

Article

Enzymatic Transformation of Secondary Metabolites in *Abeliophyllum distichum* Extract by Viscozyme[®] L Treatment

Chang-Dae Lee ¹, Eun-A Kim ² , Ho Sik Rho ³ and Sanghyun Lee ^{1,4,*} 

¹ Department of Plant Science and Technology, Chung-Ang University, Anseong 17546, Republic of Korea; great7321@naver.com

² R&D Center, Cleanhill Co., Ltd., Hwaseong 18330, Republic of Korea; caramari@hanmail.net

³ Department of Chemical Engineering, Suwon University, Hwaseong 18323, Republic of Korea; thiocarbon@naver.com

⁴ Natural Product Institute of Science and Technology, Anseong 17546, Republic of Korea

* Correspondence: slee@cau.ac.kr; Tel.: +82-31-670-4688; Fax: +82-31-676-4686

Abstract

Abeliophyllum distichum is rich in polyphenols and flavonoids with various bioactivities; however, studies on enzymatic modifications to enhance its functional properties remain limited. This study investigated the effect of Viscozyme[®] L treatment on the secondary metabolite profile of *A. distichum* leaves. Phytochemical profiling using liquid chromatography–electrospray ionization tandem mass spectrometry revealed a decrease in the total number of detectable compounds, from 26 in the untreated extract to 16 in the enzyme-treated extract. Following Viscozyme[®] L treatment, a notable shift in metabolite composition was observed, with significant enrichment of flavonoid glycosides, pyranone derivatives, and amino acid-related metabolites. Quantitative high-performance liquid chromatography analysis showed significant reductions in glycosylated compounds such as rutin (1), acteoside (2), and isoacteoside (3), while the aglycone quercetin (4) content increased more than four-fold compared to the control. These results indicate that Viscozyme[®] L facilitates the deglycosylation of flavonoid glycosides into their aglycone forms. This enzymatic transformation suggests a potential strategy to enhance the bioavailability and functional value of *A. distichum* leaf extracts for nutraceutical and pharmaceutical applications.

Keywords: enzymatic biotransformation; flavonoid glycoside; HPLC; LC-ESI/MS; quercetin



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1. Introduction

Abeliophyllum distichum, commonly known as white forsythia or Korean abeliophyllum, is a rare, monotypic, and endemic plant species belonging to the Oleaceae family [1]. Although native to the Korean Peninsula, it was first introduced to the horticultural world following its discovery in 1919 [2,3]. In traditional Korean folk medicine, various parts of *A. distichum* have long been used as remedies for ailments such as gastrointestinal disorders, inflammation [4]. Recent scientific evidence attributes these therapeutic properties to its bioactive components, notably the phenylpropanoid glycoside acteoside [3]. The mechanism for its anti-inflammatory and gastroprotective effects involves the modulation of key signaling pathways such as nuclear factor- κ B and mitogen-activated protein kinases [5], which suppresses the production of pro-inflammatory mediators such as inducible nitric oxide synthase and various cytokines [6].

Several studies have identified *A. distichum* as an excellent source of polyphenols and flavonoids, with the leaves typically containing higher concentrations of these compounds

than the stems [7,8]. One study reported polyphenol and flavonoid contents of 50.64 mg/g and 13.53 mg/g, respectively, in the leaves [4]. In addition to its anti-inflammatory effects, research has provided evidence for the antioxidant, anticancer, and anti-osteoporotic properties of this plant [9,10]. Furthermore, safety evaluations have shown that the plant is non-genotoxic, with a reported LD₅₀ of 2000 mg/kg, indicating very low toxicity [4].

Despite its promising therapeutic potential, research on the use of enzymatic modification aimed at enhancing or altering the bioactive metabolite profile of *A. distichum* remains limited. Enzyme-assisted biocatalysis using commercial enzyme complexes such as Viscozyme[®] L (a multi-enzyme formulation containing cellulase, hemicellulase, and pectinase) is an effective method for degrading plant cell walls, thereby facilitating the release of bound phenolic compounds and altering the structural composition of secondary metabolites [11]. Studies on other medicinal plants have demonstrated that enzymatic treatment can increase the yield of extractable aglycones and enhance physiological activity [12]. The present study investigates the potential effects of Viscozyme[®] L treatment on the secondary metabolome of *A. distichum* leaves [13,14].

Using high-performance liquid chromatography (HPLC) and liquid chromatography–electrospray ionization tandem mass spectrometry (LC-ESI/MS), we characterize and compare the phytochemical profiles of untreated and enzyme-treated extracts, with a particular focus on phenolic and flavonoid compounds. This study provides insights into the bioconversion potential of Viscozyme[®] L and its applicability in enhancing the functional properties of *A. distichum* leaves as a natural health-promoting material.

