{1}H NMR spectra of water: Ethanol (3:7) and ethanol extracts. As described in **Figure S1**, the intense signals of this compound were distinctly observed in the spectra of both extracts, signifying its prominence as a major flavonoid. Moreover, this result also supports the designation of tiliroside as the marker compound of *G. ulmifolia* in the Indonesian Herbal Pharmacopoeia [21].

Table 1 Characteristic ^{1}H NMR signals of the identified compounds in the *G. ulmifolia* leaves. The identification was further confirmed by COSY spectrum analysis. NA: Not applicable.

No.	Compound	Chemical shift (ppm)	COSY Correlation	Water: Ethanol extracts			
				10:0	7:3	3:7	0:10
1.	Oleic acid	0.90 (H-18, *t*), 1.30 (H-4 to H-7 and H-12 to H-17, *m*), 1.62 (H-3, *m*), 2.04 (H-8 and H-11, *m*), 2.25 (H-2, *t*), 5.35 (H-9 and H-10, *m*)	+	–	–	+	+
2.	Palmitic acid	0.94 (H-16, *t*), 1.30 (H-4 to H-15, *m*), 1.66 (H-3, *m*), 2.32 (H-2, *t*)	+	–	–	+	+
3.	Valine	0.96 (H-4, *d*), 1.05 (H-5, *d*)	NA	+	+	+	–
4.	Lactic acid	1.34 (H-3, *d*), 4.27 (H-2, *m*)	+	+	+	+	+
5.	Alanine	1.50 (H-3, *d*), 3.80 (H-2, *m*)	+	+	+	+	+
6.	Acetic acid	1.93 (H-2, *s*)	NA	+	+	+	+
7.	Chlorogenic acid	5.34 (H-1, *m*), 6.28 (H-3', *d*), 6.85 (H-5", *d*), 7.02 (H-6", *d*), 7.53 (H-2', *d*)	+	–	–	+	+
8.	GABA	1.92 (H-3, *t*), 2.28 (H-2, *t*), 3.04 (H-4, *t*)	+	+	+	–	–
9.	Succinic acid	2.44 (H-2 and H-3, *s*)	NA	+	+	+	+
10.	Citric acid	2.60 (H-1a, H-3a *d*), 2.72 (H-1b, H-3b, *d*)	+	+	+	+	+
11.	Malonic acid	3.14 (H-2, *s*)	NA	+	+	+	+
12.	Choline	3.24 ($N(CH_3)_3$, *s*), 3.51 (H-1, *t*), 3.91(H-2, *m*)	+	+	+	+	+
13.	Betaine	3.27 (-N-$(CH_3)_3$, *s*), 3.91 (H-2, *s*)	NA	+	+	+	+
14.	*Myo*-inositol	3.28 (H-2, *t*), 3.55 (H-5, *t*), 3.70 (H-1 and H-3, *dd*), 4.03 (H-4, *t*)	+	+	+	+	+
15.	Trigonelline	4.46 (N-CH_3, *s*), 9.13 (H-2, *s*)	NA	+	+	–	–
16.	Catechin	2.88 (H-4, *m*), 3.97 (H-3, *m*), 4.56 (H-2, *d*), 5.86 (H-6, *brs*), 5.93 (H-8, *brs*), 6.70 (H-6', *m*), 6.75 (H-5', *d*),	+	+	+	+	+
17.	Beta-glucose	3.26 (H-2, *m*), 3.39 (H-4, *m*), 3.47 (H-5, *m*), 3.48 (H-3, *m*), 3.74(H-6a, *m*), 3.88 (H-6b, *m*), 4.65 (H-1, *d*)	+	+	+	–	–
18.	Alpha-glucose	3.42 (H-4, *m*), 3.55 (H-2, *m*), 3.75 (H-3, *m*), 3.76 (H-6a, *m*), 3.86 (H-5, *m*), 3.84 (H-6b, *m*), 5.25 (H-1, *d*)	+	+	+	–	–
19.	Sucrose	5.42 (H-1, *d*), 3.60 (H-2, m)	+	+	+	–	–

No.	Compound	Chemical shift (ppm)	COSY Correlation	Water: Ethanol extracts			
				10:0	7:3	3:7	0:10
20.	Tiliroside	3.48 (H-2", *m*), 4.20 (H-6"a, *d*), 4.30 (H-6"b, *d*), 5.24 (H-1", *d*), 6.14 (H-6, *d*), 6.28 (H-8, *d*), 5.99 (H-8"', *d*), 6.83 (H-3"' and H-5"', *d*), 6.84 (H-3' and H-5', *d*), 7.32 (H-7"', *d*), 7.35 (H-2"' and H-6"', *d*), 8.01 (H-2' and H-6', *d*).	+	–	–	+	+
21.	Luteolin	6.20 (H-6, *d*), 6.48 (H-8, *d*), 6.54 (H-3, *s*), 6.92 (H-5', *d*), 7.40 (H-6', *dd*)	+	–	–	–	+
22.	Apigenin	6.78 (H-3, *s*), 6.93 (H-3' and H-5', *d*), 7.93 (H-2' and H-6', *d*)	+	–	–	–	+
23.	Kaempferol	6.15 (H-6, *d*), 6.42 (H-8, *d*), 6.90 (H-3' and H-5', *d*), 8.04 (H-2' and H-6', *d*)	+	–	–	–	+
24.	Quercetin	6.18 (H-6, *d*), 6.39 (H-8, *d*), 6.89 (H-5', *d*), 7.65 (H-6', *dd*), 7.73 (H-2', *d*)	+	–	–	–	+
25.	Rutin	6.24 (H-6, *d*), 6.42 (H-8, *d*), 6.95 (H-5', *d*), 7.66 (H-6, *dd*), 7.70 (H-2', *d*)	+	–	+	+	+
26.	Quercitrin	6.21 (H-6, *d*), 6.40 (H-8, *d*), 6.91 (H-5', *d*), 7.23 (H-6', *dd*), 7.34 (H-2', *d*)	+	+	–	+	+
27.	Fumaric acid	6.53 (H-2 and H-3, *s*)	NA	+	+	+	+
28.	Gallic acid	7.03 (H-2 and H-6, *s*)	NA	+	+	+	–
29.	Phenylalanine	7.32 (H-2' and H-6', *d*), 7.36 (H-4', *m*), 7.42 (H-3' and H-5', *m*)	+	+	+	+	+
30.	Formic acid	8.46 (H-1, *s*)	NA	+	+	+	–
31.	Tyrosine	6.91 (H-3' and H-5', *d*), 7.20 (H-2' and H-6', *d*)	+	+	+	–	–

Multivariate data analysis of *G. ulmifolia* leaf extracts

Multivariate data analysis was employed to explore the metabolic profiles of *G. ulmifolia* leaves subjected to various extraction solvents. The data derived from the ^{1}H NMR spectra of all *G. ulmifolia* leaf extracts were evaluated using a PCA model, an unsupervised approach. Following Pareto scaling, the PCA model encompassed four principal components, which accounted for 97.0% of the cumulative variance (R^2X) and 94.3% of the cumulative cross-validated variance (Q^2) in the spectral data. The validity of the PCA model was evaluated based on the cumulative R^2X and Q^2 values, together with the score plot and loading plot. The high R^2X and Q^2 values indicate that the PCA model captured most of the spectral variation and showed good cross-validated predictive reliability.

