α-Glucosidase Inhibitory Activity and Quantitative Contribution of Phenolic Compounds From Vietnamese Aquilaria crassna Leaves

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Abstract
Aquilaria crassna Pierre ex Lecomte, Thymelaeaceae, is cultivated for producing resinous heartwood, also called agarwood. Its leaves are a source of herbal tea in Vietnam due to its rich content of polyphenols. However, the α-glucosidase inhibition activity and the contents of phenolic compounds in leaves of different ages have not yet been determined. In the current study, 7 polyphenols [iriflirone 3,5-C-β-diglucoside (1), iriflirone 3-C-β-d-glucoside (2), mangiferin (3), iriflirone 2-O-α-rhamnose (4), genkwanin 5-O-β-primeveroside (5), genkwanin 4′-methyl ether 5-O-β-primeveroside (6), and genkwanin (7)] were isolated from the leaves of A. crassna. Among them, genkwanin (7), an O-methylated flavone, was the most active compound that inhibited α-glucosidase activity, with an IC₅₀ value of 24.0 µM. Molecular docking studies were performed to understand the binding interactions of the active compounds. In addition, a reliable and straightforward reversed-phase HPLC method was developed to determine the content of compounds in different leaves of A. crassna. Mangiferin (3) showed the highest content. The contents of 1-4 contributed to the total polyphenolic contents and significantly decreased from the youngest to the oldest leaf. The contents of 5-7 fluctuated through various ages of leaves. Compounds 5 and 6 showed a low accumulation in the first and second leaves, then obtained high contents among middle leaves and declined in the oldest. These results suggested that A. crassna and its polyphenols may prevent the development and progression of diabetes through α-glucosidase inhibition. Also, the analysis of the polyphenol content in A. crassna may be helpful for tea product manufacture.

Keywords
Aquilaria crassna, leaves, phenolic compounds, docking simulation, α-glucosidase

Introduction
Aquilaria is one of the most widespread genera of the Thymelaeaceae family, which is native to Southeast Asian countries such as Cambodia, China, Malaysia, Indonesia, Laos, Thailand, and Vietnam. Three species—Aquilaria crassna, Aquilaria malaccensis, and Aquilaria sinensis—are primarily being cultivated on thousands of hectares to produce agarwood, which comes from the resinous heartwood.¹ In 1993, the Rainforest Project Foundation began to support Vietnamese farmers in cultivating Aquilaria plants in several provinces in southern Vietnam. That project supported plantations of more than one hectare; later the scheme was expanding into central Vietnam. The primary purpose of the planting was to produce the precious commercial product (used in perfumes and incense) known as agarwood, which is almost extinct, from 5-
Materials and Methods

Plant Materials and Chemicals

Branches of *A. crassna* were collected from the Evergreen Forest Co. Ltd in Dong Phu District, Binh Phuoc Province, Vietnam (11°37′42.2″N 106°59′34.3″E) in March 2015 and identified by Dr Vo Van Chi, Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City. The leaves were detached and sorted into sets of 10, from the youngest (first leaf) to oldest (tenth leaf) leaves that were then dried at 50 °C. The first 3 leaves were used for extraction and isolation. Voucher specimens (UMP-2015-AQ001-010) were deposited at the Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam. Each leaf (from the first to the tenth) was weighed, and then pulverized into powder of <355 μm. For extraction and isolation purposes, leaves were directly extracted without pulverization. The HPLC solvents were purchased from Merck and Labscan.