2. Materials and Methods

2.1. Plant Materials

The leaves of *A. distichum* Nakai were harvested on 20 June 2024, from Misun Farm Co., Ltd., Goesan, Republic of Korea (Figure 1).

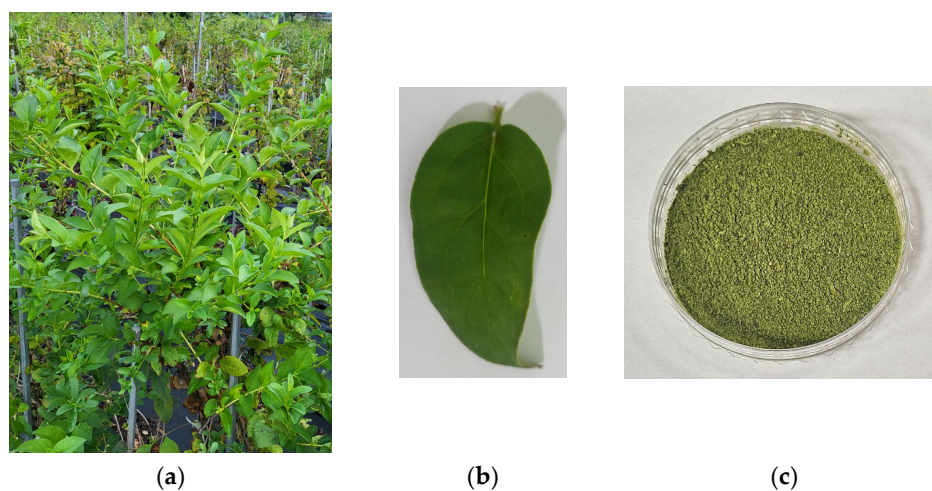


Figure 1. Whole plant (a), leaf (b), and leaf powders (c) of *A. distichum*.

2.2. Instruments and Reagents

Metabolite profiling was conducted using a Thermo Vanquish Flex ultra high-performance liquid chromatography (UHPLC) system coupled with a Thermo Q-Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). HPLC analysis was performed using an Agilent 1260 Infinity II Quaternary Pump (Santa Clara, CA, USA) equipped with a variable wavelength detector (Agilent VWD), a pump, and an autosampler. HPLC-grade solvents, including water and methanol (MeOH), were purchased from Honeywell (Burdick and Jackson, Muskegon, MI, USA), while acetonitrile (ACN) was sourced

from Scharlau (Barcelona, Spain). Trifluoroacetic acid (TFA) and formic acid were obtained from Thermo Fisher Scientific (Cleveland, OH, USA). Standard compounds—rutin (1), acteoside (2), isoacteoside (3), and quercetin (4)—were procured from the Natural Product Institute of Science and Technology (www.nist.re.kr; accessed on 7 March 2025), Anseong, Republic of Korea (Figure 2).

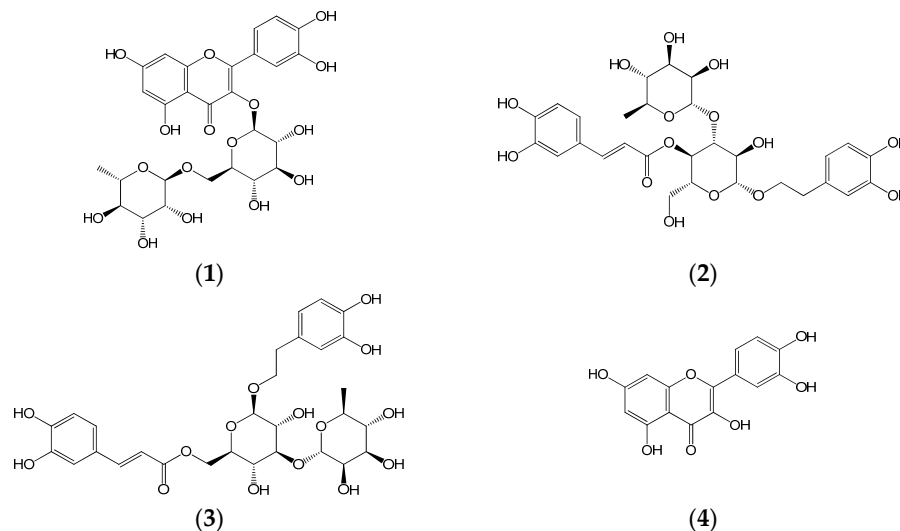


Figure 2. Chemical structures of rutin (1), acteoside (2), isoacteoside (3), and quercetin (4).