The PCA score plot, which combines principal component 1 (PC1) (55.7%) and PC2 (30.9%), successfully captured 86.6% of the variation in the data (**Figure 3(A)**). Score plot of this model (**Figure 3(A)**) clearly separated all samples according to their extraction solvent type. The first component (PC1) successfully separated all samples, while the second

component (PC2) distinguished the water: Ethanol (3:7) extract from the others. As seen in **Figure 3(A)**, the cluster position of water extract was very close to the water: Ethanol (7:3) extract cluster, suggesting both extracts had similar metabolic profiles. Meanwhile, the cluster corresponding to the ethanol extract was positioned far from the others, indicating it possessed a unique metabolic profile. On the PC1 axis, the cluster of water: Ethanol (3:7) extract was situated in the middle between the ethanol and water extracts. It suggested that metabolite profile of water: Ethanol (3:7) extract had an intermediate characteristic between both extracts.

This separation pattern may be related to differences in solvent polarity. Water has a higher polarity index than ethanol and tends to extract highly polar compounds, whereas ethanol, with lower polarity, is more effective for extracting semi-polar compounds, including many phenolics and flavonoids. The water:ethanol mixtures provide intermediate polarity conditions and may extract compounds with different solubility characteristics. Thus, the close position of the water and water: Ethanol (7:3) extracts, the intermediate position of the water: Ethanol (3:7) extract, and the distinct position of the ethanol extract suggest that solvent polarity strongly influenced the metabolite profiles of *G. ulmifolia* leaf extracts.

To reveal the responsible buckets for the sample separation in the PCA score plot, the associated loading plot was evaluated. As depicted in **Figure 3(B)**, buckets at δ 0.84 - 0.90, 1.24 - 1.30, 1.30 - 1.36, 1.55 - 1.61, 1.63 - 1.69, and 2.01 - 2.07 ppm corresponded to oleic acid and palmitic acid, were discovered contributing to the ethanol extract classification. It suggested that both are key discriminant compounds for this extract. Meanwhile, buckets belong to betaine (δ 3.25 - 3.28 ppm), lactic acid (δ 4.07 - 4.13 ppm), sucrose (δ 3.96 - 4.01, 4.01 - 4.07, 4.13 - 4.19, and 4.19 - 4.25 ppm), and glucoses (δ 3.73 - 3.75, 3.78 - 3.84, and 3.84 - 3.90 ppm were detected influencing the discrimination of the water and water: Ethanol (7:3) extracts. It suggested that these metabolites are characteristic compounds of both extracts. The hierarchical cluster analysis (HCA) was employed to reveal the cluster hierarchy of *G. ulmifolia* leaf extracts. As depicted in **Figure 3(C)**, HCA clearly classified the *G. ulmifolia* samples according to their extraction solvents. HCA showed that the water and water: Ethanol (7:3) extracts were in the close cluster lines, whereas the ethanol extract was in a different cluster line. These results indicate that the metabolite profile of the water extract is more similar to that of the water: Ethanol (7:3) extract than to the ethanol extract, thereby supporting the PCA analysis described above.

To obtain better understanding concerning the metabolic profiles of the water extract and water: Ethanol (7:3) extract of *G. ulmifolia* leaves, a two-class OPLSDA model of both extracts was created. This OPLS-DA model consisted of two components, explaining 88.2% of the variation in the metabolite data matrix ($R^2X = 0.882$) and 99.6% of the variation in sample classification ($R^2Y = 0.996$). These values indicate that the model captured most of the metabolite variation and showed strong separation among the sample groups. The model also showed a high predictive ability, as indicated by the cross-validated Q^2 value of 0.990. This suggests that the model had excellent classification performance and strong predictive reliability. Furthermore, the two-component OPLS-DA model was validated using a permutation test with 400 iterations, as shown in **Figure S6**. The permutation results indicated that the model was not overfitted and supported its predictive reliability. The score plot of this model successfully separated both extracts as described in **Figure 3(D)**. The S-plot of this two-class OPLSDA model (**Figure 3(E)**) revealed that the water extract was characterized by the buckets belonging to betaine (δ 3.25 - 3.28 ppm), sucrose (δ 3.47 - 3.52, 3.96 - 4.01, and 4.13 - 4.19 ppm), and lactic acid (δ 1.30 - 1.36 ppm). It indicated that these metabolites were characteristic markers of the water extract. Meanwhile, the water: Ethanol (7:3) extract was distinguished by tyrosine buckets (6.86 - 6.92 and 7.17 - 7.23 ppm) and a chlorogenic acid bucket (5.31 - 5.37 ppm). This result suggested that both as the discriminant compounds of the water: Ethanol (7:3) extract. The VIP plot of the two-class OPLS-DA model (**Figure S5**) confirmed that these compounds were the major discriminant metabolites contributing to the differentiation between the water extract and the water: Ethanol (7:3) extract. These results highlight the solvent-dependent extraction of polar versus semi-polar compounds, consistent with the chemical nature of the metabolites identified [22].