Apparatus

Preparative HPLC was performed on an LC-8A Shimadzu instrument, using a Discovery HS C18 column (25 cm × 21.2 mm, 10 μm). The injection volume was 10 mL, and the analysis was performed at room temperature. The flow rate was maintained at 10 mL/min, and the absorbance was measured at a wavelength of 330 nm. HPLC was performed on an ACQUITY UPLC H-Class system (Waters), equipped with a PDA detector (330 nm) and a Phenomenex Gemini C18 (150 mm × 4.6 mm, i.d., 3 μm) (Phenomenex), connected to Empower software. The separation was achieved using a mobile phase of 0.2% acetic acid/acetonitrile (A) and 0.2% acetic acid (B) in the following sequence: 0 to 1 min: 10% A; 1 to 5 min: 13% A; 5 to 10 min: 13% A; 10 to 15 min: 25% A; 15 to 18 min: 25% A; 18 to 22 min: 35% A; 22 to 26 min: 65% A; 26 to 30 min: 85% A; and 30 to 35 min: 10% A. The column temperature was set at 40 °C. The HPLC flow rate was 1 mL/min. A 4-μL sample solution was injected into the ultra-performance liquid chromatography (UPLC) system. TLC was performed using silica gel F254 (40-63 μm, Merck). Compounds were detected by UV light at 254 nm and 365 nm, and then were sprayed with 10% H2SO4 in EtOH, followed by heating. Column chromatography (CC) was performed using silica gel (200-300 mesh), along with liquid chromatography using LiChroprep RP-18 silica gel (40-63 μm) (Merck). Silica gel 60 (40-63 μm, 230-400 mesh ASTM) for the CC was purchased from Scharlau. The other chemicals were of the highest grade available. 1H and 13C NMR spectra were recorded in DMSO-d6 using a Bruker AM-500 (500 MHz) spectrometer. ESI-MS were recorded on a MSQ_PLUS mass spectrometer (THERMO).

Extraction and Isolation of Standard Compounds

The isolation of polyphenolic compounds was targeted, with major polyphenol peaks from the total extract from UPLC chromatography occurring at 330 nm. The dried young leaves of *A. crassna* (0.9 kg) were extracted under reflux using 100% MeOH (2 × 10 L) at 65 °C. The total MeOH extract was then cooled. A yellow precipitate resulted, which was filtered...
to obtain 70 g of residue. This was then crystallized several times using hot MeOH to obtain mangiferin (50 g, 3). The filtered extract was evaporated in vacuo to produce a MeOH extract (200 g), which was dissolved in water, and partitioned using CH2Cl2. A precipitate appeared in the CH2Cl2 extract, and was filtered and crystallized in CHCl3-MeOH (8:2) to obtain genkwanin (15 mg, 7). The CH2Cl2 fraction was evaporated in vacuo to obtain 25 g of extract. The water layer was extracted using EtOAc. The precipitate appeared after removing 50% of the solvent. It was then filtered to yield 1.5 g of residue (Fraction E1). The EtOAc fraction was evaporated in vacuo to obtain 45 g of extract. The water layer was extracted using watersaturated n-butanol to give 15 g of n-butanol extract. The aqueous layer was then purified using Diaion HP-20, and eluted using 100% water and 100% methanol. The methanol eluate was evaporated in vacuo to obtain 12 g of dried extract. E1 (1.5 g) was passed through a silica gel column and eluted using CHCl3:MeOH (85:15) to obtain 7 fractions (E1-E7). Fraction E7 was continuously chromatographed on a silica-gel column and eluted with MeOH-H2O (4:6) to obtain 6 further fractions (Bu5.1-Bu5.6). Fraction Bu5.6 (55 mg) was further purified by prep-HPLC, using MeOH-H2O (24:74) as a mobile phase, to yield iriophenone 3-C-β-D-glucoside (32 mg, 1). After confirming the purity (>$95\%$) by HPLC-UV analysis and recorded 1H and 13C NMR spectra, each isolated compound was authorized as an analytical standard.

**Preparation of Standard Solution**

A stock solution containing iriophenone 3,5-C-β-diglucoside 1 (273.97 µg/mL), iriophenone 3-C-β-glucoside 2 (614.11 µg/mL), mangiferin 3 (1462.80 µg/mL), iriophenone 2-O-β-rhamnoside 4 (449.64 µg/mL), genkwanin 5-O-β-primeveroside 5 (134.94 µg/mL), genkwanin 4’-methoxy-5-O-β-primeveroside 6 (30.67 µg/mL) and genkwanin 7 (24.75 µg/mL) in methanol was prepared and diluted to 6 concentrations for the establishment of calibration curves. The limit of detection and quantitation under the chromatographic conditions used were determined based on the response at signal-to-noise ratios of approximately 2 to 10, respectively.

