Isolation and Quantification of Palmatine from *Fibraurea tinctoria* Lour: In Vitro Antioxidant Activity and In Silico Antidiabetic Activity Evaluation

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**Purpose:** This study aimed to isolate and characterize palmatine from *Fibraurea tinctoria* Lour stems, quantify its content, and determine its antioxidant and antidiabetic activities.

**Patients and Methods:** Palmatine was isolated from the methanol extract of *Fibraurea tinctoria* Lour stems by silica gel column chromatography. Structural elucidation of the isolated compounds was performed using spectral data analysis and comparison with the literature. High-Performance Liquid Chromatography (HPLC) was used to quantitatively determine palmatine in the crude methanol extract and fractions. The DPPH and non-enzymatic SOD mimic methods were used to assess the antioxidant activity of the methanol extract, fractions, and isolated compounds. The antidiabetic activity was evaluated in silico by the molecular docking method of alpha-glucosidase and DPP-IV enzymes. Palmatine was used as a test ligand and was compared with berberine and its native ligand or standard compounds.

**Results:** The isolated compound was identified as palmatine. Quantification of palmatine compound by HPLC showed that palmatine was found in the extract and all fractions. In the in vitro antioxidant activity test using the DPPH method, fraction 4 showed the highest activity, with an IC₅₀ value of 91 ppm. In contrast, using the non-enzymatic SOD mimic method, the methanol extract, fraction 5, and isolated compound (palmatine) exhibited very strong antioxidant activity, with IC₅₀ values of 18, 20, and 28 ppm, respectively. The in silico antidiabetic activity of palmatine is thought to have the potential to inhibit these two enzymes.

**Conclusion:** These results showed that *Fibraurea tinctoria* Lour stems have potential as an antioxidant and antidiabetic agent. Further research on phytochemical and pharmacological is required to validate the use of this plant species for the treatment of various diseases, especially diabetes mellitus.

**Keywords:** *Fibraurea tinctoria* Lour, berberine, palmatine, antioxidant, antidiabetic

**Introduction**

The prevalence of diabetes mellitus has significantly increased worldwide. Globally, 537 million people have diabetes, which is projected to increase to 643 million by 2030, and this figure is expected to rise by 783 million or approximately 46% by 2045. Diabetes mellitus is a chronic metabolic disease characterized by elevated blood glucose levels and abnormalities in the metabolism of carbohydrates, proteins, and fats owing to insufficient insulin production or inadequate insulin activity.

Oxidative stress and diabetes mellitus have been related in several studies. Oxidative stress is a condition in which there is an imbalance between the amounts of ROS and antioxidant capacity. ROS mainly encompass free radicals such as superoxide anion radicals (O₂⁻), hydroxyl radicals (•OH), and non-free-radical species such as hydrogen peroxide.
(H₂O₂) and singlet oxygen (¹O₂). Numerous studies have demonstrated that oxidative stress, mediated mainly by the hyperglycemia-induced generation of free radicals along with a decrease in antioxidant enzyme activity, contributes to the development and progression of diabetes and its complications. Therefore, it became clear that ameliorating oxidative stress through antioxidant treatment may be an effective strategy for improving diabetes and reducing diabetic complications.

Recently, there has been considerable interest in identifying natural antioxidants in plant materials. Extensive studies have been conducted on the antioxidant effects of various substances, including plant-derived antioxidants. It was found that the phytochemical components of medicinal plants exhibit antioxidant activity and synergistic effects with hypoglycemic drugs; thus, they are highly effective in diabetes treatment. Antioxidant therapy defends beta cells against oxidative stress-induced apoptosis and preserves the function of beta cells. Previous studies have shown that antioxidants diminish diabetes-related complications and improve insulin sensitivity.

_Fibraurea tinctoria_, or what is known as Akar Kuning (Yellow Root) in Kalimantan, is a yellow vine and has long been used by indigenous tribes in Kalimantan for the traditional treatment of various diseases, including diabetes, jaundice, and malaria. The parts often used for treatment are leaves, roots, stems, and bark. Several studies have been conducted on the pharmacological activity of _Fibraurea tinctoria_ and have shown that this plant has anti-inflammatory, anti-malarial, anti-microbial, and anti-proliferative in cervical, oral, and liver cancers. According to the taxonomy, this plant belongs to the _Menispermaceae_ family, which is known to produce approximately 22 different types of alkaloids. One of them is protoberberine alkaloids, such as berberine, which has been reported to have various pharmacological properties, including antioxidant and antidiabetic properties.

Berberine, a quaternary ammonium salt from the protoberberine group of isoquinoline alkaloids, is a yellow alkaloid found in several plants. Berberine has several derivatives, including palmatine. Berberine and palmatine have similar molecular structures, with only a slight difference in substitution of the isoquinoline moiety. In contrast to berberine, which has a methylenedioxy moiety at positions C2 and C3 in its tetracyclic structure, palmatine is substituted with four methoxyl groups (Figure 1).

Several studies have reported that palmatine has pharmacological effects, including antioxidant and blood glucose regulatory effects. Palmatine reduced blood glucose levels, increased insulin levels, and improved oxidative stress in STZ-induced diabetic animal models, as indicated by decreased MDA levels and increased enzymatic antioxidant levels. Palmatine can activate proteins related to antioxidant activity, which protects cells from reactive oxygen species and endoplasmic stress in STZ-induced diabetic animal models. These pharmacological effects indicate that palmatine is valuable for preventing and treating certain diseases, including diabetes mellitus and its complications. To the best of our knowledge, quantification and evaluation of the antioxidant and antidiabetic activities of palmatine, especially from _Fibraurea tinctoria_ Lour, have not been extensively investigated. This study aimed to isolate, characterize, and determine the palmatine content from the stems of _Fibraurea tinctoria_ Lour and investigate their antioxidant and antidiabetic activities.

Figure 1 Palmatine and Berberine structure.
Material and Methods

Materials

The plant material was collected in August 2021 from Menua Sadap Village, West Kalimantan Province, Indonesia. It was identified by Mrs. Mukarlina of the Department of Biology, Faculty of Mathematics and Natural Science, Tanjung Pura University, Indonesia (No. 119/A/LB/FMIPA/UNTAN/2021).