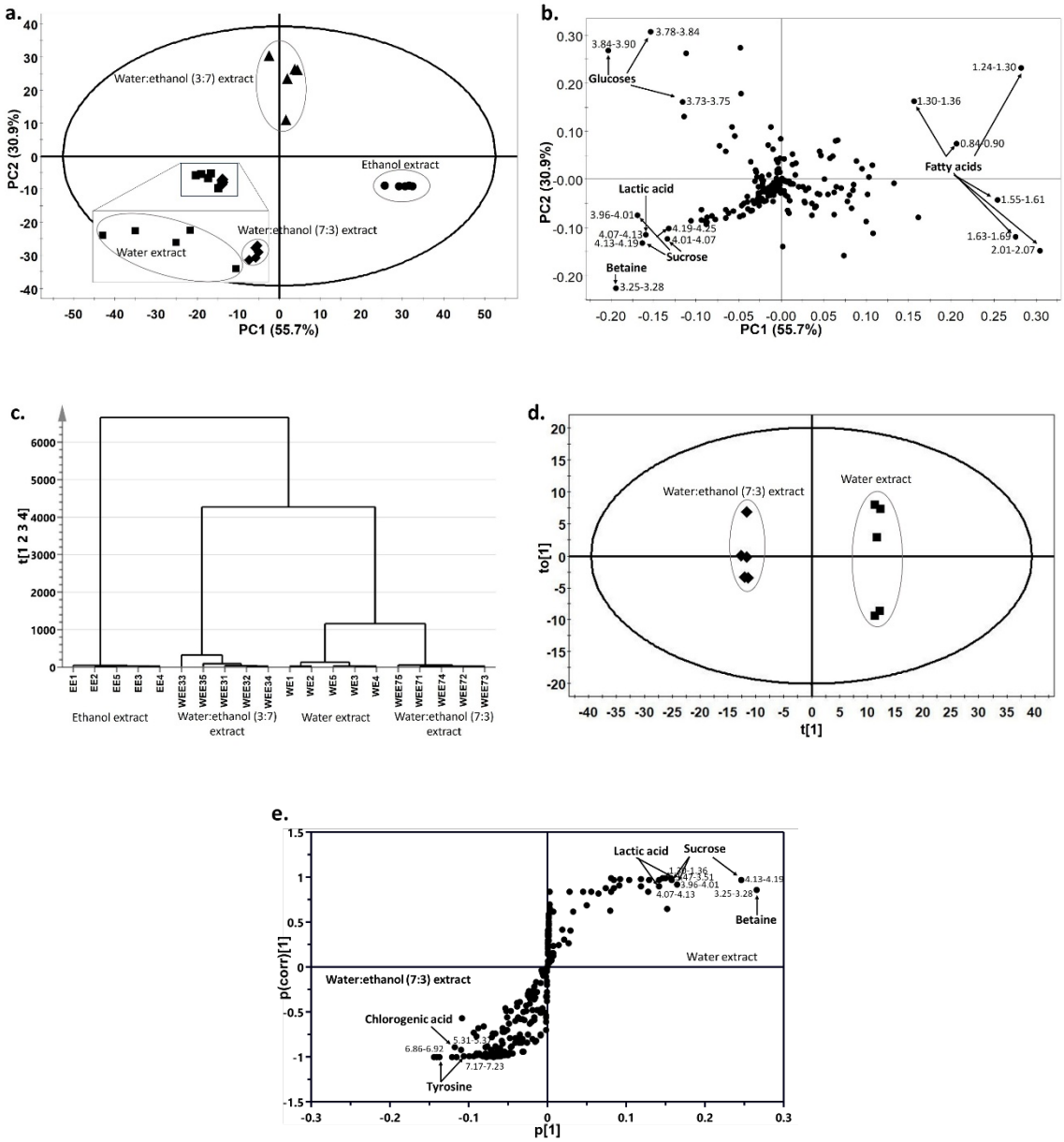


Figure 3 Multivariate data analysis calculated for *G. ulmifolia* leaf extracts. (a) PCA score plot separated the samples based on the extraction solvents yielding 4 independent clusters. (b) PCA loading plot revealed the important buckets contributing to the classification. (c) HCA plot showed the relationship within *G. ulmifolia* leaf extracts. (d) score plot of the two-class OPLSDA model computed for the water extract and water: Ethanol (7:3) extract. (e) S-plot of the two-class OPLSDA model revealed the discriminant compounds for each extract.

Total phenolic content and total flavonoid content

Table 2 presents the Total Phenolic Content (TPC) of *G. ulmifolia* leaves extracted using various solvents. The TPC of *G. ulmifolia* leaves ranged from 35.82 ± 2.53 mg GAE/g to 58.73 ± 4.14 mg GAE/g crude extract. The variation in TPC values among the water: Ethanol (7:3) extract, water: Ethanol (3:7) extract, and water extract was relatively slight, as shown in **Table 2**. Nevertheless, the water: Ethanol (7:3) extract had the highest TPC value (58.73 ± 4.14 mg GAE/g). Meanwhile, the extraction with ethanol yielded the lowest phenolic content, registering at 35.82 ± 2.53 mg GAE/g crude extract.

The Total Flavonoid Content (TFC) of *G. ulmifolia* leaves extracted using various solvents is depicted in **Table 2**. The TFC ranged from 14.72 ± 0.66 mg QE/g to 33.12 ± 2.14 mg QE/g crude extract. The

highest flavonoid content was observed in the extraction using 100% ethanol, recording at 33.12 ± 2.14 mg QE/g crude extract. Conversely, the extraction with water showed the lowest flavonoid content, at 14.72 ± 0.66 mg QE/g crude extract. Meanwhile, TFCs of water: Ethanol (7:3) extract and water: Ethanol (3:7) extract were 18.61 ± 1.69 mg QE/g, and 31.59 ± 3.03 mg QE/g crude extract, respectively.

Plants produce a wide variety of phenolic compounds which can be categorized into flavonoids, lignin precursors, and phenolic acids. These phenolic compounds are valuable for estimating antioxidant capacity and significantly contribute to the overall water-soluble antioxidant activity [23,24]. As described in **Table 2**, the highest TPC value was discovered in the water: Ethanol (7:3) of G. ulmifolia leaves. Meanwhile, the TFC analysis revealed a progressive increase in flavonoid content with the rising proportion of ethanol in the extraction solvents, suggesting that higher ethanol concentrations enhance the solubility and recovery of flavonoids. This observation is consistent with a previous study reporting that ethanol provides optimal polarity for flavonoid extraction [25].

Table 2 The results of bioactivity assays, the total phenolic content, and the total flavonoid content obtained from *G. ulmifolia* leaf extracts[a].

Assay/Parameter	*G. ulmifolia* leaf extracts				*p*-value
	Water	Water:ethanol (7:3)	Water:ethanol (3:7)	Ethanol	
IC_{50} of DPPH[*] (µg/mL)	396.07 ± 0.48[a]	258.07 ± 8.02[b]	255.80 ± 1.81[b]	199.46 ± 2.55[c]	<0.05
AAI of DPPH[**]	0.18	0.27	0.27	0.35	-
EC_{50} of FRAP[*] (µg/mL)	214.46 ± 0.64[a]	145.26 ± 2.21[b]	121.84 ± 2.37[c]	105.60 ± 1.95[d]	<0.05
The percentage inhibition of HMG-CoA reductase (%)[***]	29.67 ± 4.87[d]	47.34 ± 4.23[c]	65.74 ± 4.61[b]	82.79 ± 3.04[a]	<0.05
Alpha-glucosidase inhibitory activity (IC_{50}, mg/mL)[****]	125.99 ± 5.25[c]	176.43 ± 10.43[a]	162.86 ± 4.82[ab]	150.33 ± 6.39[b]	<0.05
Total phenolic content (mg GAE/g)	54.33 ± 4.45[a]	58.73 ± 4.14[a]	54.41 ± 3.37[a]	35.82 ± 2.53[b]	<0.05
Total flavonoid content (mg QE/g)	14.72 ± 0.66[c]	18.61 ± 1.69[b]	31.59 ± 3.03[a]	33.12 ± 2.14[a]	<0.05

[a] The provided values represent mean ± standard deviation (SD) with 5 replications. [a - d] According to Tuckey test, differences in the superscripts of the mean values for each metabolite indicate statistical significance. [*] Ascorbic acid was used as the standard compound for DPPH and FRAP assays with IC_{50} and EC_{50} values of 4.40 and 5.39 µg/mL, respectively. [**] Antioxidant activity index (AAI) values were below 0.5, indicating poor antioxidant activity. [***] The standard used in the inhibition test of HMG-CoA reductase, was pravastatin with the inhibition percentage of 51.10 ± 8.20%. [****] Acarbose was used as the reference compound in the alpha-glucosidase inhibitory test with IC_{50} value of 33.95 ± 1.48 mg/mL.