**Optimization Extraction Solvent for HPLC**

To obtain better extraction rates for the 7 polyphenols under investigation from *A crassna* leaves, different solvents and
Table 1. Extraction Methods.

| No. | Solvent | Method\(a\) | Temperature (°C) | Duration (mins) |
|-----|---------|-------------|-----------------|----------------|
| 1   | H₂O     | Reflux      | 100             | 60             |
| 2   | MeOH    | Reflux      | 60              | 60             |
| 3   | MeOH    | Sonication  | 55              | 30             |
| 4   | MeOH    | Reflux      | 65              | 60             |
| 5   | MeOH    | Sonication  | 55              | 30             |
| 6   | MeOH    | Reflux      | 75              | 60             |
| 7   | MeOH    | Sonication  | 55              | 30             |
| 8   | MeOH    | Reflux      | 80              | 60             |
| 9   | MeOH    | Sonication  | 55              | 30             |

\(a\)Each extraction was conducted in triplicate.

Method Validation

The precision of the analytical method was determined by intra- and inter-day variations. A 100 mg aliquot of A crassna leaf powder for each different age was placed in a volumetric flask, to which 25 mL MeOH 100% was added. This was then shaken on a Vortex Genie (Scientific Industries, Inc) for 10 s before being subjected to ultrasonication (Branson 5510) for 30 min at 55 °C. The solution was filtered through a 0.22-µm membrane before injection into the UPLC.

Preparation of HPLC Samples

A 100 mg aliquot of A crassna leaf powder for each different age was placed in a volumetric flask, to which 25 mL MeOH 100% was added. This was then shaken on a Vortex Genie (Scientific Industries, Inc) for 10 s before being subjected to ultrasonication (Branson 5510) for 30 min at 55 °C. The solution was filtered through a 0.22-µm membrane before injection into the UPLC.

\[ Ri = \frac{Sr}{St} \times Cr \times \frac{25}{10^6 \times m} \]

where \( Ri \) is the polyphenol content (\%) in the leaf, \( St \) is the peak area of polyphenol (\%) in the sample solution, \( Sr \) is the peak area of polyphenol (\%) in the standard solution, \( Cr \) is the polyphenol content in the standard solution, and \( m \) is the weight of the sample.

\( \alpha \)-Glucosidase Inhibitory Assay

\( \alpha \)-glucosidase (EC 3.2.1.20) isolated from Saccharomyces cerevisiae (750 UN) and p-nitrophenyl-\( \alpha \)-d-glucopyranoside were obtained from Sigma Chemical Co., and acarbose and dimethyl-sulfoxide from Merck. The inhibitory activity of \( \alpha \)-glucosidase was determined following a modified published method.\(^{13} \)

Aliquots of 1.5 mM \( p \)-nitrophenyl-\( \alpha \)-d-glucopyranoside (50 \( \mu \)L) and 0.1 U/mL \( \alpha \)-glucosidase (50 \( \mu \)L) in 0.01 M phosphate buffer (pH = 7.0) were added to the sample solution (525 \( \mu \)L) to start the reaction. Each reaction was allowed to occur at 37 °C for 30 min and was then stopped by adding 0.1 M Na₂CO₃ (375 \( \mu \)L). An inhibitory assay quantified the enzymatic activity by measuring the absorbance at 401 nm. One unit of \( \alpha \)-glucosidase activity was defined as the amount of enzyme liberating \( p \)-nitrophenol at 1.0 \( \mu \)M/min. The IC₅₀ value was defined as the concentration of \( \alpha \)-glucosidase inhibitor that inhibited 50% of the \( \alpha \)-glucosidase activity. Acarbose, a known \( \alpha \)-glucosidase inhibitor, was used as the positive control.

Protein and Ligand Preparation

The 3D structure of \( \alpha \)-glucosidase (Protein Data Bank [PDB 2ZE0]) was obtained from the PDB (http://www.rcsb.org/pdb). The selected 2ZE0 was fixed using a CHARMM (Chemistry at Harvard Macromolecular Mechanics) force field in DS 2.5 (DS, Accelrys Software), which added up the hydrogen atoms, partial charges, and missing residues that are appropriately used for the processes of molecular docking. The 3D structures of 3 natural compounds—mangiferin (3), genkwanin-5-O-\( \beta \)-D-primeveroside (5), and genkwanin (7)—from the A crassna leaves were retrieved from the PubChem database or prepared by MarvinSketch (ChemAxon). In the final step, all ligands were converted into dockable pdbqt format, utilizing the Open Babel toolbox.