Technical-grade solvents were used for extraction and column chromatography after distillation. Silica gel 60 (Merck, 0.063–0.200 mm and 0.200–0.500 mm) was used for column chromatography (CC), whereas precoated silica gel 60 F254 (Merck) was used for thin-layer chromatography (TLC). The DPPH reagent was obtained from Wako (Japan). The reference standard of berberine chloride (purity ≥98.0%, batch No. SLCB1670) was provided by Sigma-Aldrich (St. Louis, MO, USA). Palmatine chloride (purity ≥98.0%, batch No. SLCM3523) was supplied from Merck (Darmstadt, Germany). HPLC-grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany), whereas trifluoroacetic acid (TFA) was acquired from Sigma-Aldrich (St. Louis, MO, USA).

In silico antidiabetic activity prediction, molecular docking was performed between alpha-glucosidase and dipeptidyl peptidase (DPP-IV) enzymes with palmatine, berberine, and their native ligands or standard compounds. The X-ray crystallographic structures of alpha-glucosidase (PDB ID: 3W37) and DPP-IV (PDB ID: 6B1E), bound with their co-crystallized ligands were obtained from the Protein Data Bank (https://www.rcsb.org/). The 3D structures of berberine and palmatine were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/).

Instrument

The UV-Vis spectra were measured using a Shimadzu UV-1800 ultraviolet-visible spectrophotometer (Shimadzu, Co., Ltd., Kyoto, Japan), and the IR spectra were recorded using a Perkin-Elmer Spectrum 100 FT-IR spectrometer (PerkinElmer, Inc., USA). Meanwhile, mass spectra (MS) were performed on a mass spectrometer Waters Acquity TQ detector using ESI + mode (Waters Corporation, Milford, MA, USA), and NMR spectra were recorded on a JEOL JNM ECZRS500 MHz spectrometer (JEOL, Japan) with TMS as an internal standard. HPLC analysis was performed on a Waters Alliance e2695 HPLC system (Waters Corporation, Milford, MA, USA) with column Merck LiChroCART (250 mm x 4.6 mm). The chromatograph was equipped with a UV-Vis 2489 detector (Waters Corporation, Milford, MA, USA). The antioxidant activity test was measured using Biochrom EZ Read 400 Plus Microplate Reader (Biochrom Ltd., Cambridge, UK).

Isolation Compound from Extract of Fibraurea tinctoria Lour

All dried samples were pulverized and passed through a 40-mesh sieve before extraction. The air-dried and powdered stems (1.00 kg) were extracted with MeOH at room temperature (4 × 4 L, 24 h each). Subsequently, the solvent was evaporated, and the MeOH extract (22.3 g) was obtained. The MeOH extract was then partitioned by silica gel column chromatography with stepwise elution of n-hexane, EtOAc, and MeOH with a gradient of 50%, successively to yield five fractions (Fr.1–5). The fraction obtained was then evaporated to obtain Fr.1 (0.3092 g), Fr.2 (1.5289 g), Fr.3 (2.2309 g), Fr.4 (9.5288 g) and Fr.5 (9.7322 g). Fr.5 was further separated on a silica gel column chromatography with EtOAc/MeOH at a gradient of 10% to provide eleven fractions (Fr.5.1–11). Fr.5.8 was chromatographed on silica gel with eluent using EtOAc/MeOH at a gradient of 5% to obtain twenty-five fractions (Fr.5.8.1–25). Fr.5.8.21 (0.0611 g) was acidified using 25 mL of 1% hydrochloric acid and then basified with ammonium hydroxide to a pH of 9.5–10. The alkaloids were then extracted with chloroform. The organic phase from Fr.5.8.21 was then subjected to further TLC testing using silica gel 60 F254 with EtOAc: MeOH (1:1, v/v) solvent and one drop of ammonia to obtain the compound 1 (7 mg).

Structure Determination of Isolated Compound

The chemical structure of the isolated compound was established based on spectroscopic data, such as Ultraviolet-Visible (UV-Vis), Infrared (IR), 1D-NMR (1H, 13C, DEPT 135), and 2D-NMR (HMQC, COSY, HMBC) plus Mass Spectroscopy (MS) data. The HMQC and DEPT 135 experiments helped to assign the 13C, while HMBC and COSY established the
connectivities of the molecular fragments. The spectra and structure elucidation analyses were further aided by comparing the observed and published $^1$H and $^{13}$C NMR data.

**Determination of Palmatine Contents**

Chromatographic analysis of palmatine was performed on a Waters Alliance system consisting of an e2695 separation module and a UV-Vis 2489 detector (Waters Corporation, Milford, MA, USA) and equipped with column Merck LiChroCART (250 mm x 4.6 mm). The mobile phase comprised 0.1% trifluoroacetic acid in water (solvent A) and acetonitrile (solvent B). The gradient elution was applied: 0–1 min, 20% B; 1–6 min, 20–30% B; 6–12 min, 30–40% B, 12–12.01 min, 20% B, 12.01–15 min 20% B. The eluent flow rate and injection volume were 1.0 mL/min and 10 µL, respectively. Detection for quantification was performed at 346 nm.