Bioactivity assessments

The results of DPPH assay are recorded as IC_{50} values and presented in **Table 2**. The IC_{50} values of DPPH assay varied from 199.46 ± 2.55 µg/mL to 396.07 ± 0.48 µg/mL and categorized. Based on the Antioxidant Activity Index (AAI) classification [26], all extracts were categorized as having poor antioxidant activity, with AAI values ranging from 0.177 to 0.351. In the DPPH assay, the ethanol extract exhibited the highest activity with an IC_{50} value of 199.46 ± 2.55 µg/mL, whereas the water extract showed the lowest activity with an IC_{50} value of 396.07 ± 0.48 µg/mL. Meanwhile,

the DPPH activity of water: Ethanol (7:3) extract and water: Ethanol (3:7) extract did not differ significantly, with IC_{50} values of 258.07 ± 8.02 µg/mL and 255.80 ± 1.81 µg/mL, respectively. The results of FRAP assay are reported as EC_{50} values and depicted in **Table 2**. The EC_{50} values of FRAP assay varied from 105.60 ± 1.95 µg/mL to 214.46 ± 0.64 µg/mL. The ethanol extract achieved the highest FRAP capacity with the EC_{50} value of 105.60 ± 1.95 µg/mL, followed by those extracted with water: Ethanol (3:7), water: Ethanol (7:3) and water, which had EC_{50} values of 121.84 ± 2.37 µg/mL, 145.26 ± 2.21 µg/mL, and 214.46 ± 0.64 µg/mL, respectively.

Antioxidant compounds in plants sources, including leaves, reduce the harmful effects of free radicals [27]. Specifically, antioxidants stabilize free radicals or inhibit their oxidation, thereby mitigating potential damage to biological molecules like DNA, proteins, and lipids [28]. This scavenging activity helps in protecting against various diseases and physiological conditions linked to oxidative stress. In this study, the DPPH radical scavenging and FRAP assays were utilized to assess the antioxidant capacity of *G. ulmifolia* leaves. The results showed that the ethanol extract possessed the strongest DPPH and FRAP activities (**Table 2**). Interestingly, the activity patterns of DPPH and FRAP were in accordance with the trend of TFC values. The ethanol extract exhibited both the highest flavonoid content and the most potent antioxidant properties. The Pearson correlation coefficient test was carried out to confirm the relationship statistically between the antioxidant activity with the TFC. The results revealed that the TFC had positive relationships with the DPPH ($r = 0.826$, p-value = 0.174) and FRAP ($r = 0.940$, p-value = 0.060) insignificantly as described in **Table S1**. These findings suggest a possible association between flavonoid content and the antioxidant activity of *G. ulmifolia* leaf extracts, which is in agreement with previous reports [29-31]. Further studies with larger sample sizes and more detailed compound identification are needed to confirm the contribution of flavonoids to the observed antioxidant activity.

The alpha-glucosidase inhibitory effects of the *G. ulmifolia* leaf extracts are presented in **Table 2** as IC_{50} values. The leaf extracts inhibited the alpha-glucosidase with IC_{50} values ranging from 125.99 ± 5.25 mg/mL to 176.43 ± 10.43 mg/mL. These activities were lower than that of acarbose with an IC_{50} value of 33.95 ± 1.48 mg/mL, which was used as the positive control. Among the tested samples, the water extract of *G. ulmifolia* leaves demonstrated the highest alpha-glucosidase inhibitory activity (IC_{50} value = 125.99 ± 5.25 mg/mL), followed by the ethanol extract (150.33 ± 6.39 mg/mL), water: Ethanol (3:7) extract (162.86 ± 4.82 mg/mL), and the water: Ethanol (7:3) extract (176.43 ± 10.43 mg/mL). These results indicate that the alpha-glucosidase inhibitory activity of *G. ulmifolia* leaf extracts did not increase linearly with ethanol concentration. This suggests that the activity was not determined solely by ethanol concentration but may depend on the selective extraction of compounds with different polarity and molecular characteristics. Plant-derived alpha-glucosidase inhibitors are chemically diverse, including phenolics, flavonoids, glycosides, alkaloids, and other secondary metabolites [32]. The stronger activity of the aqueous extract may therefore be associated with relatively polar and water-soluble compounds, such as polar phenolics, glycosylated flavonoids, or tannins. Differences in solvent polarity can influence the extracted phytochemical profile and the resulting alpha-glucosidase inhibitory activity [33,34].

Obesity, which currently affects approximately 600 million people worldwide, is strongly associated with comorbidities such as diabetes and cardiovascular diseases [1]. Previous studies reported that the extracts of *G. ulmifolia* leaves showed potential anti-obesity [35,36]. One therapeutic approach for managing postprandial hyperglycemia in obese and diabetic patients is the inhibition of alpha-glucosidase enzyme, an intestinal enzyme responsible for carbohydrate digestion, thereby delaying glucose absorption and moderating blood sugar levels [37,38]. In this study, *G. ulmifolia* leaf extracts were evaluated with alpha-glucosidase inhibitory assay. The extracts inhibited the alpha-glucosidase enzyme with IC_{50} values ranging from 125.99 ± 5.25 mg/mL to 176.43 ± 10.43 mg/mL. These results confirmed the previous work reporting the alpha-glucosidase inhibitory activity of *G. ulmifolia* extracts [39]. The observed inhibitory activity may be associated with the presence of bioactive phytochemicals in *G. ulmifolia* leaves, particularly flavonoids [33,34]. These compounds are known to

contribute to antidiabetic activity through several possible mechanisms, including interaction with the active site of alpha-glucosidase, reduction of carbohydrate hydrolysis, and modulation of oxidative stress related to metabolic disorders [33,34].

The inhibitory activity of *G. ulmifolia* leaf extracts against HMG-CoA reductase is reported as the percentage inhibition and summarized in **Table 2**. The inhibition of HMG-CoA reductase ranged from 29.67 ± 4.87 % to 82.79 ± 3.04%. Notably, the ethanol extracts showed the highest inhibitory activity (82.79 ± 3.04%), which was significantly greater ($p < 0.05$) than those of the other extracts. Moreover, the percentage of HMG-CoA reductase inhibition of the ethanol extract was higher than that of pravastatin (51.10 ± 8.20%), which was used as the positive control. Meanwhile, the lowest percentage of HMG-CoA reductase inhibition was the water extract (29.67 ± 4.87%). The inhibitory percentage of the water: Ethanol (7:3) extract and water: Ethanol (3:7) extract against HMG-CoA reductase were 47.34 ± 4.23% and 65.74 ± 4.61%. Statistical analysis confirmed significant differences ($p < 0.05$) between the extracts.

The enzyme HMG-CoA reductase plays a critical role in the synthesis of cholesterol in the liver. Inhibiting this enzyme reduces blood cholesterol levels, thereby lowering the risk of cardiovascular diseases. In this study, antihypercholesterolemic activity of *G. ulmifolia* leaf extracts, was evaluated with HMG-CoA reductase inhibitory assay. The results revealed that ethanol extract had the highest HMG-CoA reductase inhibitory. Furthermore, the results of HMG-CoA reductase inhibitory assay mirrored the trends seen in antioxidant activity and TFC results explained previously. The Pearson correlation coefficient test was performed to find the relationship between HMG-CoA reductase inhibitory activity and TFC statistically. The results showed that HMG-CoA reductase inhibitory activity possessed significantly a positive relationship with TFC ($r = 0.959$, p-value = 0.041) as depicted in **Table S1**. Therefore, it suggested that the flavonoid compounds may contribute to the HMG-CoA reductase inhibitory activity of *G. ulmifolia* leaf extracts. This suggestion is aligned with the previous research [40]. Further investigations to identify the specific bioactive compounds and clarify the underlying mechanisms are expected to provide deeper insight into the potential of *G. ulmifolia* leaf extracts as HMG-CoA reductase inhibitors.