Molecular Docking

Virtual screening of the bioactive compounds on \( \alpha \)-glucosidase [PDB 2ZE0] was carried out using AutoDock Vina software.\(^{14,15} \)

A grid box covering the active protein site was generated using the following parameters: center_x = 8.24; center_y = 26.90; center_z = 23.69; size_x = 25; size_y = 25; and size_z = 25. The docking scores are reported in kcal/mol, and the compounds were
ranked by their docking scores. Finally, the molecular interactions between the proteins and selected ligands were visualized using Discovery Studio Visualizer software.

**Statistical Analysis**

The statistical significance was evaluated using an analysis of variance and SPSS software version 20. A P value of <.05 was considered to be statistically significant.

**Results and Discussion**

**Isolation of Phenolic Compounds**

Seven polyphenolic compounds were isolated from *Aquilaria crassna* leaves using liquid–liquid partitioning, followed by normal-phase CC, RP-18 silica-gel CC, and/or semi-prep HPLC. The structures of the isolated compounds [iriflophenone 3,5-C-β-diglucoside (1), iriflophenone 3-C-β-glucoside (2), mangiferin (3), iriflophenone 2-O-β-rhamnoside (4), genkwanin 5-O-β-primeveroside (5), genkwanin 4’-methoxy-5-O-β-primeveroside (6), and genkwanin (7) (Figure 2)] were identified by comparing their spectroscopic data with literature data. The compounds were all significant components in the UPLC chromatogram at 330 nm, and each indicated high purity of more than 95%. Mangiferin (3) was isolated from *A crassna* leaves in a high yield of ~5.5% using a straightforward approach. Its poor solubility allowed it to be easily collected, as it precipitated after cooling the MeOH total extract. Using HPLC-guided isolation, iriflophenone 3,5-C-β-diglucoside (1) was found in the aqueous layer following liquid–liquid partitioning of n-BuOH, due to its high polarity, and could then be purified by RP-18 CC, followed by prep-HPLC.

**α-Glucosidase Inhibitory Activity of the Isolated Compounds and Extracts**

The α-glucosidase inhibitory activity of compounds 1-7 was examined. Compound 7 (genkwanin) exhibited the most

![Figure 2. Structures of major polyphenols isolated from *Aquilaria crassna* leaves.](image)
potent inhibitory effect, with an IC\textsubscript{50} value of 24.0 ± 3.6 µM, which was 4 times greater than that of acarbose, with an IC\textsubscript{50} value of 105.2 ± 11.7 µM. Genkwanin-5-O-β-D-primeveroside (5) presented significant α-glucosidase inhibitory activity, with an IC\textsubscript{50} value of 76.5 ± 6.8 µM, whereas the activity of mangiferin (3) was 217.6 ± 10.5 µM. Interestingly, genkwanin-4′-methoxy-5-O-β-D-primeveroside (6), a derivative of genkwanin-5-O-β-D-primeveroside (5), showed no inhibitory effect. Genkwanin-4′-methoxy-5-O-β-D-primeveroside (6) contains one methoxy group at C-4 of the genkwanin skeleton. This linkage may reduce the α-glucosidase inhibitory activity of these flavone glycosides. Compounds 1, 2, and 4 showed no activity.

In the next study, the extracts from leaves 1 to 10 were tested for their α-glucosidase inhibitory activity using 2 concentrations of 1500 and 750 µg/mL. The results are presented in Table 2.

The α-glucosidase inhibitory activity of the leaves indicates a gradual decrease in activity from leaf 1 to leaf 10 in both of the 2 concentrations (1500 and 750 µg/mL). These results are understandable because the highest polyphenol contents were found in leaves 1 and 2, and gradually decreased to leaf 10. The younger leaf samples (1-3) showed α-glucosidase inhibition of 89.5%, 92.4%, and 73.7%, respectively. This might indicate a correlation between the polyphenol content of the leaf samples and α-glucosidase inhibitory activity. Since leaves 1 to 3 showed the most potent α-glucosidase inhibitory activity, they were subsequently tested for their IC\textsubscript{50} values.