**Evaluation of the Antioxidant Activity**

The antioxidant activities of extract, fractions, and isolated compound was determined using the DPPH and non-enzymatic SOD mimic methods, following standardized protocols. Briefly, for the DPPH test, the sample solution and methanol were added to the microplate wells. DPPH solution was added to each sample, incubated for 30 min in the dark, and then measured at 517 nm. A blank solution containing DPPH in methanol was prepared. The absorbance value of each concentration variation was recorded, and the IC$_{50}$ value was calculated.$^{29}$ For the non-enzymatic SOD mimic method, sample and bioassay reagent solutions were prepared (the riboflavin solution was dissolved in EDTA and NBT solutions and then homogenized and diluted with phosphate buffer). Working solutions I (containing riboflavin) and II (without riboflavin) were prepared. The test solution was prepared in a microplate well and irradiated in a light box for 15 min. The measurements were performed at 550 nm.$^{30}$

**Molecular Docking Simulations**

The molecular docking method was used to investigate the prediction of antidiabetic activity in silico. Autodock 4.0 was the set of tools used. The Chimera program was used to separate the alpha-glucosidase and DPP-IV enzymes from their native ligands and stored in the PDB format. Using Autodock 4.0, alpha-glucosidase and DPP-IV free from native ligands were molecularly anchored to berberine and palmatine. To set the grid box and docking area, the receptors and ligands kept in pdbqt format were opened and stored in gpf format. The ligand-receptor binding that was achieved with the help of genetic algorithm parameters was recorded in the dpf format. Using the Autogrid4 and Autodock4 formulas, both files were docked and saved on the command prompt. The conformation with the lowest binding affinity was selected as the best conformation. The interactions between alpha-glucosidase and DPP-IV enzymes with berberine and palmatine were visualized using the BIOVIA Discovery Studio program.

**Results**

**Structural Determination of Isolated Compound**

Compound 1 was isolated as a yellow solid with a molecular formula of C$_{21}$H$_{24}$NO$_4$ as determined by MS (m/z 352.68 [M-H]$^+$. The IR spectrum showed the presence of H-C-$sp^2$ stretch (3344 cm$^{-1}$), H-C-$sp^3$ stretch (2849 and 2910 cm$^{-1}$), benzene stretch (1230 and 1278 cm$^{-1}$), -CO stretch (1103 cm$^{-1}$), and -CN stretch (1037 cm$^{-1}$). Further supporting data from the UV-Vis spectrum showed absorption at 225, 267, 348, and 429 nm together with IR absorption bands, indicating the presence of a diene group, a benzenoid band, an extension of the conjugation of benzene with diene, and the presence of an auxochrome group attached to benzene conjugated with diene, respectively.

The $^{13}$C NMR spectrum determined that there are twenty-one carbon signals consisting of four methyls (δC 55.3, 56.3, 55.7, and 61.2), two methylenes (δC 26.5 and 56.0), six methines (δC 108.5, 110.9, 119.9, 123.2, 126.6, and 145.0), and nine quaternary carbons (δC 119.1, 121.9, 128.7, 133.9, 138.4, 144.3, 149.5, 150.6, and 152.4). The $^1$H NMR spectrum showed a proton signal at δH 8.09 (1H, d, $J = 8.5$ Hz) and δH 8.00 (1H, d, $J = 9$ Hz). The coupling constant values of 8.5 and 9 Hz indicated the ortho position of the aromatic ring. Furthermore, there is a shift at δH 3.25 (2H, d, $J = 6.5$ Hz) and δH 4.91 (2H, d, $J = 7$ Hz), which indicates that the two protons are adjacent (vicinal hydrogens). The
other four protons are at δH 7.63 (1H, s), 7.02 (1H, s), 9.74 (1H, s), and 8.79 (1H, s), indicating a para position. In addition, one proton singlet at δH 9.74 indicated that the corresponding carbon (C-8) was directly attached to the quaternary nitrogen\(^{31}\) (Table 1).

The COSY spectrum showed correlation between H-1 at δH 7.63 (1H, s) with H-4 at δH 7.02 (1H, s), H-11 at δH 8.09 (1H, d, \(J = 8.5\) Hz) with H-12 at δH 8.00 (1H, d, \(J = 9\) Hz), H-5 at δH 3.25 (2H, d, \(J = 6.5\) Hz) with H-6 at δH 4.91 (2H, d, \(J = 7\) Hz). The position of 3-OCH\(_3\) was confirmed by the HMBC correlation between the proton at δH 4.08 (3H, s, 3-OCH\(_3\)) and the carbon at δC 150.6 (C-3). In addition, the position of 2-OCH\(_3\) was confirmed by the COSY correlation of the proton at δH 3.91 (3H, s, 2-OCH\(_3\)) with the proton at δH 4.08 (3H, s, 3-OCH\(_3\)), suggesting that the position of 2-OCH\(_3\) is adjacent to 3-OCH\(_3\). The position of 9-OCH\(_3\) was confirmed by the HMBC correlation between the proton at δH 4.19 (3H, s, 9-OCH\(_3\)) and the carbon at δC 144.3 (C-9). Moreover, the position of 10-OCH\(_3\) was confirmed by the COSY correlation of the proton at δH 3.97 (3H, s, 10-OCH\(_3\)) with the proton at δH 4.19 (3H, s, 9-OCH\(_3\)), which suggested that the position of 10-OCH\(_3\) is adjacent to 9-OCH\(_3\). Based on spectral data analysis and comparison with published papers,\(^{32}\) compound 1 was identified as palmatine, which has a molecular weight of 352 g/mol. The Key COSY and HMBC correlations of compound 1 are shown in Figure 2.