As described in the multivariate data analysis, oleic acid was identified as a key characteristic compound of the ethanol extract of *G. ulmifolia* leaves. Interestingly, oleic acid was reported to inhibit HMG-CoA reductase [41,42]. This supports the suggestion that oleic acid may contribute to the HMG-CoA reductase inhibitory activity of ethanol extract. Given the key role of HMG-CoA reductase in cholesterol synthesis, these findings indicate that ethanol extract of *G. ulmifolia* leaves possesses high potential as a cholesterol-lowering agent.

Among the putatively identified compounds, catechin, luteolin, and quercetin have been previously associated with HMG-CoA reductase inhibitory activity. Catechin isolated from *Uncaria gambir*, has shown direct *in vitro* HMG-CoA reductase inhibition [43]. Meanwhile, luteolin and quercetin have been reported to modulate HMG-CoA reductase activity or related cholesterol biosynthesis pathways [44,45]. Other flavonoids, such as apigenin and tiliroside, may also contribute to lipid-related bioactivity through regulation of lipid metabolism pathways [46,47], although direct evidence for HMG-CoA reductase inhibition remains limited. Therefore, the HMG-CoA reductase inhibitory activity of *G. ulmifolia* leaf extracts may reflect the combined contribution of multiple flavonoids and related compounds.

This study has several limitations. Metabolite identification was mainly based on ^{1}H NMR analysis, which provides useful metabolite profiling but has limited sensitivity for detecting minor compounds and confirming detailed structures. Further studies using complementary techniques such as LC-MS/MS or HPLC are therefore needed to obtain more comprehensive phytochemical characterization. In addition, the biological activities were evaluated only through *in vitro* assays, including antioxidants, alpha-glucosidase inhibitory, and HMG-CoA reductase inhibitory. Future studies should include additional *in vitro* assays to further explore the underlying mechanisms, such as enzyme kinetics, molecular docking, cellular glucose uptake, lipid metabolism, and oxidative stress markers. Moreover, *in vivo* studies are required to confirm the efficacy, safety, bioavailability, and potential therapeutic effects of *G. ulmifolia* extracts.

Conclusions

In this study, phytochemical profiles of *G. ulmifolia* leaves extracted with different solvents were evaluated successfully with ^{1}H NMR-based metabolomics. In total, 31 metabolites were annotated putatively. PCA score plot effectively classified the *G. ulmifolia* leaf samples based on the extraction solvents used, with the loading plot highlighting glucose, sucrose, lactic acid, betaine, and fatty acids as key contributors to this classification. Total flavonoid content analysis indicated an increase in flavonoid concentration with higher ethanol composition in the extraction solvent. Ethanol extract of *G. ulmifolia* leaves possessed the best antioxidant activity and the highest HMG-CoA reductase inhibitory property. These bioactivities had a positive relationship with the total flavonoid content statistically. Our findings also suggested that the ethanol extract of *G. ulmifolia* leaves has significant potential as a cholesterol-lowering agent by inhibiting HMG-CoA reductase.

Acknowledgements

This research was funded by LPDP (Indonesia Endowment Fund for Education Agency) via the RISPRO Program with contract number: PRJ-1/LPDP/LPDP.4/2023.

Declaration of Generative AI in Scientific Writing

The authors declare that no generative AI tools were used in the writing or preparation of this manuscript.

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Supplementary Materials

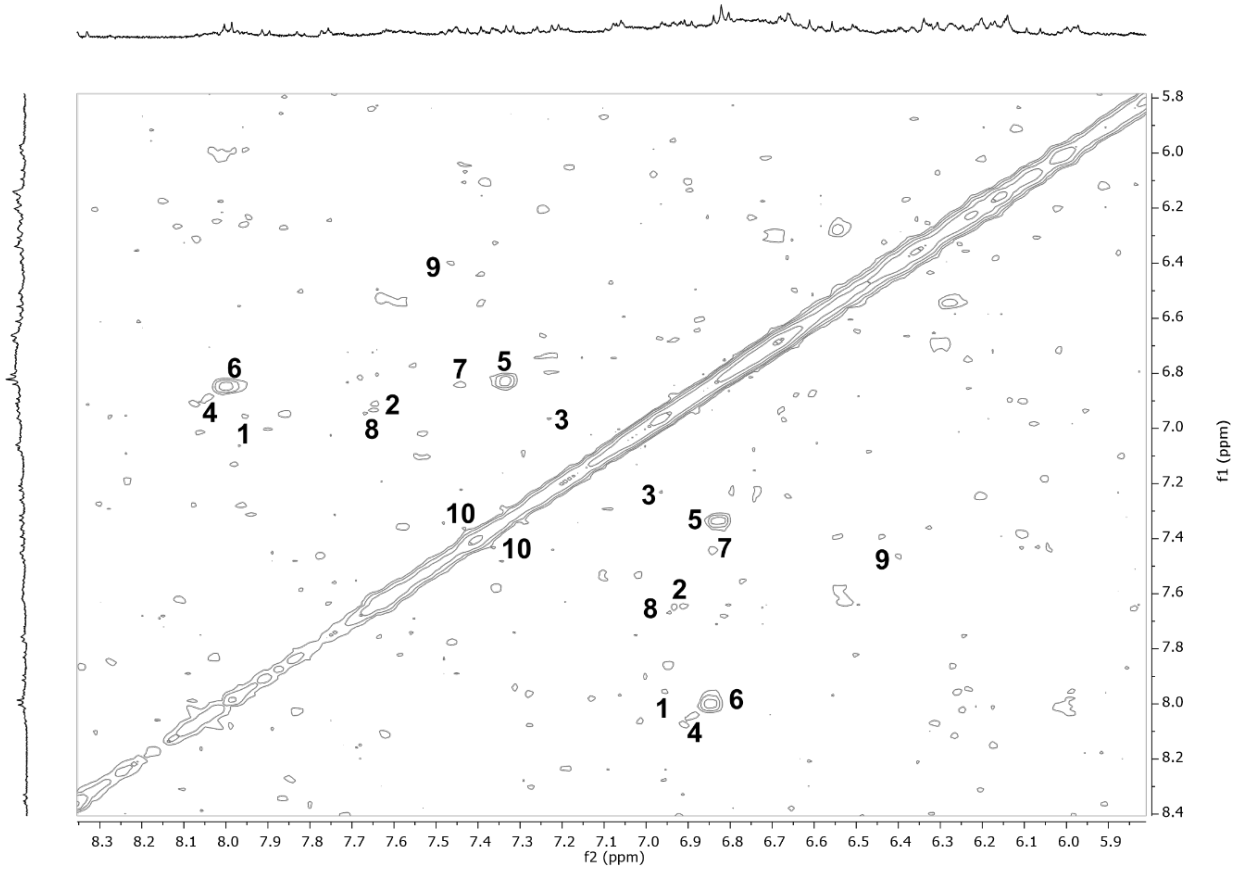
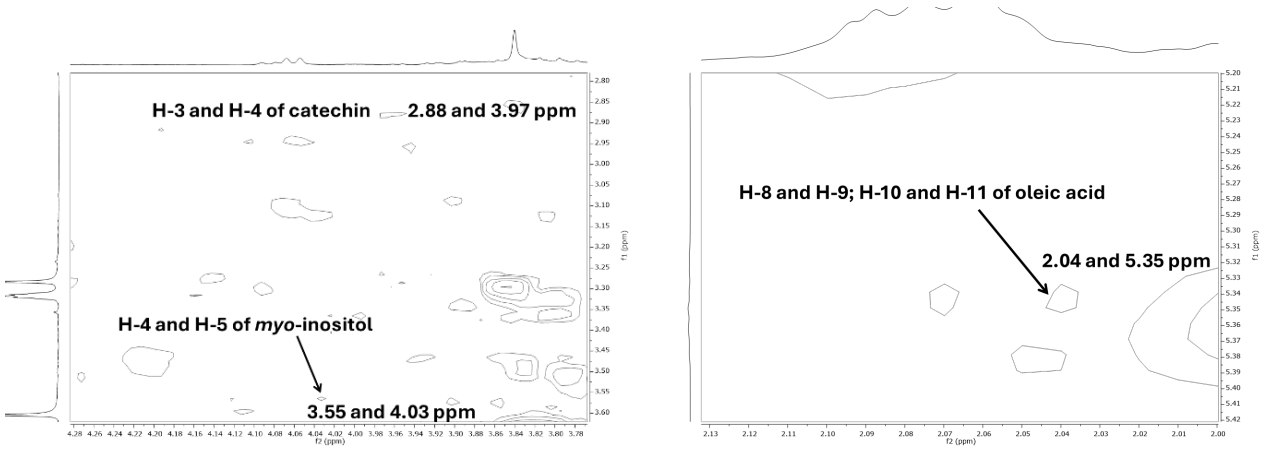