2.3. Extraction and Preparation of Viscozyme[®] L-Treated *A. distichum* Leaves

Immediately after harvesting on 20 June 2024, the leaves of *A. distichum* were dried at 38 °C for 24 h. This temperature was selected based on research indicating that drying conditions in the range of 30–40 °C are often ideal for minimizing the degradation of heat-sensitive metabolites, such as polyphenols, and preserving their chemical integrity [15]. The dried leaves were ground using a blender, and 6.7 g of powdered material was transferred to a 250 mL Erlenmeyer flask containing 40 mL of distilled water. Then, 1 mL of Viscozyme[®] L was added, and the mixture was incubated in a shaking water bath (BS-11, JeioTech, Seoul, Republic of Korea) at 70 °C for 6 h. A similar treatment, replacing Viscozyme[®] L with distilled water, has been applied as a control assay. To inactivate the enzyme, the mixture was heated at 99 °C for 5 min. Following enzymatic hydrolysis, 70% ethanol was added, and the mixture was extracted by immersion in a shaking water bath at 80 °C for 24 h. The resulting extract was filtered and stored at −20 °C until further analysis.

2.4. LC-ESI/MS Conditions

LC-ESI/MS profiling was performed using LC-Orbitrap analysis with a Thermo Q-Exactive mass spectrometer (San Jose, CA, USA) coupled with a Thermo Vanquish UHPLC system, equipped with a heated electrospray ionization source (H-ESI) operating in positive mode. The analytical column used was a Waters Cortecs T3 (2.1 × 150 mm, 1.6 μm; Milford, MA, USA), maintained at 45 °C. The injection volume was 4 μL, and the flow rate was 0.3 mL/min. The mobile phases were (A) 0.1% formic acid in water and (B) 0.1% formic acid in ACN. Gradient elution was performed as follows: 0–0.1 min 5% B; 10 min 10% B; 50–55 min 95% B; 55.1 min, returned to 5% B. Mass spectrometric detection was conducted in both positive and negative ionization modes over an *m/z* range of 100–1500, with a resolution of 70,000 (MS1). Spray voltage was set at 3.5 kV (positive mode) and 2.5 kV (negative mode), with a capillary temperature of 320 °C. Sheath gas, auxiliary gas, and sweep gas were set to 50, 10, and 1 arb units, respectively. ESI/MS fragmentation was performed using stepped normalized collision energies of 10, 30, and 50.

2.5. HPLC Conditions

Quantitative analysis of the chemical composition of *A. distichum* leaves was performed using a reverse-phase HPLC system. Chromatographic separation was achieved using a YMC Pack Pro C18 column (4.6 × 250 mm, 5 µm; Kyoto, Japan). The analysis was conducted using a gradient method with 0.1% TFA in water (solvent A) and ACN (solvent B). The gradient elution system was as follows: 0–10 min, 93% A; 15–20 min, 77% A; 30 min, 50% A; 31–35 min, 0% A; 50–60 min, 83% A. The column temperature was maintained at 30 °C. The injection volume was 10 µL, and the flow rate was set to 1.0 mL/min. Detection was carried out at a wavelength of 254 nm. The HPLC analysis was performed with reference to a previously reported method, with minor modifications [16].

2.6. Calibration Curve

A stock standard solution was prepared by dissolving four compounds in MeOH at a concentration of 1 mg/mL. The working solutions used to construct calibration curves were prepared by serial dilution of the stock solution to the desired concentrations. Leaf samples of *A. distichum* were dissolved in MeOH at 10 mg/mL. Both standard and sample solutions were passed through 0.20 µm polyvinylidene fluoride filters prior to analysis. Compound concentrations in the samples were quantified based on the calibration curves. The calibration function for each compound was calculated using peak area (*Y*) versus concentration (*X*, µg/10 µL) (Table 1).

Table 1. Calibration curve equations for compounds 1–4.

Compound	<i>t_R</i> ¹	Calibration Equation ²	Correlation Factor, <i>r</i> ² ³
1	20.7	$Y = 10,494.1X + 4.7$	1.0000
2	21.2	$Y = 5641.9X - 2.0$	1.0000
3	23.0	$Y = 3583.3X + 26.9$	0.9996
4	30.3	$Y = 24,334.1X + 56.0$	0.9999

¹ *t_R* = retention time; ² *Y* = peak area; *X* = standard concentration (mg/mL); ³ *r*² = correlation coefficient for the five data points in the calibration curve; rutin (1), acteoside (2), isoacteoside (3), and quercetin (4).

2.7. Statistical Analysis

All results are reported as mean ± standard deviation and were derived from three independent trials (*n* = 3). Statistical comparisons were performed using one-way analysis of variance (ANOVA), followed by Tukey’s post hoc test for multiple comparisons, using GraphPad Prism software (version 8.0.2; GraphPad Software, Boston, MA, USA). Metabolite set enrichment analysis (MSEA) was conducted using the enrichment analysis module in MetaboAnalyst 6.0 [<https://www.metaboanalyst.ca/>] (accessed on 7 July 2025)].