**Table 1** \(^1\)H and \(^{13}\)C NMR Spectroscopic Data of Compound 1 (CD\(_3\)OD; 500 Hz; δ in Ppm)

<table>
<thead>
<tr>
<th>Position</th>
<th>δ(^1)H (ΣH, mult, (J) in Hz)</th>
<th>δ(^{13})C, type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.63 (1H, s)</td>
<td>108.5 (CH)</td>
</tr>
<tr>
<td>2</td>
<td>–</td>
<td>149.5 (C)</td>
</tr>
<tr>
<td>3</td>
<td>–</td>
<td>150.6 (C)</td>
</tr>
<tr>
<td>4</td>
<td>7.02 (1H, s)</td>
<td>110.9 (CH)</td>
</tr>
<tr>
<td>4a</td>
<td>–</td>
<td>128.7 (C)</td>
</tr>
<tr>
<td>5</td>
<td>3.25 (2H, d, (J = 6.5) Hz)</td>
<td>26.5 (CH(_2))</td>
</tr>
<tr>
<td>6</td>
<td>4.91 (2H, d, (J = 7) Hz)</td>
<td>56.0 (CH(_2))</td>
</tr>
<tr>
<td>8</td>
<td>9.74 (1H, s)</td>
<td>145.0 (CH)</td>
</tr>
<tr>
<td>8a</td>
<td>–</td>
<td>121.9 (C)</td>
</tr>
<tr>
<td>9</td>
<td>–</td>
<td>144.3 (C)</td>
</tr>
<tr>
<td>10</td>
<td>–</td>
<td>152.4 (C)</td>
</tr>
<tr>
<td>11</td>
<td>8.09 (1H, d, (J = 8.5) Hz)</td>
<td>126.6 (CH)</td>
</tr>
<tr>
<td>12</td>
<td>8.00 (1H, d, (J = 9) Hz)</td>
<td>123.2 (CH)</td>
</tr>
<tr>
<td>12a</td>
<td>–</td>
<td>133.9 (C)</td>
</tr>
<tr>
<td>13</td>
<td>8.79 (1H, s)</td>
<td>119.9 (CH)</td>
</tr>
<tr>
<td>13a</td>
<td>–</td>
<td>138.4 (C)</td>
</tr>
<tr>
<td>13b</td>
<td>–</td>
<td>119.1 (C)</td>
</tr>
<tr>
<td>2-OCH(_3)</td>
<td>3.91 (3H, s)</td>
<td>55.3 (CH(_3))</td>
</tr>
<tr>
<td>3-OCH(_3)</td>
<td>4.08 (3H, s)</td>
<td>56.3 (CH(_3))</td>
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<tr>
<td>9-OCH(_3)</td>
<td>4.19 (3H, s)</td>
<td>61.2 (CH(_3))</td>
</tr>
<tr>
<td>10-OCH(_3)</td>
<td>3.97 (3H, s)</td>
<td>55.7 (CH(_3))</td>
</tr>
</tbody>
</table>
Determination of Palmatine Contents

HPLC analysis of palmatine using RP-C18 stationary phase and gradient elution with 0.1% trifluoroacetic acid in water (solvent A) and acetonitrile (solvent B). The absorbance at 346 nm was used to characterize the chromatogram and quantify palmatine content. The retention time of the standard palmatine chloride was 15.773 min, and the HPLC chromatogram is shown in Figure 3.

The formulas LOD = 3.3σ/S and LOQ = 10σ/S, where σ and S represent the standard deviation of the response and the slope of the calibration curve, respectively, were used to compute the limits of detection (LOD) and limit of quantification (LOQ). The lowest concentration of the analyte in a sample that can be detected using the developed method is known as the limit of detection. On the other hand, the lowest concentration that can be measured in the operating conditions is represented by the limit of quantification. Table 2 shows the palmatine retention time data, calculated calibration curve, linearity, LOD, and LOQ.

Table 2 Retention Time, Calibration Curves, Linearity, LOD, and LOQ HPLC Analysis for Palmatine

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention time</th>
<th>Calibration Curve</th>
<th>$r^2$</th>
<th>LOD (µg/mL)</th>
<th>LOQ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmatine</td>
<td>15.773</td>
<td>$y = 42890x + 743.9$</td>
<td>0.9998</td>
<td>0.1683</td>
<td>0.5101</td>
</tr>
</tbody>
</table>

Figure 2 Key $^1$H-$^1$H COSY and HMBC correlations in compound 1.

Figure 3 HPLC chromatogram of standard palmatine chloride at 8 µg/mL.
Afterwards, the stems of *Fibraurea tinctoria* Lour were subjected to extraction using methanol and fractionated using *n*-hexane, ethyl acetate, and methanol with a gradient of 50% to obtain five fractions (fractions 1–5). The extract and fractions were analyzed using HPLC to determine the palmatine content.

Quantification of palmatine compounds by HPLC in extract and fractions from the stems of *Fibraurea tinctoria* Lour showed that palmatine was found in the extract and all fractions. In the methanol extract, the palmatine content was 1.5398%. On the other hand, the palmatine content was determined to be a high amount at 2.8523% in fraction 5, and the lowest content of palmatine was detected in fraction 1 at 0.0027%, as seen in Table 3.

**Evaluation of the Antioxidant Activity**

The extract, fractions, and the isolated compound from *Fibraurea tinctoria* Lour were examined for their antioxidant activity using the DPPH and non-enzymatic SOD mimic (mSOD) methods, and Table 4 presents the results. A substance is categorized as a very strong (IC\(_{50}\) value <50 ppm), strong (IC\(_{50}\) value <50-100 ppm), moderate (IC\(_{50}\) value <101-150 ppm), and weak (IC\(_{50}\) value >150 ppm) antioxidant, according to Molyneux (2004). 34

In an in vitro antioxidant activity test using the DPPH method, fraction 4 showed the highest activity, with an IC\(_{50}\) value of 91 ppm. In contrast, using the mSOD method, the methanol extract and fraction 5 showed very strong antioxidant activity, with IC\(_{50}\) values of 18 and 20 ppm, respectively. The isolated compound (palmatine) showed very

### Table 3 Palmatine Contents in *Fibraurea tinctoria* Lour

<table>
<thead>
<tr>
<th>Samples</th>
<th>Yield (%)</th>
<th>MeOH extract</th>
<th>Fraction 1</th>
<th>Fraction 2</th>
<th>Fraction 3</th>
<th>Fraction 4</th>
<th>Fraction 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2.23</td>
<td>0.04</td>
<td>0.15</td>
<td>0.22</td>
<td>0.95</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.3983</td>
<td>0.0269</td>
<td>0.0343</td>
<td>0.0667</td>
<td>0.0756</td>
<td>28.5229</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5398</td>
<td>0.0027</td>
<td>0.0034</td>
<td>0.0067</td>
<td>0.0076</td>
<td>2.8523</td>
</tr>
</tbody>
</table>

### Table 4 Antioxidant Activity of *Fibraurea tinctoria* Lour

<table>
<thead>
<tr>
<th>Extract/Fractions/Compound</th>
<th>IC(_{50}) (ppm)</th>
<th>DPPH method</th>
<th>Non-enzymatic SOD mimic method</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH extract</td>
<td>113</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Fraction 1</td>
<td>46,057</td>
<td>&gt; 15,000</td>
<td></td>
</tr>
<tr>
<td>Fraction 2</td>
<td>1,113</td>
<td>545</td>
<td></td>
</tr>
<tr>
<td>Fraction 3</td>
<td>212</td>
<td>210</td>
<td></td>
</tr>
<tr>
<td>Fraction 4</td>
<td>91</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Fraction 5</td>
<td>516</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Compound 1 (palmatine)</td>
<td>1,835</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Berberine chloride (standard)</td>
<td>5,881</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Quercetin (standard)</td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>
strong antioxidant activity using the mSOD method, with an IC<sub>50</sub> value of 28 ppm. In contrast, the DPPH method showed very weak antioxidant activity, with an IC<sub>50</sub> value of 1,835 ppm.