Figure S1 The signal correlation of apigenin, quercetin, quercitrin, kaempferol, tiliroside, luteolin, chlorogenic acid, phenylalanine, and tyrosine in the COSY spectrum of the ethanol extract. 1: H-2'/H-3' and H-5'/H-6' of apigenin, 2: H-5'/H-6' of quercetin, 3: H-5'/H-6' of quercitrin, 4: H-2'/H-3' and H-5'/H-6' of kaempferol, 5: H-2"'/H-3"' and H-5"'/H-6"' of tiliroside, 6: H-2'/H-3' and H-5'/H-6' of tiliroside, 7: H-5'/H-6' of luteolin, 8: H-5'/H-6' of rutin, 9: H-2'/H-3' of chlorogenic acid, 10: H-3'/H-4' of phenylalanine.



(A) (B)

Figure S2 The signal correlation of catechin and *myo*-inositol (A), oleic acid (B) in the COSY spectrum of ethanol extract.

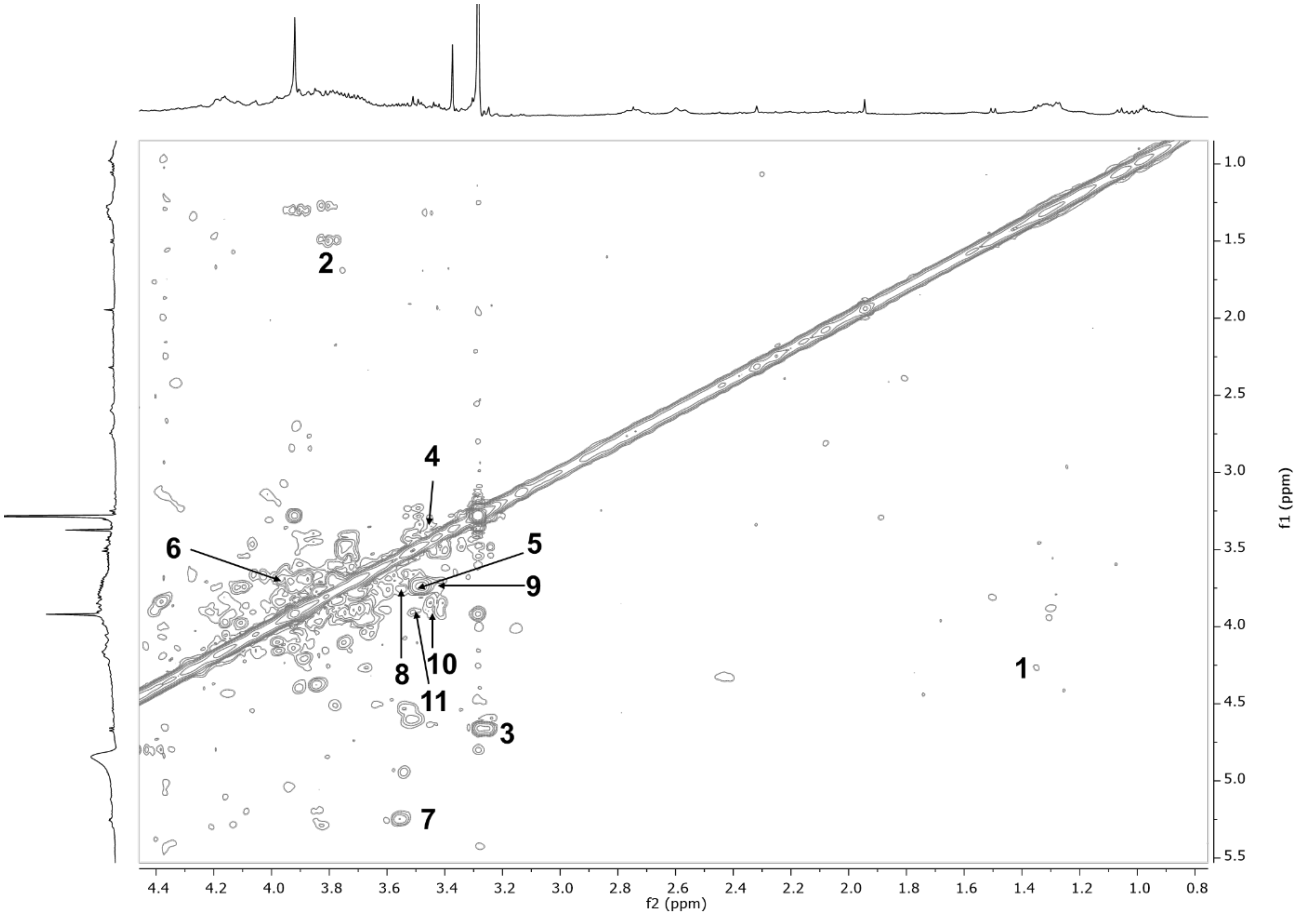


Figure S3 The signal correlation of lactic acid, alanine, alpha-glucose, beta-glucose, and choline in the COSY spectrum of water extract. 1: H-2/H-3 of lactic acid, 2: H-2/H-3 of alanine, 3: H-1/H-2 of beta-glucose, 4: H-3/H-4 of beta-glucose, 5: H-5/H-6a of beta-glucose, 6: H-6a/H-6b of beta-glucose, 7: H-1/H-2 of alpha-glucose, 8: H-2/H-3 of alpha-glucose, 9: H-3/H-4 of alpha-glucose, 10: H-4/H-5 of alpha-glucose, 11: H-1/H-2 of choline.