3. Results

3.1. LC-ESI/MS Profiling and MSEA for Chemical Structure Classification

The metabolite profiles of *A. distichum* leaf extracts, both before and after enzyme treatment, were analyzed using LC-ESI/MS (Figure 3). In the untreated control group, a total of 26 compounds were identified. In contrast, only 16 compounds were detected in the Viscozyme® L-treated group, indicating a reduction in the overall number of compounds (Tables 2 and 3).

To better understand the biological significance of the metabolic changes induced by Viscozyme® L treatment in *A. distichum* leaves, enrichment analysis was performed using the differentially accumulated metabolites identified via LC-ESI/MS profiling. The enrichment module conducts MSEA using several libraries, comprising approximately 6300 metabolite sets. For this analysis, we selected the “chemical structures” library, which

includes sub-classes (1250 sub-chemical class metabolite sets or lipid sets). The MSEA results for chemical structure classification of the key metabolites in the *A. distichum* leaf extracts revealed classification into 12 sub-classes in the untreated extract, and 10 sub-classes in the Viscozyme® L-treated extract (Figure 4).

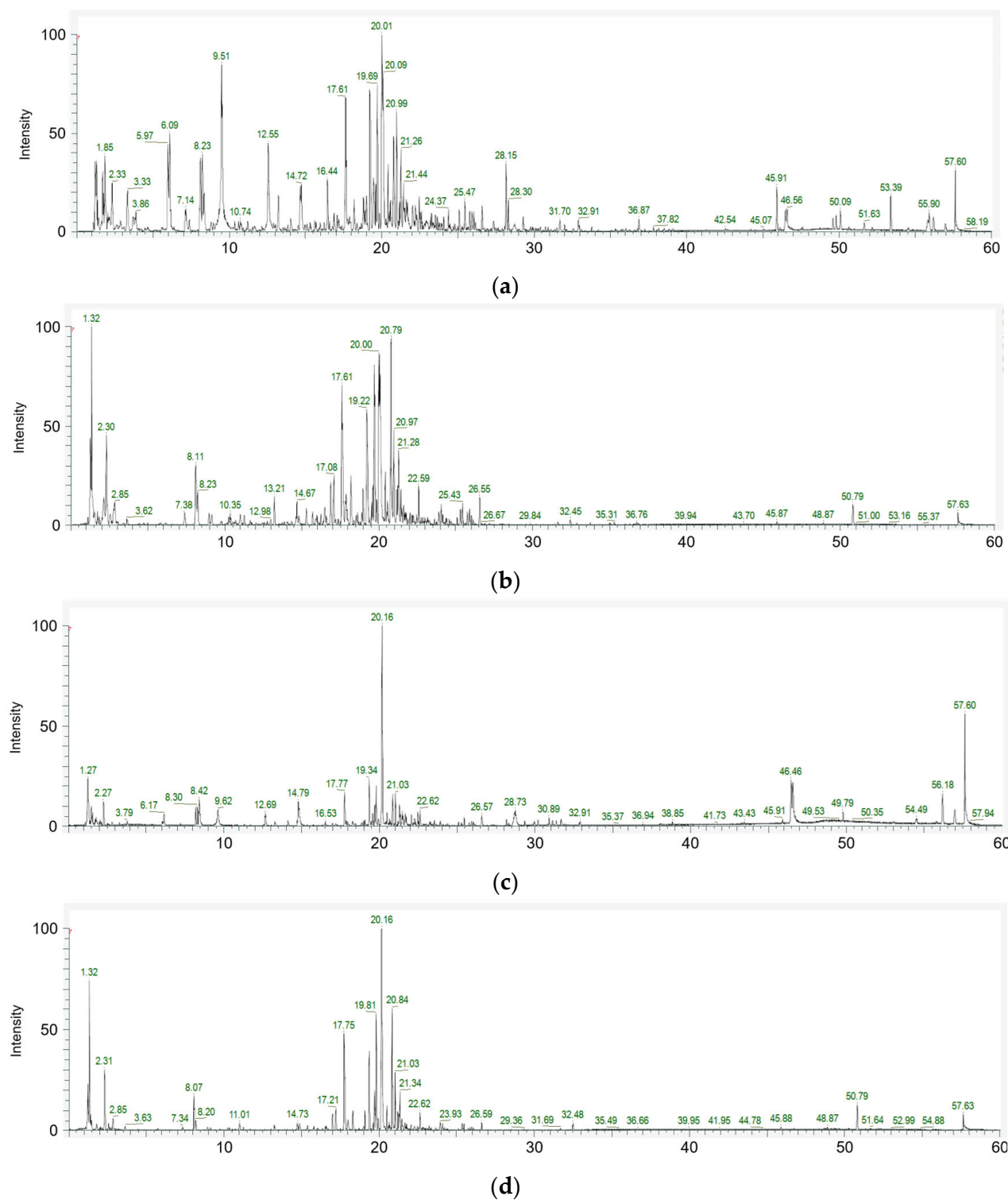


Figure 3. Total ion chromatogram of the positive (a) and negative (b) modes of *A. distichum* leaf extract; positive (c) and negative (d) modes of Viscozyme® L-treated *A. distichum* leaf extract.

