Identification and Determination of Berberine from Arcangelisia Flava, East Borneo

Riski Sulistiarini

Pharmacy Faculty of Mulawaran University
Corresponding author: riski@farmasi.unmul.ac.id

Abstract

Berberine is a compound that has various benefits but also has dangerous toxic effects. In Indonesia, the Regulation of the Head of the Food and Drug Supervisory Agency No. 10 of 2014 concerning the Prohibition of Producing and Circulating Traditional Medicines and Health Supplements Containing Coptis Sp, Berberis Sp, Mahonia Sp, Chelidonium Majus, Phellodendron Sp, Arcangelica Flava, Tinosporae Radix, and Catharanthus Roseus. Regulation No. 7 of 2018 (BPOM, 2018) also prohibits the presence of berberine in processed food. This research was conducted to determine the content of berberine compounds from the extract and stem fraction of Arcangelisia flava. The research was conducted by identifying the content of berberine by TLC method compared with Rf Berberine sulfate and determination of berberine content by HPLC method (High-Performance Liquid Chromatography) using column C-18 (ODS). Berberine content of methanol extract, n-hexane fraction, ethyl acetate fraction and Arcangelisia Flava methanol-water fraction were 0.0040, respectively; 0.0010; 0.0041, 0.0044%.

Keywords: Berberine, TLC, HPLC, Arcangelica Flava

Submitted: 28 September 2021   Accepted: 25 December 2021   DOI: https://doi.org/10.25026/jtpc.v5i4.372

1 Introduction

Berberine is a plant alkaloid used in Ayurvedic and traditional Chinese medicine. Berberine can be found in Tinosporae Radix Chelidonium Majus, Phellodendron Sp, Hydrastis Canadensis [1], Coptis chinensis, Berberis aquifolium, Berberis vulgaris, Berberis aristata [2], Arcangelisia flava, Coscinium fenestratum, Acalypha Indica Linn [3], Scutellaria baicalensis [4] and several other plant species.

Berberine [18,5,6-dihydro -9,10-dimethoxybenzo(g)-1,3-benzodioxolo (5,6-a) quinolizinium], is a bright yellow crystalline form found in roots, stems, rhizomes, bark wood
and fruit. Currently, berberine is also commercially available in various salts such as berberine chloride and hemisulfate. The chemical structure of berberine can see in Figure 1.

Berberine exhibits significant antimicrobial activity against various organisms, including bacteria, viruses, fungi, protozoa, and worms. Berberine has also been reported to have hepatoprotective activity [6], neuroprotective [2], [7], pulmonary fibrosis [8], and the most popular is anti-DM type 2 activity. In DM patients [9]. In China, berberine is an over-the-counter drug for the treatment of bacterial diarrhea. In 1988, a hypoglycemic effect of berberine was reported, and currently, berberine has been prescribed to treat type 1 diabetes mellitus (type-1 diabetes). The mechanism of action of berberine in the treatment of type 1 DM is activating AMPK - Adenosine Monophosphate Protein Kinase, increasing insulin sensitivity, and stimulating pancreatic beta cell regeneration [10, p. 200]. In addition, information on the mechanism of action by repairing normal gastrointestinal flora [11] and regulation of PI3K/Akt and Nrf2 signaling [12] are the reasons for the mechanism of action of berberine in improving hyperglycemic conditions.

Berberine has also been reported to have antiparasitidal activity with the mechanism of action of inhibiting telomerase activity and in combination with pyrimethamine, showing better Plasmodium inhibition when compared to treatment using a combination of pyrimethamine and tetracycline or pyrimethamine and cotrimoxazole in patients with chloroquine-resistant malaria [13]. Berberine founds in the roots, rhizomes, and bark of plants Coptis sp., Berberis sp., Mahonia sp., Chelidonium Majus, Phellodendron sp., Arcangelisia Flava, in the roots of Tinosporae sp. [14] and on the stems of F. tinctoria and C. fenestratum [15]. On August 5, 2014, the Regulation of the National Food and Drug Agency No. 10 of 2014 concerning the Prohibition of Producing and Circulating Traditional Medicines and Health Supplements Containing Coptis Sp, Berberis Sp, Mahonia Sp, Chelidonium Majus, Phellodendron Sp, Arcangelica Flava, Tinosporae Radix, and Catharanthus Roseus. Regulation No. 7 of 2018 [16] also prohibits the presence of berberine in processed food. These regulations are a follow-up to the 2012 ASEAN TMHS Scientific Committee (ATSC) meeting on the negative list and restricted list of plants containing berberine and Catharanthus roseus for use in traditional medicines and health supplements in developing countries ASEAN members.