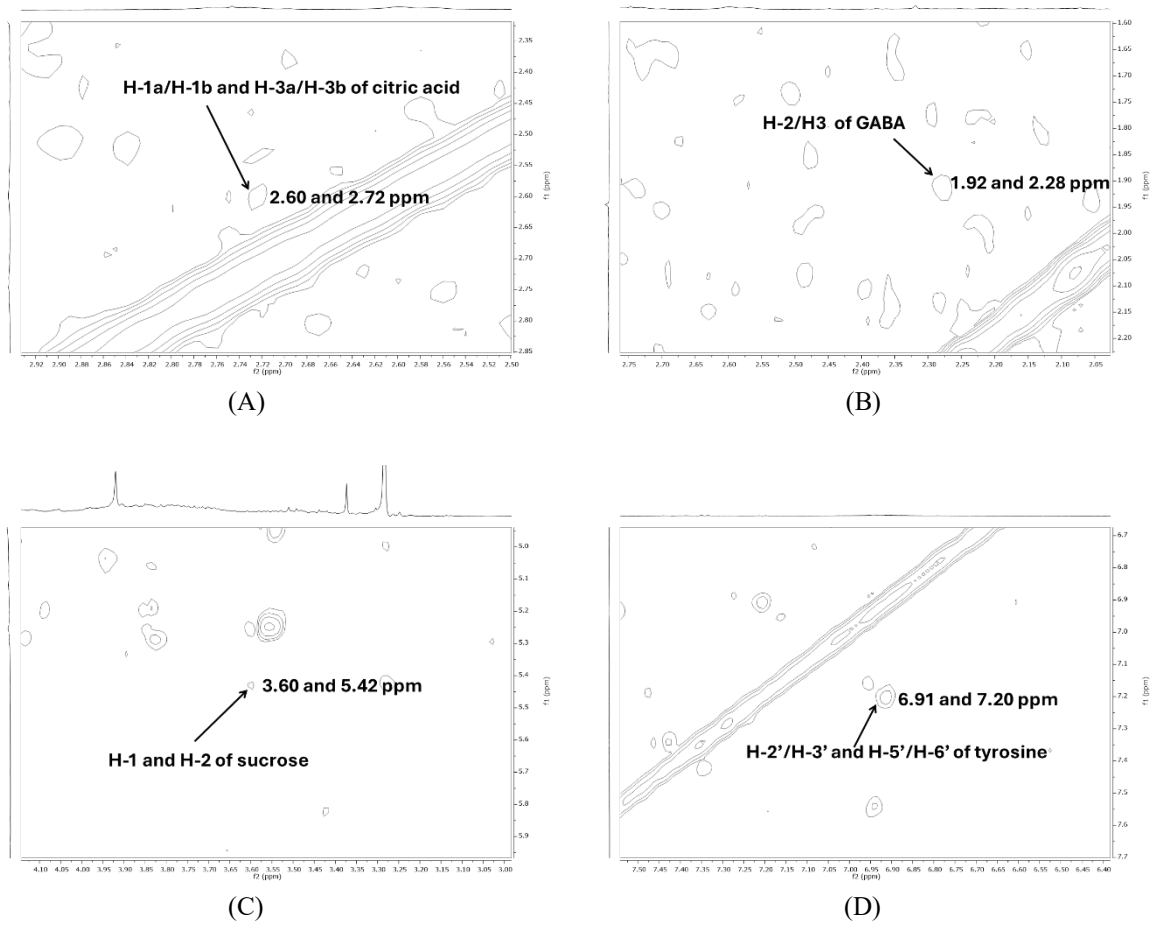


Figure S4 The signal correlation of citric acid (A), GABA (B), sucrose (C), and tyrosine (D) in the COSY spectrum of water extract.

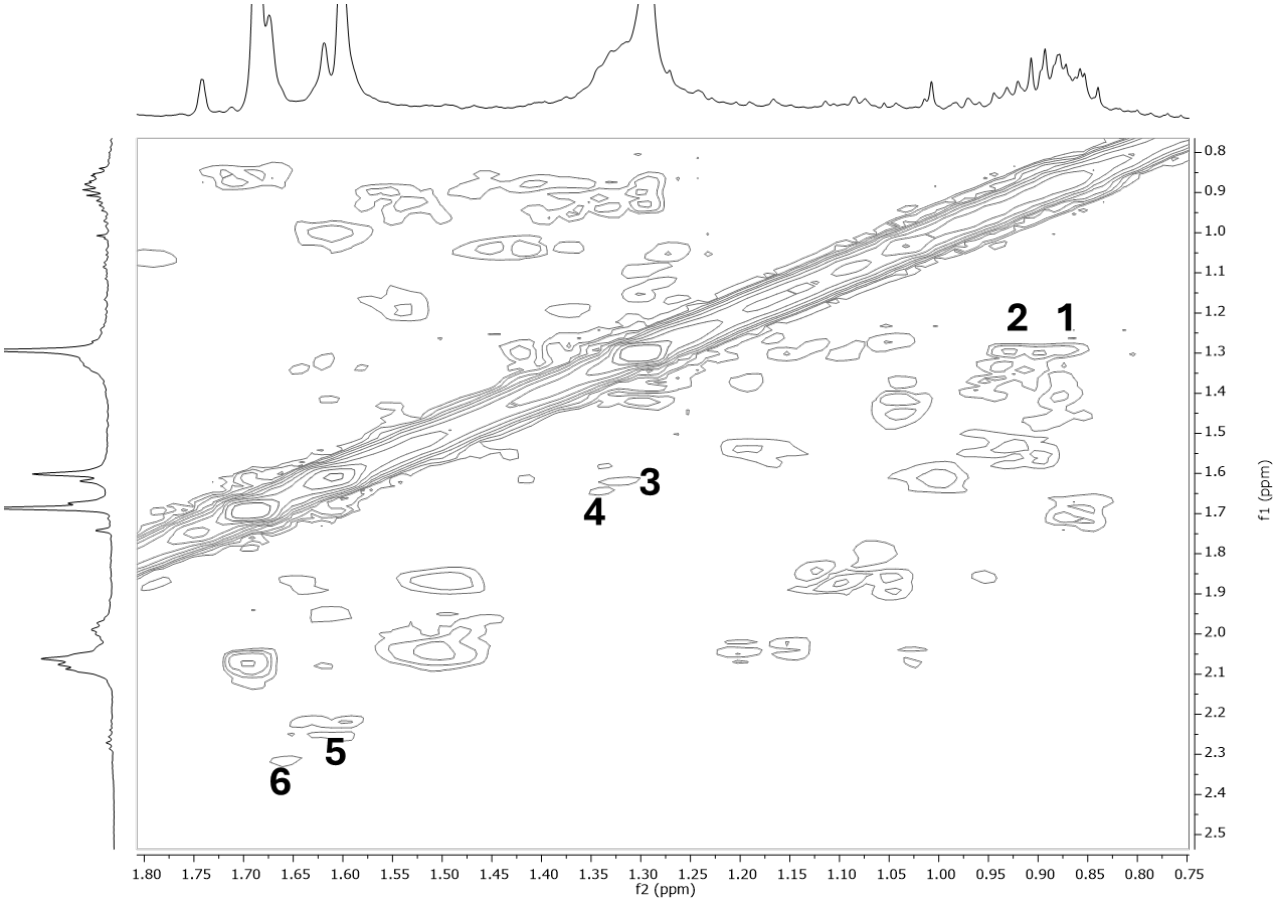


Figure S5 The signal correlation of oleic acid and palmitic acid in the COSY spectrum of the ethanol extract. 1: H-18 and H-17 of oleic acid, 2: H-16 and H-15 of palmitic acid, 3: H-3 and H-4 of oleic acid, 4: H-3 and H-4 of palmitic acid, 5: H-2 and H-3 of oleic acid, 6: H-2 and H-3 of palmitic acid.