In this study, berberine and quercetin were used as standards. Berberine is a well-known protoberberine alkaloid widely used in treating diabetes mellitus and is reported to have antioxidant activity. Meanwhile, quercetin is a major plant flavonoid known as a potent antioxidant. Berberine showed very strong antioxidant activity when measured using the mSOD method, with an IC<sub>50</sub> of 12 ppm, but showed very weak antioxidant activity using the DPPH method. In contrast, quercetin showed very strong antioxidant activity in the DPPH and mSOD methods, with IC<sub>50</sub> values of 4 and 5 ppm, respectively.

Molecular Docking Simulations

The prediction of antidiabetic activity was evaluated in silico using molecular docking. A docking study was performed to predict the mode of interaction between the identified compounds with alpha-glucosidase and DPP-IV enzymes. The binding interactions of palmatine were compared with those of berberine and its native ligands or standard compounds. The docking results of the compounds are presented in Table 5. The lower or more negative value of the binding energy indicates a higher binding affinity within the active site of the respective protein target.

In silico molecular docking of palmatine, berberine, and its native ligands or standard compounds (acarbose, vildagliptin, and sitagliptin) was performed on the active site of the alpha-glucosidase (PDB ID 3W37) and DPP-IV (PDB ID 6B1E) enzymes. The docking studies results of palmatine, berberine, and the standard for alpha-glucosidase (acarbose) showed binding affinity of −6.1, −6.8, and −8.0 kcal/mol, while those for DPP-IV (vildagliptin and sitagliptin) were −7.1, −7.4, −6.4, and −8.6, respectively.

Discussion

Diabetes mellitus is currently the most common health problem worldwide. Since oxidative stress is known to play a role in the pathogenesis of diabetes and its complications, antioxidant therapies have potential value in its treatment. Currently, research related to drug discovery in diabetes not only focuses on insulin-centric targets but also includes glucose-centric strategies, such as the antioxidant protection of beta cells. Most medicinal plants with antidiabetic properties possess antioxidant activity. Several studies have shown that antioxidants deliver promising results as a useful complementary therapeutic approach for the treatment of diabetes and for reducing complications.

*Fibraurea tinctoria* is a traditional medicine used by native tribes of Kalimantan to treat diabetes mellitus. Palmatine is one of the main bioactive compounds present in several representatives of different botanical genders, such as *Fibraurea* spp., *Coptis* spp., *Phellodendron* spp., *Corydalis* spp., *Berberis* spp., *Papaver* spp., *Enantia* spp., and others. Palmatine has been reported to have several pharmacological properties, including antidiabetic and antioxidant properties.

In this study, palmatine was isolated from natural sources using silica gel column chromatography, and its chemical structure was established based on spectral data, such as UV-Vis, IR, NMR, and MS, and compared with previously published papers. The analysis of palmatine content was performed using the HPLC method. No quantification of palmatine in *Fibraurea tinctoria* Lour

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<th>Receptor</th>
<th>Ligand</th>
<th>Binding energy (Kcal/mol)</th>
<th>Hydrogen bond</th>
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<td>Alpha-glucosidase enzyme</td>
<td>Acarbose</td>
<td>−8.0</td>
<td>Arg552, His626, Asp568, Asp232, Asp469</td>
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<td></td>
<td>Berberine</td>
<td>−6.8</td>
<td>Arg552, Lys506</td>
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<td></td>
<td>Palmitate</td>
<td>−6.1</td>
<td>Arg552, Asp568</td>
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<td>DPP-IV enzyme</td>
<td>Vildagliptin</td>
<td>−6.4</td>
<td>Tyr547, Ser630</td>
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<td></td>
<td>Sitagliptin</td>
<td>−8.6</td>
<td>Gln553, Tyr666, Glu206, Phe357</td>
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<td></td>
<td>Berberine</td>
<td>−7.4</td>
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has been performed to date. According to this study, the methanol extract from the stems of *Fibraurea tinctoria* Lour contained a significant quantity of palmatine (15.3983 mg/g). Determination of the palmatine content in fractions showed that as the polarity of the solvent increased, the palmatine content also increased. Among the five fractions, fraction 5 had the highest palmatine content (28.5229 mg/g). The results of this study indicate that the stem extract composition is based on alkaloids and that palmatine is one of the main constituents. A thorough HPLC-based quantification of palmatine and berberine from *Coptis chinensis* was published by Li et al.\(^{39}\) In their study, this plant was also found to contain protoberberine alkaloids, with palmatine and berberine contents of 16.2412 and 57.3066 mg/g, respectively. Based on this study, our results showed a slightly lower palmatine content than *Coptis chinensis*.