This prohibition based on a reasonably narrow therapeutic index and toxic levels of berberine. The LD50 of berberine was 1,280 and 520 mg/kg of extracts of Berberis vulgaris in rats and mice, respectively, for toxicity. Berberine sulfate isolated from Berberis aristata on intraperitoneal administration to rats had an LD50 value of 205 mg/kg. However, administration of 50 mg/kg Berberine sulfate caused diarrhea in 40% of rats which directly affected the gastrointestinal tract [17]. In cats, 100 mg/kg (oral) berberine caused vomiting within 6-8 hours and, at the same dose for 8-10 days, caused the death of all test animals. In cats, 50/100 mg/kg for ten days oral administration of berberine sulfate causes hemorrhagic inflammatory problems in the small and large intestines. Some mild symptoms of low amounts of berberine and its toxicity have been seen in dogs. These symptoms are drooling, nausea, diarrhea, vomiting, muscle tremors, and sometimes paralysis also appear in dogs [17]. Sub-acute toxicity of berberine indicates gastric ulcer [18], Freund-induced chronic arthritis, enlarged liver and kidneys, increased body weight (up to 30%) [19], decreased binding of bilirubin protein in adult mice [20]. [21] have reported several immunotoxic effects of
berberine. It was reported that 10 mg/kg administration of berberine was responsible for reducing the number of leukocytes, neutrophils, lymphocytes (blood cell count), and spleen weight. Significant reductions in the generation/differentiation of B and T cells and splenic CD19+ B cells, CD4+ and CD8+ T cells were also associated with berberine. In total, 5 mg/kg berberine was only responsible for influencing lymphocyte proliferation and delayed-type hypersensitivity responses, while 10 mg/kg berberine was responsible for suppressing cellular and humoral immune functions [17].

In East Borneo, the use of Arcangelisia Flava as an herb which is known as "bajakah kuning", has recently been widely used. With the many benefits of treatment and the potential for toxic effects, this study was conducted to analyze the levels of berberine in the extract and fraction of Arcangelisia flava.

2 Materials and Methods

2.1 Tools and Materials

In this study, simplicia preparation and extraction were first carried out. Furthermore, identification of berberine content was carried out using the thin layer chromatography method by comparing the Rf of berberine chloride with the Rf produced from selected plant fractions. In addition to using TLC, identification was also carried out using High-Performance Liquid Chromatography (HPLC) by analyzing the levels of berberine in the extract and its subsections.

The materials used were simplicia Arcangelisia Flava, TLC plate, organic solvent with HPLC standard, Milli-Q-water, and column C-18. The tools used in the process of preparing simplicia and extraction as well as fractions are cutting tools, dryers, grinders, distillers, reflux devices and rotary evaporators, and separating funnels. For the identification process, the tools needed are chromatographic column, TLC chamber, and HPLC Waters e2695 and 2489 UV/Vis instruments.

2.2 Identification of berberine by TLC

Identification was carried out using the TLC and HPLC methods. In the TLC method, the stains that emerged from all fractions were compared with the stains of the standard berberine HCl using ethyl acetate, n-hexane, methanol, and formic acid in a ratio of 6:4:0.2:2.

2.3 Identification of Berberine by HPLC

2.3.1 Preparation of extract stock solutions, fractions, and berberine standards

A total of 2 mg of extract, and the fraction dissolved in 5 ml of HPLC methanol with standard HPLC quality, then ultrasonicated and filtered with a 0.2 millipore filter.