Leaf sample 1 exhibited the most potent α-glucosidase inhibiting effect, with an IC\textsubscript{50} value of 169.8 µg/mL, followed by leaf 2, with an IC\textsubscript{50} value of 248.0 µg/mL, and leaf 3, with an IC\textsubscript{50} value of 507.2 µg/µg/mL (acarbose, positive control 50.0 µg/mL). These results are consistent with the screening results that indicated that the α-glucosidase inhibitory effect increased with the polyphenol content in the leaf samples. In terms of tea quality, this suggests that for a high polyphenol content and vigorous α-glucosidase inhibitory activity, the leaves should be collected from the ends of the branches.

### Molecular Docking Results

α-Glucosidase inhibitors are common oral antidiabetic drugs used to control carbohydrates, typically by converting them into simple sugars, allowing them to be absorbed by the intestines. The 3D structures of the most potent compounds, such as mangiferin (3), genkwanin-5-O-β-D-primeveroside (5), and genkwanin (7), were constructed and used in the docking process with the α-glucosidase active site 2ZE0. In previous studies, acarbose has been shown to inhibit α-glucosidase. Therefore, we decided to choose acarbose as a reference inhibitor. The ligand bound with the α-glucosidase protein showed the crucial amino acid residue (Arg197) in the localized active site. Acarbose docking with α-glucosidase indicated that the model structure of the ligand–protein interaction between acarbose and α-glucosidase had formed 3 interactions of the ligand molecules with Asp199, Arg197, and Asn61, as illustrated in Figure 2A and B for the 2D and 3D structures, respectively. The key point is that, among these 3 compounds, genkwanin 5-O-β-D-primeveroside showed the highest binding affinity to the α-glucosidase active site 2ZE0, having a docking score of −10.5 Kcal/mol. Genkwanin 5-O-β-D-primeveroside showed interaction with α-glucosidase through hydrogen bonds at Tyr63, Asp199, Arg197, Glu256, and His203, and through van der Waals interactions at Val383, Asn61, Asp326, Asp98, His325, Asn324, Trp49, Phe282, Asn258, Phe225, Met229, ILE143, Phe163, Gln167, Asp60, and Val383. The interaction analysis revealed that genkwanin and mangiferin also formed several significant interactions with α-glucosidase. As shown in Figure 2 and Table 4, genkwanin formed 2 hydrogen bonds with Glu256 and Arg411, forming Ala200, Asp199, Gln167, Asp60, Phe144, Asp382, Arg407, Asn61, and Arg417 through van der Waals interactions. Meanwhile, mangiferin formed 2 hydrogen bonds, with Asp60 and Arg411, and had several hydrophobic interactions with Asp199, Ala200, His103, Gln167, Phe144, Ser384, Val383, Ala59, Asn58, Gly62, Arg407, Asp326, His325, Arg197, and Glu256. These interactions were somewhat similar to the interactions between the positive control of acarbose and 2ZE0 of this enzyme (Figure 3, Table 3).

### Polyphenol Content of Different-Aged Leaves

#### Extraction Method Development

In order to obtain an effective extraction method, ultrasonication and reflux were used in tandem with the variables involved in the procedure, including methanol at different concentrations (30%, 60%, 80%, and 100%), temperature (55, 60, 80, and 100 °C), extraction duration (30-60 min), and extract repetition (3 times). The results showed that reflux using 60% MeOH gave a better extraction.

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**Table 2**: Screening for α-Glucosidase Inhibitory Effects of the Extracts of First—10th Leaves 1 to 10.

| Samples | 1500 µg/mL | 750 µg/mL |
|---------|------------|-----------|
| L1<sup>b</sup> | 97.8 ± 0.4 | 89.5 ± 1.2 |
| L2 | 102.6 ± 0.4 | 92.4 ± 1.4 |
| L3 | 85.7 ± 0.3 | 73.3 ± 1.0 |
| L4 | 90.1 ± 0.5 | 57.4 ± 1.1 |
| L5 | 78.8 ± 0.8 | 57.3 ± 1.8 |
| L6 | 70.5 ± 0.7 | 61.0 ± 2.8 |
| L7 | 72.7 ± 0.4 | 56.2 ± 2.4 |
| L8 | 55.1 ± 0.4 | 42.9 ± 3.6 |
| L9 | 86.9 ± 1.4 | 43.6 ± 1.2 |
| L10 | 43.1 ± 0.9 | 30.0 ± 3.2 |
| Acarbose<sup>c</sup> | 50.05 ± 2.3 |