This study tested the antioxidant activity using DPPH and mSOD methods. The DPPH method is widely used to evaluate the antioxidant activity of a sample by examining its ability to remove the free radicals originating from DPPH. The single electron of the nitrogen atom in DPPH was reduced to the corresponding hydrazine by removing the hydrogen atom from the antioxidant.\(^ {40}\) Apart from the DPPH method, the mSOD method can be used to evaluate the antioxidant activity. To test mSOD activity in vitro, xanthine oxidase, which is usually used in enzymatic SOD testing as a source of superoxide anion species (O\(_2^-\)), was replaced with riboflavin via non-enzymatic photoreduction and nitroblue tetrazolium (NBT) as an indicator. The activity of mSOD was demonstrated by its ability to inhibit the reduction of active redox indicators by O\(_2^-\) species to obtain an IC\(_{50}\) value measured at \(\lambda_{\text{max}}\) 560 nm.\(^ {41}\)

The antioxidant activity of plants is closely related to their secondary metabolite contents. Phenolic compounds are the primary antioxidant-active compounds found in plants. This is because of their aromatic ring, which makes it possible to relocate and stabilize the unpaired electrons in their structure and facilitates the donation of electrons and hydrogen atoms from their hydroxyl groups.\(^ {42}\) However, in addition to phenolic compounds, other natural compounds with promising antioxidant properties, such as alkaloids, have received little attention.

Alkaloids are secondary metabolites containing one or more nitrogen atoms and are generally found in a heterocyclic ring structure. Previous studies have shown that the alkaloids have been studied as potential antioxidants. The antioxidant activity of alkaloids depends on their structures.\(^ {43,44}\) The high antioxidant activity of isoquinoline alkaloids is due to the presence of hydroxyl groups (donating hydrogen atoms) in the skeleton of the alkaloid compounds, the presence of N-containing groups, and the availability of H atoms. Furthermore, the group’s position and degree of methylation can affect the antiradical activity of these alkaloids.\(^ {45}\) Another study conducted by Yin et al showed that the presence of aryl hydroxyl/phenolic groups or vicinal triol systems in diterpenoid alkaloids is responsible for their antiradical activity.\(^ {46}\)

The antioxidant activity of aporphine alkaloids increases when the N-acetyl group at the N6 position is changed to an N-methylsulfonyl group,\(^ {47}\) whereas in non-phenolic aporphine, which does not have an -OH group, stabilization of the benzylic C-6a radical with a nitrogen lone pair strengthens its antioxidant properties.\(^ {48}\) The occurrence of double bonds, two secondary amines, and glucose residues in indole alkaloid structures is responsible for their ability to protect against ROS.\(^ {49}\) In imidazole alkaloids, the acid-base equilibrium plays an important role in the free radical scavenging activity. These deprotonated alkaloid species exhibited free radical-scavenging activity.\(^ {50}\) Research conducted by Yoon et al showed that quinoline alkaloids with two aromatic OH groups at positions 3 and 8 exhibit strong antioxidant activity. In contrast, the O-substituted quinolines at positions 3 and 4 showed very weak antioxidant activity.\(^ {51}\) Piperidine showed strong antioxidant activity owing to its oxidizable SH group.\(^ {52}\)

There have been conflicting findings from several previous investigations on the antioxidant capacity of palmatine. Research conducted by Chaves et al showed that palmatine isolated from *Guatteria friesiana* showed very strong activity against DPPH radicals, with an EC\(_{50}\) value of 3.48 μg/mL.\(^ {53}\) A similar research by Okechukwu et al demonstrated that palmatine obtained from *Coscinium fenestratum* could inhibit free radicals produced by DPPH.\(^ {54}\) In a study by Mridula et al, palmatine showed very strong antioxidant activity against DPPH radicals with an IC\(_{50}\) value of 45.4 μM.\(^ {27}\) In contrast, Jang et al revealed that palmatine isolated from *Coptis chinensis* exhibits weak or inactive antioxidant activity against DPPH radicals.\(^ {55}\)

Based on the in vitro antioxidant activity, the DPPH assay results showed that the extract and fractions of *Fibraurea tinctoria* Lour showed potential as antioxidants. On the other hand, the palmatine compound isolated from *Fibraurea tinctoria* Lour showed very weak or inactive antioxidant activity against DPPH radicals, with an IC\(_{50}\) value >1000 ppm. However, in this study, the isolated compound, palmatine, showed a higher IC\(_{50}\) value than the berberine standard.

In the mSOD method, the methanol extract, fraction 5, isolated compound (palmatine) from the stems of *Fibraurea tinctoria* Lour and the berberine standard showed very strong antioxidant activity, with IC\(_{50}\) values <50 ppm. Superoxide...
and palmatine showed good interactions with alpha-glucosidase, although their binding affinities were lower than that of high affinity is due to hydrogen bond interactions between the ligand and active site residues of the receptor. Both berberine and palmatine have the highest binding affinity value (−8.0 kcal/mol), followed by berberine (−6.8 kcal/mol) and palmatine (−6.1 kcal/mol). This indicates that they are effective in inhibiting the formation of advanced glycation end products (AGEs) that play a role in the pathological complications of diabetes mellitus.

In diabetes mellitus, there is a significant decrease in antioxidant enzyme activity and a significant increase in malondialdehyde (MDA), a lipid peroxidation product commonly known as a marker of oxidative stress. Palmatine has been reported to reduce MDA levels and increase enzymatic antioxidant activities. Palmatine (10 mg/kg for six weeks) reduced blood glucose levels and increased insulin levels in STZ-induced diabetic rats. These changes are related to reduced oxidative stress, which is characterized by reduced MDA levels and increased SOD enzyme activity. A similar study showed that palmatine at a dose of 2 mg/kg for 90 days improved oxidative stress in STZ-induced diabetes mellitus animal models, as indicated by reduced MDA levels and increased SOD, CAT, and GSH activities. Palmatine can activate proteins related to antioxidant activity, which protects cells from ROS and endoplasmic stress in STZ-induced diabetes mellitus animal models.

The strong antioxidant activity of palmatine inhibits the formation of free radicals, which play an important role in glucose auto-oxidation and non-enzymatic protein glycation, thereby weakening the antioxidant defense system and insulin resistance. In addition, palmatine shows the ability to reduce and inhibit lipid peroxidation and chelate metal ions, which are reported to be effective in inhibiting the formation of advanced glycation end products (AGEs) that play a role in the pathological complications of diabetes mellitus. Palmatine can react with the carbonyl groups of reducing sugars and dicarbonyl intermediate compounds, thereby blocking their conversion to AGEs. The anti-glycation activity of palmatine is attributed to its antioxidant properties and ability to capture reactive carbonyls. The ability of palmatine to react with carbonyls is the primary mechanism underlying the inhibition of protein glycation.