Table 2. Profiling of compounds in *A. distichum* leaf extract via LC-ESI/MS analysis.

t_R ¹	Molecular Formula	Molecular Weight	Tentative Identification
4.99	C ₇ H ₆ O ₄	154.0	Pyrocatechuic acid ²
5.82	C ₁₄ H ₂₀ O ₈	316.1	Hydroxytyrosol 4-O-glucoside ²
7.38	C ₇ H ₆ O ₃	138.0	Protocatechuic aldehyde
8.10	C ₂₀ H ₃₀ O ₁₂	462.2	Verbascoside ²
8.23	C ₁₄ H ₂₀ O ₇	300.1	Salidroside ²
10.59	C ₈ H ₁₀ O ₄	170.1	Penicillic acid ²
10.66	C ₁₉ H ₂₈ O ₁₁	432.2	Osmanthuside H ²
10.70	C ₉ H ₆ O ₄	178.0	Esculetin
11.00	C ₉ H ₈ O ₄	180.0	Caffeic acid
13.74	C ₁₆ H ₁₈ O ₈	338.1	4-Coumaroylquinic acid ²
14.63	C ₁₅ H ₁₄ O ₆	290.1	Epicatechin ²
14.66	C ₁₈ H ₂₂ O ₁₁	414.1	Asperuloside ²
14.80	C ₉ H ₈ O ₃	164.0	<i>o</i> -Coumarate
16.49	C ₂₃ H ₂₆ O ₁₁	478.1	Calceolarioside A
17.08	C ₂₉ H ₃₆ O ₁₆	640.2	Plantamajoside
19.22	C ₂₇ H ₃₀ O ₁₆	610.2	Rutin
19.61	C ₂₁ H ₂₀ O ₁₂	464.1	Hyperoside
20.02	C ₂₉ H ₃₆ O ₁₅	624.2	Acteoside
20.13	C ₂₉ H ₃₆ O ₁₅	624.2	Isoverbascoside
20.52	C ₂₇ H ₃₀ O ₁₅	594.2	Datiscin ²
20.85	C ₂₁ H ₂₂ O ₁₀	434.1	Prunin ²
23.90	C ₁₅ H ₁₀ O ₇	302.0	Quercetin
24.15	C ₁₁ H ₁₂ O ₄	208.1	Caffeic acid ethyl ester
25.30	C ₁₅ H ₁₂ O ₅	272.1	Naringenin
25.90	C ₁₅ H ₁₀ O ₆	286.0	Kaempferol ²
31.63	C ₃₀ H ₄₈ O ₅	488.4	Asiatic acid

¹ t_R = retention time; ² compounds were not detected in the Viscozyme[®] L-treated extract.

Table 3. Profiling of compounds in Viscozyme[®] L-treated *A. distichum* leaf extract via LC-ESI/MS analysis.

t_R ¹	Molecular Formula	Molecular Weight	Tentative Identification
7.34	C ₇ H ₆ O ₃	138.0	Protocatechuic aldehyde
10.69	C ₉ H ₆ O ₄	178.0	Esculetin
11.01	C ₉ H ₈ O ₄	180.0	Caffeic acid
14.87	C ₉ H ₈ O ₃	164.0	<i>o</i> -Coumarate
16.60	C ₂₃ H ₂₆ O ₁₁	478.1	Calceolarioside A
17.01	C ₂₉ H ₃₆ O ₁₆	640.2	Plantamajoside
17.77	C ₂₃ H ₂₆ O ₁₁	478.1	Calceolarioside B
19.08	C ₂₇ H ₃₀ O ₁₆	610.2	Quercetin
19.35	C ₂₇ H ₃₀ O ₁₆	610.2	3-O-neohesperidoside
19.69	C ₂₁ H ₂₀ O ₁₂	464.1	Rutin
20.16	C ₂₉ H ₃₆ O ₁₅	624.2	Hyperoside
20.84	C ₂₉ H ₃₆ O ₁₅	624.2	Acteoside
23.95	C ₁₅ H ₁₀ O ₇	302.0	Isoverbascoside
24.21	C ₁₁ H ₁₂ O ₄	208.1	Quercetin
25.35	C ₁₅ H ₁₂ O ₅	272.1	Caffeic acid ethyl ester
31.67	C ₃₀ H ₄₈ O ₅	488.4	Naringenin
			Asiatic acid

¹ t_R = retention time.