<sup>a</sup>Experiments carried out in 3 replicates.
<sup>b</sup>L1: first leaves.
<sup>c</sup>Positive compound.
Figure 3. Molecular docking of α-glucosidase using 2ZEO active site. (A) 2D and (B) 3D structures of acarbose. (C) 2D and (D) 3D structure of genkwanin. (E) 2D and (F) 3D structure of genkwanin 5- O-β-D-primeveroside. (G) 2D and (H) 3D structure of mangiferin.
yield than ultrasonication with MeOH 100% and was suitable for extracting almost all the major polyphenolic compounds (data not shown). However, in the reflux method using 60% MeOH, mangiferin precipitated after the extract cooled. Therefore, 100% MeOH with ultrasonication is the most appropriate extraction method for the 7 primary polyphenols in *A. crassna* leaves.

**Optimization of HPLC Conditions**

The optimal mobile phase composition for the analysis of iriﬂophenone 3,5-C-β-D-diglucoside (1), iriﬂophenone 3-C-β-D-glucoside (2), mangiferin (3), iriﬂophenone 2-O-α-D-rhamnoside (4), genkwanin 5-O-β-primeveroside (5), genkwanin-4’-methoxy-5-O-β-primeveroside (6), and genkwanin (7) from 100% MeOH with ultrasonication from *A. crassna* leaves was selected by performing several HPLC runs using various concentrations of acetonitrile in acid-buffer solutions as the mobile phase. Among the buffer solutions, separation was achieved using a mobile phase of 0.2% acetic acid/acetonitrile (A) and 0.2% acetic acid (B) in the following sequence: 0 to 1 min: 10% A; 1 to 5 min: 13% A; 5 to 10 min: 13% A; 10 to 13 min: 25% A; 13 to 14 min: 25% A; 14 to 15 min: 30% A; 15 to 18 min: 35% A; 18 to 19 min: 64% A; 19 to 22 min: 65% A; 22 to 23 min: 65% A; 23 to 28 min: 85% A; and 28 to 31 min: 10% A. The column temperature was set at 40 °C. The HPLC peaks of the standard compounds 1-7 in each leaf were verified. The retention times of iriﬂophenone 3,5-C-β-D-diglucoside (1), iriﬂophenone 3-C-β-D-glucoside (2), mangiferin (3), iriﬂophenone 2-O-α-D-rhamnoside (4), genkwanin 5-O-β-primeveroside (5), genkwanin-4’-methoxy-5-O-β-primeveroside (6), and genkwanin (7) were 6.48, 6.94, 10.92, 13.94, 16.54, 19.55, and 22.18 min, respectively.

**Validation of a Developed Method**

The detection method calibration curves exhibited good linear regressions under the optimized chromatographic conditions, as shown in Table 4.

### Table 3. Molecular Docking Simulation Results for Inhibitory Complexes Between the Ligands and Targeted Protein (PTP1P-ZZE0).

| No | Ligand* | Docking score (kcal/mol) | Hydrogen bond | Van der Waals interaction |
|----|---------|--------------------------|---------------|--------------------------|
| 1  | Genkwanin | −8.9 | Glu256, Arg411 | Ala200, Asp199, Gln167, Asp60, Phe144, Asp382, Arg407, Asn61, Asp326, Arg197 |
| 2  | Genkwanin 5-O-β-D-primeveroside | −10.5 | Tyr63, Asp199, Arg197, Glu256, His203 | Val383, Asn61, Asp326, Asp98, His325, Asn324, Trp49, Phe282, Asn258, Phe225, Met229, ILE143, Phe163, Gln167, Asp60, Val383 |
| 3  | Mangiferin | −9.7 | Asp60, Arg411 | Asp199, Ala200, His103, Gln167, Phe144, Ser384, Val383, Ala59, Asn58, Gly62, Arg407, Asp326, His325, Arg197, Glu256 |

*Binding sites of tested compounds in α-glucosidase using ZZE0 and AutoDock 4.0 molecular docking software.