Palmatine stabilizes the free radicals via the nitrogen atom of the isoquinoline moiety. SAR studies of palmatine have shown that it contains positively charged nitrogen, which is structurally unstable. The presence of conjugate double bonds and the influence of the substituent on the benzene ring, namely methoxy, push electrons to the nitrogen atom to stabilize the charge. Apart from the resonance or mesomeric effect, the induction effect of the electron-pushing group, namely, the methoxy group, directs the phi electrons to move toward the nitrogen atom. This transfer of phi electrons can potentially cause homolytic bond breakage or the formation of nitrogen radicals. During homolytic bond breakage, one radical electron is donated to the ROS or RNS radical, thereby stabilizing the ROS/RNS radical. The palmatine radicals formed can stabilize themselves by the mesomeric effect and induction of the benzene ring and benzene substituents so that they are not harmful to the body.

The antidiabetic activity was evaluated in silico by molecular docking. This method is one of the most well-known and effective structure-based in silico methods for predicting the interactions between ligands and protein targets, similar to lock-and-key ideas. This process generally begins by predicting the molecular orientation of a ligand in a receptor and then estimating its complementarity using a scoring function. The benefit of using in silico approaches is that they can investigate target structures as potential active sites to provide candidate molecules with information regarding hydrogen interactions and binding affinities. Additionally, this makes the research cost-effective and time-efficient.

Two enzymes, alpha-glucosidase and DPP-IV, which are known to play significant roles in diabetes mellitus, were used in this study. Alpha-glucosidase, the main carbohydrate digestive enzyme, is found at the brush border of the small intestine. The conversion of starch and disaccharides to glucose is catalyzed by this enzyme. The inhibition of alpha-glucosidase slows carbohydrate digestion and prevents postprandial hyperglycemia, which is a major contributor to chronic diabetes-associated complications. DPP-IV enzyme rapidly degrades glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). This major hormone plays a significant role in maintaining blood glucose levels by promoting insulin secretion. DPP-IV inhibitors block GLP-1 and GIP degradation and thus play a significant role in regulating glucose homeostasis.

The molecular docking study on the alpha-glucosidase enzyme showed that the standard compound, acarbose, has the highest binding affinity value (−8.0 kcal/mol), followed by berberine (−6.8 kcal/mol) and palmatine (−6.1 kcal/mol). This high affinity is due to hydrogen bond interactions between the ligand and active site residues of the receptor. Both berberine and palmatine showed good interactions with alpha-glucosidase, although their binding affinities were lower than that of...
acarbose. Berberine forms two hydrogen interactions with the amino acid residues Arg552 and Lys506 (Figure 4B). The proton from Arg552 showed a hydrogen interaction with the ether oxygen of berberine at carbon number 2. Meanwhile, the proton from Lys506 showed a hydrogen interaction with the methoxy oxygen of berberine at carbon number 10. In addition,
two hydrophobic interactions were observed between Trp432 with the aromatic ring and aliphatic carbon. Similarly, palmatine formed two hydrogen interactions with the amino acid residues Arg552 and Asp568 (Figure 4C). The proton of Arg552 showed a hydrogen interaction with the methoxy oxygen of palmatine at carbon number 2. In addition, the proton of Asp568 interacted with the methoxy hydrogen of palmatine at carbon number 2. Furthermore, palmatine showed six hydrophobic interactions with Trp329, Trp432, Phe601, and Ala234. Three hydrophobic interactions formed between Trp432, Phe601, and Ala234 with aromatic rings; one hydrophobic interaction formed between Trp432 and the methoxy hydrogen on carbon number 3; and two hydrophobic interactions formed between Trp329 and Trp432 with aliphatic carbon number 5.

The standard compound, acarbose, showed seven hydrogen interactions with amino acid residues Arg552, His626, Asp568, Asp232, and Asp469 (Figure 4A). The oxygen in acarbose showed a hydrogen interaction with His626 of alpha-glucosidase. The proton of Arg552 showed two hydrogen interactions with the oxygen in acarbose on carbon numbers 2 and 3’. Two other hydrogen interactions were formed between the nitrogen amine and hydrogen hydroxy groups in acarbose with Asp232. Furthermore, two hydrogen interactions were formed between Asp568 and Asp469 with hydrogen amine and nitrogen amine, respectively. In addition, acarbose showed three hydrophobic interactions between Trp329, Trp432, and Phe601 with hydrogen from the aliphatic carbon.

The docking results with the DPP-IV enzyme showed that both berberine and palmatine have good interactions with the DPP-IV enzyme where the binding affinity values of berberine (~7.4 kcal/mol) and palmatine (~7.1 kcal/mol) are higher compared to standard compound, vildagliptin (~6.4 kcal/mol), but lower than sitagliptin (~8.6 kcal/mol). Berberine forms four hydrogen interactions with the amino acid residues Tyr585, Tyr547, Tyr662, and Gln553 (Figure 5C). The proton from Tyr585 showed a hydrogen interaction with the methoxy oxygen of berberine at carbon number 9. The proton from Tyr547 showed a hydrogen interaction with the ether oxygen of berberine at carbon number 2. Two other hydrogen interactions were formed between Tyr662 and Gln553 with the ether carbon and methoxy proton at carbon number 10, respectively. In addition to forming hydrogen interactions, berberine exhibits five hydrophobic interactions. Three hydrophobic interactions were formed between Phe357 and Tyr666 with the aromatic ring of berberine and two hydrophobic interactions were formed between Phe357 with hydrogen from the aliphatic carbon. Similarly, palmatine formed four hydrogen interactions with the amino acid residues Lys554, Tyr547, Cys551, and Tyr585 (Figure 5D). The proton from Lys554 showed a hydrogen interaction with the methoxy oxygen on carbon number 2. Meanwhile, three hydrogen interactions were formed between Tyr547, Cys551, and Tyr585 with the methoxy hydrogen on carbon numbers 9 and 10. Palmatine also exhibits four hydrophobic interactions. Two hydrophobic interactions were formed between Tyr547 with the aromatic ring of palmatine and two hydrophobic interactions were formed between Tyr547 with hydrogen from the aliphatic carbon.