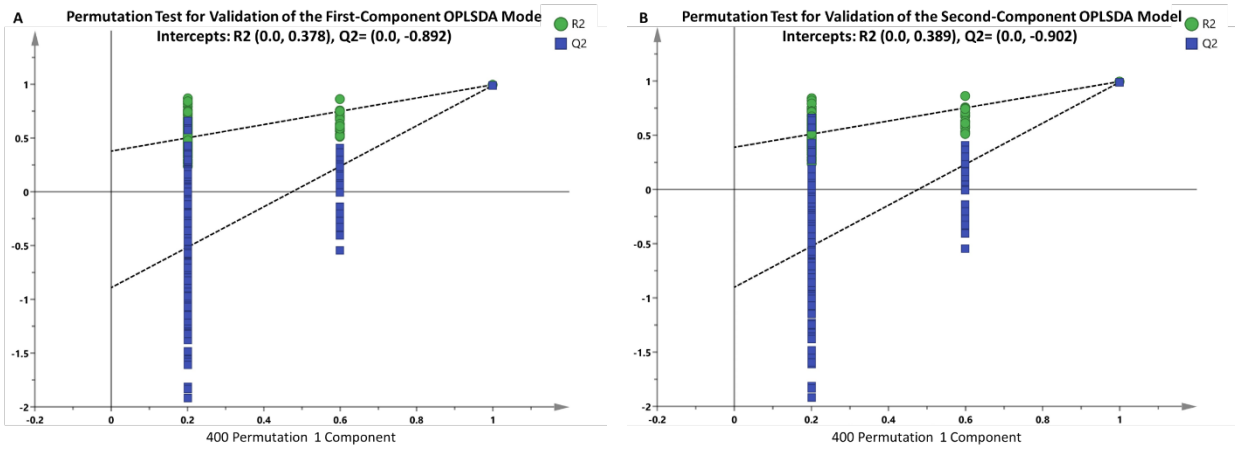


Figure S6 Permutation test results for the two-class OPLS-DA model: First component (A) and second component (B).

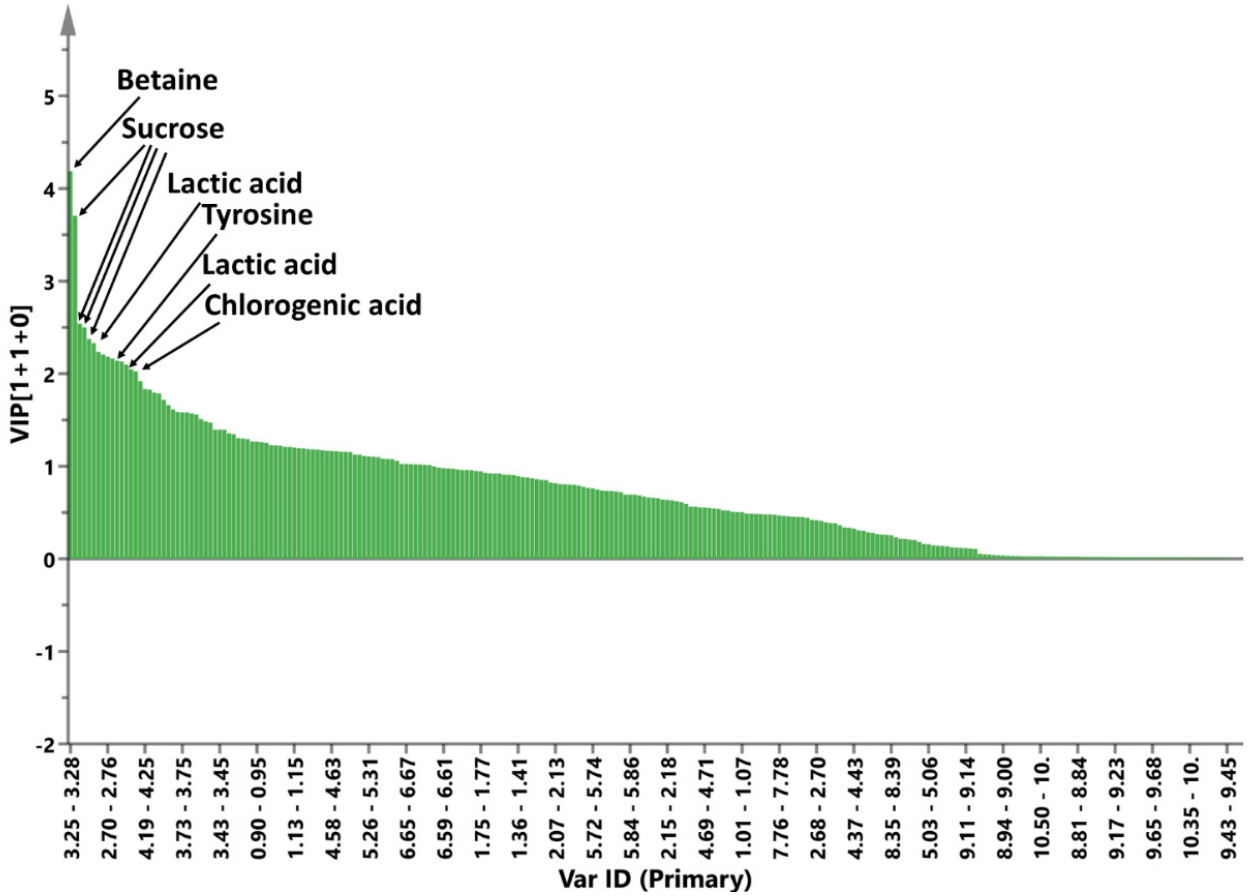


Figure S7 VIP plot of the two-class OPLS-DA model used to differentiate the water extract from the water: Ethanol (7:3) extract.

Table S1 Pearson correlation analysis of bioactivities with total flavonoid content and with total phenolic content.

Relationship	Correlation (r)	*p*-value
Total flavonoid content and HMG-CoA inhibitory activity	0.959	0.041
Total flavonoid content and alpha-glucosidase inhibitory activity	–0.384	0.616
Total flavonoid content and DPPH activity	0.826	0.174
Total flavonoid content and FRAP activity	0.940	0.060
Total phenolic content and HMG-CoA inhibitory activity	–0.751	0.249
Total phenolic content and alpha-glucosidase inhibitory activity	–0.171	0.829
Total phenolic content and DPPH activity	–0.695	0.305
Total phenolic content and FRAP activity	–0.665	0.335