### Table 4. Validated Analytical Parameters for the UPLC/UV Quantification of 7 Major Polyphenols in *Aquilaria crassna* Leaves.

| Compounds | Linear regression equation | R² | Range (mg/mL) | Accuracy (recovery) (%) | RSD (%) |
|-----------|----------------------------|----|--------------|------------------------|--------|
| 1         | $y = 1401.5x$              | 0.9981 | 3.5-274 | 101.8 | 2.83 |
| 2         | $y = 4792.8x$              | 0.9986 | 7.8-614 | 95.9 | 2.25 |
| 3         | $y = 4103x$                | 0.9963 | 18.8-1462 | 103.4 | 1.57 |
| 4         | $y = 3845.6x$              | 0.9999 | 5.8-450 | 97.4 | 2.27 |
| 5         | $y = 7770.3x$              | 0.9998 | 1.4-135 | 100.6 | 2.81 |
| 6         | $y = 26737.7x$             | 0.9999 | 0.4-30.7 | 99.4 | 1.91 |
| 7         | $y = 16546.9x$             | 0.9999 | 0.3-24.7 | 102.7 | 1.15 |

Abbreviation: UPLC, ultra-performance liquid chromatography.

### Polyphenol Content of *A. crassna* Leaves

The major polyphenolic components of the leaves included compounds 1-3, which had the highest content in the youngest leaves (leaf number 1), gradually decreasing to 25% to 35% in leaf number 5, then not changing much from leaf number 5 to leaf number 10 (the oldest). Compound 4 was present in high amounts in leaf 1, decreasing gradually in leaves 2 to 4, being reduced to about 50% in leaf 5. However, the content of 4 was relatively stable in the remaining leaves. There was a low proportion of polyphenol components 5-7, with fluctuations in the leaf contents due to different ages. Compound 7, however, had a high content in leaves 1 and 2, showed a sharp decrease in leaf 3 (by ~85%), and then, the content became relatively stable up to leaf 10. The polyphenolic components were mainly iriﬂophenone 3,5-C-β-D-diglucoside (1), iriﬂophenone 3-C-β-D-glucoside (2), mangiferin (3), and iriﬂophenone 2-O-α-D-rhamnoside (4). These compounds had their highest contents in the young leaves 1 to 3; their contents decreased as the leaves got older. Some of the contents of the minor polyphenols in the leaves—genkwanin 5-O-β-primeveroside (5), genkwanin-4’-methoxy-5-O-β-primeveroside (6), and genkwanin (7)—fluctuated with increasing leaf age; for example, compound 7 was abundant in the young leaves 1 and 2 but decreased as the leaves aged. The others (5 and 6) had low concentrations in the young leaves 1 and 2, but increased in the middle-aged (3-7) leaves before slightly decreasing in the old (8-10) leaves (Table 5).
Conclusion

In this study, 7 phenolic compounds [iritilphenon 3,5-C-β-D-diglucoside (1), iritilphenon 3-C-β-D-glucoside (2), mangiferin (3), iritilphenon 2-O-α-D-rhamnoside (4), genkwanin 5-O-β-primeveroside (5), genkwanin-4’-methoxy-5-O-β-primeveroside (6), and genkwanin (7)] were isolated from the leaves of A. crassna. They were subjected to HPLC to determine standards for quantification. A reliable and straightforward reversed-phase HPLC method was performed to determine the content of the secondary metabolites in different-aged A. crassna leaves. The contents of 1-4 contributed to the total polyphenolic contents in all leaves. In addition, their contents significantly decreased from the youngest (leaf 1) to the oldest leaf (leaf 10). The total polyphenol contents were higher in the first 4 leaves. This study showed that the content of the main phenolic contents in the different leaves of A. crassna changed with the age of the leaves. This result suggests that young agarwood leaves should be harvested to obtain the higher polyphenol contents. Genkwanin (7) was found to be the most active compound for glucosidase inhibition. A molecular docking analysis, using AutoDock 4.0 software, was performed in order to understand the binding interactions of the active compounds and locate the active sites of the enzymes. These results suggested that A. crassna and its polyphenols may prevent the development and progression of diabetes through α-glucosidase inhibition and be a good source of anti-diabetes herbal materials. The method of analysis of polyphenols content in the leaves of A. crassna helps orient the standardization of this plant material, as well as for the collection, classification, and processing of materials to ensure the development of products with high polyphenol content.

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Authors’ Note

NVTL, PLN, and MHT designed the research and revised the manuscript; TNTN, TDL, DHN, HVTN, and TNN performed the experiments; and TKN, NVTL, and MHT wrote the article together. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

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