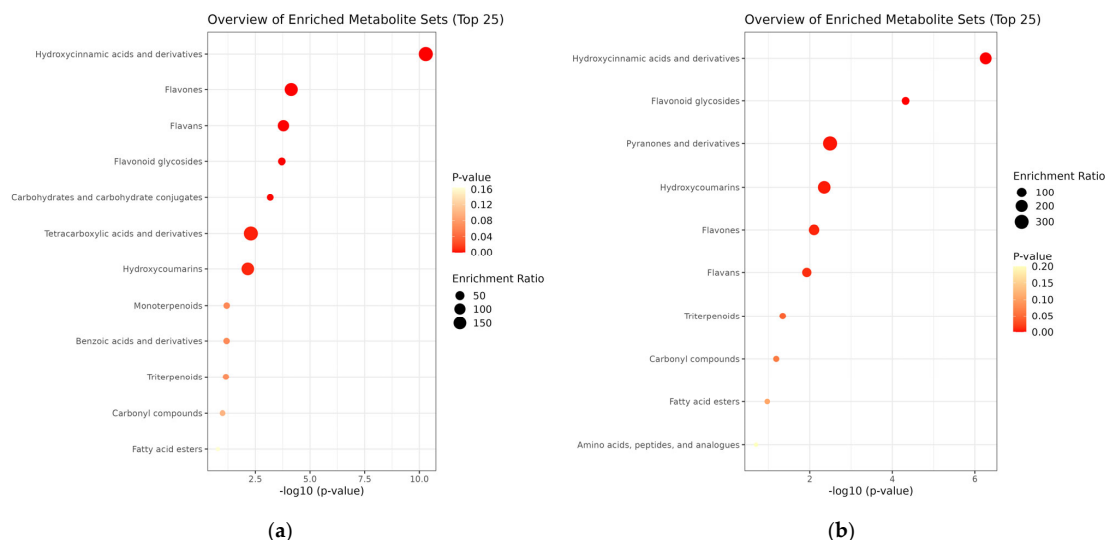


Figure 4. MSEA-based chemical structure sub-class classification (1250 sub-chemical class metabolite or lipid sets) for *A. distichum* extract (a) and Viscozyme® L-treated *A. distichum* leaf extract (b).

3.2. HPLC Quantitative Analysis

To evaluate the impact of enzymatic treatment on the phytochemical composition of *A. distichum* leaf extract, comparative HPLC analysis was conducted on extracts prepared with and without Viscozyme® L (Figure 5; Table 4). The HPLC results for glycosylated compounds showed that the total content of rutin (1), acteoside (2), and isoacteoside (3) decreased from 402.52 mg/g DW in the control extract to 350.76 mg/g DW in the Viscozyme® L-treated sample. Among these, acteoside (286.65 mg/g DW) and isoacteoside (65.66 mg/g DW) were the predominant constituents, followed by rutin (50.21 mg/g DW). Upon Viscozyme® L treatment, the total glycosylated compounds content decreased to 350.76 mg/g DW, indicating an overall reduction in the quantified metabolites. Specifically, the levels of rutin (1), acteoside (2), and isoacteoside (3) significantly decreased to 36.72, 273.66, and 40.38 mg/g DW, respectively, compared to those in the control. In contrast, the amount of aglycone quercetin (4) markedly increased to 0.29 mg/g DW in the enzyme-treated sample, showing a more than fourfold increase relative to the control (0.07 mg/g DW).

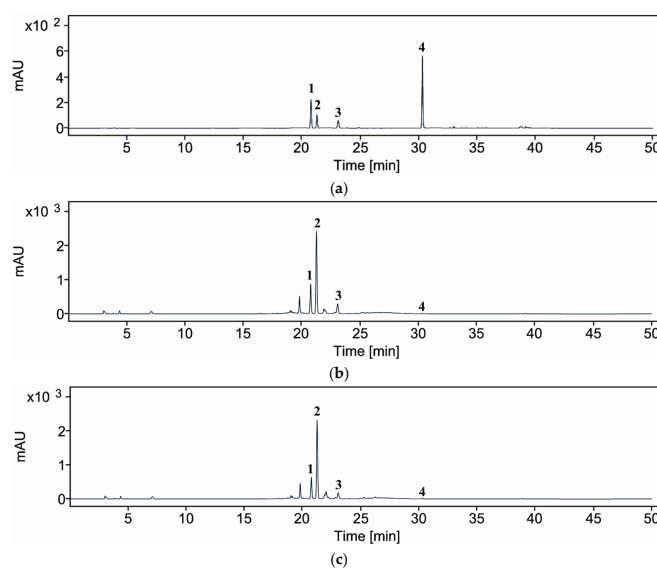


Figure 5. HPLC/UV chromatograms of compounds 1–4 (a), *A. distichum* leaf extract (control) (b), and Viscozyme® L-treated *A. distichum* leaf extract (c); rutin (1), acteoside (2), isoacteoside (3), and quercetin (4).