A total of 25 mg of standard berberine HCl was dissolved in 25 ml of methanol with standard HPLC quality, then ultrasonicated and filtered using a 0.2 millipore filter. Variations of standard concentrations of berberine were made with concentrations of 1, 5, 10, 20, 40, and 80 ppm.

2.3.2 Mobile Phase and Stationary Suitability Test

The mobile phase adjustment method was carried out based on modifications from previous studies (Pasrija et al., 2010). The stationary phase used was C-18, while the mobile phase used was a mixture of 1.36 grams of potassium hydrogen phosphate dissolved in 1000 ml of Milli-Q water. The pH adjusted to 2.5 with the addition of phosphoric acid at a flow rate of 1 ml/min. with an injection volume of 10 L. each.

2.3.3 Berberine Standard Calibration Curve Actions

The stationary phase, mobile phase, and standard solution of berberine were prepared with various concentrations. Each standard solution of berberine HCl was injected into the HPLC column as much as 10 L at a flow rate of 1 ml/min. Detection was carried out at a wavelength of 346 nm. The repetition is done five times. From the measurement data, a calibration curve is made with the equation y = ax + b.

2.3.4 Determination of Berberine Levels

The extract and the three fractions that have been made were injected into the HPLC column as much as 10 L with a flow rate of 1
ml/min. Detection was carried out at a wavelength of 346 nm. The test results are recorded. The test data were analyzed by using the standard curve equation, with the Y value being the AUC of the sample and X being the concentration of berberine in the test sample. The concentration of the injected material was determined.

3 Results and Discussion

After the sample preparation and extraction process was carried out, the extract was ready to be tested. The extract and the three fractions obtained were then identified for the content of berberine using Thin Layer Chromatography (TLC). TLC results can be seen in Figure 2.

TLC results showed that the methanol extract, water-methanol fraction, and ethyl acetate fraction had stains parallel to the berberine stain with Rf 0.1.

These results were confirmed using HPLC with the C-18 stationary phase and phosphate-buffered mobile phase. Before the identification process is carried out, a validation process is carried out on the berberine standard. The results of the berberine calibration curve with an R-value of 0.99 can be seen in Figure 3.

Figure 2. Results of identification of berberine in subfraction using TLC. Eluent: n-hexane:ethylacetate:methanol (3:7:2). The order of stains left to right: 1. F. tinctoria methanol extract, 2. Methanol-water fraction, 3. n-hexane fraction, 4. Ethyl acetate, and 5. Berberine

Figure 3. Berberine HPLC calibration curve
From the calibration curve, the equation $y = 29947x - 10694$ was obtained. Subsequently, the berberine content was determined in the water-methanol, n-hexane, and ethyl acetate fractions of A. Flava. The results of the assay using HPLC can be seen in Table 1.

Table 1. Berberine content in the extract and fraction Arcangelis Flava

<table>
<thead>
<tr>
<th>Sample</th>
<th>Berberine (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol extract of A. Flava</td>
<td>0.0040</td>
</tr>
<tr>
<td>n-hexane fraction</td>
<td>0.0010</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>0.0041</td>
</tr>
<tr>
<td>Methanol-water fraction</td>
<td>0.0044</td>
</tr>
</tbody>
</table>

4 Conclusions

From the results of identification and assay, it is known that berberine is contained in the methanol extract and the n-hexane fraction, ethyl acetate fraction, and the methanol-water fraction Arcangelis Flava. Berberine content of methanol extract, n-hexane fraction, ethyl acetate fraction, and Arcangelis Flava methanol-water fraction were 0.0040, respectively; 0.0010; 0.0041, 0.0044%.

5 Acknowledgments

Author thanks to Pharmacy Faculty of Mulawarman University.

6 Conflicts of Interest

Author declare no conflict of interest.

7 References


[12] Y. Dou et al., ‘Oxyberberine, an absorbed metabolite of berberine, possess superior
Identification and Determination of Berberine from Arcangelisia Flava, East Borneo