Vildagliptin and sitagliptin were used as standard compounds. Sitagliptin exhibited higher binding affinity than vildagliptin. Vildagliptin showed two hydrogen interactions with amino acid residues Ser630 and Tyr547 (Figure 5A). The proton of Ser630 showed a hydrogen interaction with the carbonyl oxygen of vildagliptin and the oxygen of Tyr547 showed a hydrogen interaction with the amine proton of vildagliptin. In addition, vildagliptin showed three hydrophobic interactions between Tyr547, Trp629, and Tyr666 with aliphatic carbon. On the other hand, the other standard compound, sitagliptin, formed five hydrogen interactions with the amino acid residues Gln553, Tyr666, Glu206, and Phe357 (Figure 5B). Three hydrogen interactions were observed between Gln553 and Tyr666 with two fluorine groups. Meanwhile, two other hydrogen interactions were formed between Glu206 with an aliphatic carbon and Phe357 with an amine hydrogen. Additionally, three hydrophobic interactions were observed between Tyr547 with the phi electrons on the aromatic ring and the amide ring and Tyr666 with aliphatic carbon.

Several in vitro tests of palmatine against alpha-glucosidase have been performed and have shown different results. Research conducted by Tang et al showed that palmatine isolated from *Rhizoma coptidis* was found to possess potent alpha-glucosidase inhibitory (IC$_{50}$ 2.13 mg/mL) in relation to acarbose (IC$_{50}$ 4.98 mg/mL). In contrast, palmatine (IC$_{50}$ 9.39 µM) demonstrated lower inhibitory activity toward alpha-glucosidase compared with acarbose (IC$_{50}$ 1.31 µM). Likewise, the alpha-glucosidase inhibitory activity assay results showed that the inhibitory ability of berberine (IC$_{50}$ 0.511 g/L) was lower than that of acarbose (IC$_{50}$ 0.379 g/L). The in silico findings of this study strengthen the previously reported in vitro evaluation of alpha-glucosidase inhibitory activity. Theoretically, inhibitory ligands have the potential to deactivate enzymes by occupying their
Figure 5  (A) 2D and 3D images of docked conformations of DPP-IV enzyme and vildagliptin. (B) 2D and 3D images of docked conformations of DPP-IV enzyme and sitagliptin. (C) 2D and 3D images of docked conformations of DPP-IV enzyme and berberine. (D) 2D and 3D images of docked conformations of DPP-IV enzyme and palmatine.
active sites and hindering access to the active pocket.\textsuperscript{74} Palmitine and berberine were able to occupy the active pocket of alpha-glucosidase, although they were still unable to match the standard acarbose. Molecular docking of alpha-glucosidase revealed that palmitine has almost the same binding affinity as berberine, but palmitine has more hydrophobic interactions than berberine. Hydrophobic bonds are known to greatly contribute to the conformational stability of alpha-glucosidase, so they can be the main key in directing its pharmacological properties.\textsuperscript{75,76}

Furthermore, molecular docking of DPP-IV protein revealed that neither palmitine nor berberine could surpass sitagliptin as a standard. These docking results aligned with previously reported in vitro and in vivo evaluations of DPP-IV inhibitory activity, which showed that palmitine and berberine weakly inhibited DPP-IV compared with the standard drug sitagliptin.\textsuperscript{72,77,78} However, in this study, it was found that both palmitine and berberine had better binding affinities than the standard vildagliptin. Palmitine and berberine had more hydrogen and hydrophobic interactions than vildagliptin did. Vildagliptin is a potent, selective, competitive, and reversible DPP-IV inhibitor widely used in various countries. Vildagliptin blocks DPP-IV through substrate-like binding to the active site of the enzyme for an extended time, and compared with other DPP-IV inhibitors, only vildagliptin has been shown to block the inactivation of GLP-1 and GIP between meals and overnight.\textsuperscript{79,80} Vildagliptin has demonstrated efficacy as a single drug and has shown a synergistic effect, which can enhance efficacy and cause few adverse reactions when combined with other antidiabetic drugs or insulin.\textsuperscript{81–84}

Overall, molecular docking plays a key role in drug development, allowing researchers to understand the complex molecular interactions and design more effective and potent compounds. Although the results of this in silico study indicate that palmitine has not shown satisfactory results as an antidiabetic by molecular docking, this observation provides valuable insight into the potential of this compound. From this analysis, it can be concluded that further research using molecular dynamic simulations is needed to understand the accurate and comprehensive mechanisms and interactions between these compounds and their target proteins.

In addition, it is important to note that existing research does not specifically discuss the antioxidant potential of palmitine, especially from the \textit{Fibraurea tinctoria} Lour. Diabetes mellitus is closely associated with oxidative stress. Therefore, it is important to understand the potential of this compound not only as an antidiabetic agent but also as an antioxidant. Finally, the results obtained from this computational study can provide a solid foundation for future in vitro tests and will assist experimental researchers in validating the antidiabetic activity related to the antioxidant properties of these compounds.

**Conclusion**

In the present study, palmitine was successfully isolated from \textit{Fibraurea tinctoria} Lour. Analysis of the palmitine content showed that the stems of \textit{Fibraurea tinctoria} Lour contained a significant quantity of palmitine. In vitro antioxidant tests showed that \textit{Fibraurea tinctoria} Lour and its isolated compound (palmitine) possessed potent antioxidant activity. An in silico study showed that palmitine has potential as an antidiabetic agent by inhibiting alpha-glucosidase and DPP-IV enzymes. Considering that oxidative stress plays a key role in the pathogenesis of diabetes and its complications, the antioxidant properties of palmitine add value to its antidiabetic properties. Further research on phytochemicals and pharmacology is required to validate the use of this plant species in the treatment of various diseases, particularly diabetes mellitus.

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**Disclosure**

The authors report no conflicts of interest in this work.
References


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