Table 4. Content of compounds 1–4 in the samples.

Sample	Contents (mg/g DW) ****				
	1	2	3	4	Total
Control	50.21 ± 0.01 ^a	286.65 ± 0.04 ^a	65.66 ± 0.07 ^a	0.07 ± 0.02 ^b	402.59
Viscozyme [®] L	36.72 ± 0.03 ^b	273.66 ± 0.31 ^b	40.38 ± 0.45 ^b	0.29 ± 0.01 ^a	351.05

^{a,b} different letters indicate significant differences; **** indicates significance at $p < 0.0001$; rutin (1), acteoside (2), isoacteoside (3), and quercetin (4).

4. Discussion

A. distichum, a traditional medicinal herb, has been actively investigated for its diverse biological activities, including anti-diabetic and anti-inflammatory effects [17]. It is well recognized that enzymatic biotransformation plays a critical role in activating numerous prodrugs [18,19] and in enhancing the bioactivity of natural products across various applications [20]. In previous studies, cell wall-degrading enzymes such as Pectinex[®] Ultra SP-L and Viscozyme[®] L have been reported to improve polyphenol recovery and enhance radical-scavenging activity in plant extracts [21]. Similarly, in the case of mulberry leaves, enzymatic biotransformation has been shown to increase tyrosinase inhibitory activity in leaf extracts [22]. These findings collectively underscore the potential of enzyme-assisted processing as an effective strategy for modulating and enhancing the functional properties of herbal extracts.

In the initial stage of this study, enzymatic biotransformation of *A. distichum* leaf extract was performed using the hydrolytic enzyme complex Viscozyme[®] L, followed by metabolite profiling of the transformed samples through LC-ESI/MS and targeted HPLC analysis. A key observation in this study was the reduction in the total number of detectable metabolites following Viscozyme[®] L treatment, from 26 in the control to 16. While this might initially suggest a loss of chemical diversity, the quantitative analysis indicates this is not a net loss of bioactivity but rather a targeted biotransformation that enhances the extract's functional value. This process is driven by the selective enzymatic conversion of glycosides into their corresponding aglycones. Specifically, the hydrolysis of glycosidic bonds led to a significant decrease in compounds like rutin (1) and acteoside (2), which was accompanied by a more than four-fold increase in the highly bioactive aglycone, quercetin (4). This conversion is crucial, as aglycones are well-documented to be more readily absorbed in the gastrointestinal tract, thus possessing greater bioavailability and often higher pharmacological potency than their glycoside precursors [23–25]. Therefore, this enzymatic treatment should be interpreted not as a degradation of active compounds, but as a strategic modulation designed to enhance the overall therapeutic potential of the *A. distichum* extract.

To systematically interpret the complex metabolite profiles and identify biologically relevant patterns, MSEA was conducted using LC-ESI/MS data. The results revealed distinct differences in metabolite enrichment patterns between the untreated and Viscozyme[®] L-treated *A. distichum* extracts. MSEA of the key metabolites in the *A. distichum* extracts showed that metabolites in the untreated extract were distributed across 12 sub-classes, while those in the Viscozyme[®] L-treated extract were distributed across 10 sub-classes (Figure 4). In the untreated extract, metabolites belonging to the flavonoid class—particularly flavans, flavones, and flavonoid glycosides—along with hydroxycinnamic acid and coumarin derivatives, were significantly enriched. This profile aligns with the well-documented secondary metabolite composition of *A. distichum*, which is known to be rich in polyphenolic compounds [26]. Interestingly, following Viscozyme[®] L treatment, a notable shift in metabolite composition was observed. Specifically, flavonoid glycosides, pyranone derivatives, and amino acid-related metabolites showed significant enrichment.

These findings suggest that enzymatic hydrolysis facilitated the cleavage of glycosidic bonds, thereby generating a broader range of low-molecular-weight metabolites. In particular, the marked increase in flavonoid aglycone enrichment implies that enzymatic treatment may enhance the bioavailability of glycosylated flavonoids by producing more accessible or soluble forms. This chemical profile shift underscores the potential of enzymatic biotransformation to modulate the functional properties of *A. distichum* leaf extracts.

In addition to qualitative metabolite profiling by LC-ESI/MS, targeted quantitative analysis was conducted using HPLC to compare the concentrations of representative glycosylated and aglycone compounds. Four major compounds were selected to assess the changes in glycoside and aglycone content between untreated and Viscozyme® L-treated *A. distichum* leaf extracts. This complementary approach enabled a more precise evaluation of the enzymatic effects on chemical composition, particularly the conversion of glycosides into their corresponding aglycones. Notably, a seemingly paradoxical phenomenon was observed: although the overall number of detected compound types decreased, the content of a specific bioactive aglycone, quercetin (4), increased significantly. HPLC analysis revealed that quercetin (4) content rose from 0.07 mg/g DW in the control to 0.29 mg/g DW in the Viscozyme® L-treated sample, while the levels of glycosylated compounds such as rutin (1), acteoside (2), and isoacteoside (3) declined. These findings indicate that enzymatic hydrolysis effectively converted glycosidic forms into their corresponding aglycones.

The limited bioavailability of flavonoid glycosides, such as rutin (1), is well-documented and is primarily attributed to their low intestinal permeability and partial absorption in the gastrointestinal tract [23–25]. In the small intestine, glycosides can undergo enzymatic hydrolysis by brush-border lactase-phloridzin hydrolase or intracellular β -glucosidases, releasing aglycones that more readily diffuse across epithelial membranes [27]. However, unabsorbed glycosides pass into the colon, where gut microbiota further metabolize them into smaller phenolic acids, which are subsequently absorbed and excreted [28,29]. Such enzymatic deglycosylation improves bioavailability and modulates biological activity, as aglycones are often more pharmacologically potent yet chemically less stable [23]. Supporting this, previous studies have shown that enzymatic treatment of rutin with α -L-rhamnosidase produces isoquercitrin, a glucoside derivative with improved antioxidant and anti-proliferative activity compared to rutin itself [30]. In this context, the present findings—that *A. distichum* leaves are enriched with flavonoid glycosides—suggest that targeted enzymatic hydrolysis, using multi-enzyme complexes such as Viscozyme® L, could be employed to enhance the therapeutic potential of these phytochemicals by releasing their corresponding aglycones or more bioactive glycoside forms.

The observed biotransformation can be attributed to the dual effect of Viscozyme® L, a multi-enzyme complex from *Aspergillus aculeatus* [13,31,32]. Its enzymatic composition facilitates a two-step process: initially, cellulase, hemicellulase, and xylanase activities degrade the plant cell wall, maximizing the release of phytochemicals. Subsequently, the complex's notable β -glucosidase activity directly modifies these released compounds by cleaving β -1,4-glycosidic bonds [33]. This deglycosylation converts stable glycosides into their corresponding aglycones, as evidenced by the increase in quercetin (4). This process explains how the treatment leads to not just an enhanced release but a targeted modulation of the extract's chemical makeup, favoring more bioavailable and bioactive forms [34]. Indeed, previous studies have demonstrated that flavone glycoside levels significantly decrease following enzymatic treatment, while aglycone concentrations initially increase but may subsequently decline with prolonged exposure due to secondary degradation [9,35]. Experimental findings confirm that treatment with Viscozyme® L leads to a significant reduction in flavone glycosides in plant extracts, accompanied by a transient increase in their corresponding aglycones [31,36]. These results suggest that Viscozyme® L selectively

hydrolyzed glycosidic forms of flavonoids such as rutin (quercetin-3-O-rutinoside) (1), potentially releasing the corresponding aglycone quercetin (4), while concurrently degrading or transforming other glycosylated compounds such as acteoside (2) and isoacteoside (3).

Collectively, these findings underscore the potential of enzyme-assisted biotransformation—particularly using Viscozyme® L—as an effective strategy to modulate chemical composition, enhance bioactivity, and improve the pharmacological relevance of *A. distichum* extracts by converting glycosides into more bioavailable and bioactive forms.

5. Conclusions

This study demonstrated that enzymatic biotransformation of *A. distichum* extracts using the multi-enzyme complex Viscozyme® L effectively modulated their metabolite composition by converting glycosylated forms into their corresponding aglycones. LC-ESI/MS-based metabolite profiling revealed a reduction in the overall diversity of detectable compounds, alongside an enrichment of specific classes—particularly flavonoid glycosides and aglycones—as confirmed by targeted HPLC quantification. MSEA further identified a distinct sub-class distribution pattern between the untreated and enzymatically treated extracts, highlighting the structural and functional changes induced by enzymatic processing. The significant decrease in glycosylated flavonoids such as rutin (1), acteoside (2), and isoacteoside (3), accompanied by an increase in the bioactive aglycone quercetin (4), suggests that Viscozyme® L selectively hydrolyzed glycosidic bonds to yield more bioavailable and pharmacologically relevant metabolites. These findings highlight the utility of enzyme-assisted processing as a promising approach for enhancing the functional and therapeutic potential of *A. distichum* by improving the bioavailability and biological activity of its polyphenolic constituents. Future studies evaluating the in vivo pharmacokinetics and biological efficacy of transformed metabolites are warranted to further validate these benefits.

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