Health benefits of aqueous and ethanolic extracts of Medinilla speciosa blume

Florensa Rosani Purba¹,²,⁵, Ika Rahayu¹,³,⁶, Susana Elya Sudradjat¹,⁴, Kris Herawan Timotius¹,³,⁵

¹Research Center for Jamu and Herbal Medicine (JaHe), Indonesia
²Department of Informatics, Universitas Kristen Krida Wacana (UKRIDA), Indonesia
³Department of Biochemistry, Universitas Kristen Krida Wacana (UKRIDA), Indonesia
⁴Department of Pharmacology, Universitas Kristen Krida Wacana (UKRIDA), Indonesia
⁵Center for Biomedical Imaging and BioInformatics (CCBIB), Universitas Kristen Krida Wacana (UKRIDA), Indonesia

*Corresponding author: ika.rahayu@ukrida.ac.id

**ABSTRACT**

Medinilla speciosa (local name: parijoto) has many pharmacological benefits, including for treating female infertility. This study aimed to identify bioactive compounds of the aqueous and ethanolic extracts of Medinilla speciosa; to find information on their pharmacological benefits, and to do its docking profile with the protein phosphatase 1, which is associated with the enhancement of female fertility. Articles were searched from PubMed. The components of Medinilla speciosa were analyzed with LC-MS/MS. In silico study was conducted based PubChem, Protein Data Base, and Swiss ADME. Pyrx 0.8 and Discovery Studio Visualizer v21.1 were used to predict the interaction. Four flavonoids were identified, namely fisetin, robinetin, luteolin, and kaempferol. Except for robinetin, they exist in glycosidic form. One polyphenol, ellagic acid, was also identified. Literature studies showed they have various pharmacological benefits, such as antioxidants, anti-inflammation, anticancer, antidiabetes, organ protection, and antimicrobial. However, no information is available on its potential for fertility enhancement. Docking analysis showed that the bioactive compounds interact with the A and C chains of the catalytic domain of protein phosphatase 1 (PP1). Aqueous and ethanolic extracts of Medinilla speciosa possess fisetin, robinetin, luteolin, kaempferol, and ellagic acid that bind to catalytic chains of protein phosphatase 1.

Keywords: Parijoto, Fisetin, Luteolin, Kaempferol, Ellagic Acid, Fertility

Copyright © 2023, Purba et al. This is an open access article under the CC–BY-SA license.
INTRODUCTION

In Indonesia, mainly on Java Island and Bali, Medinilla speciosa (Melastomaceae) is an ornamental and medicinal plant. Traditionally, this plant is commended for various health care, such as fertility enhancement, diabetes, anticancer, diarrhea, mouth sores (sariawan), antiinflammation, anticancer, and antibacterial. Several species of Medinilla are traditionally used as various healthcare products. Its fruits and leaves are edible and good for female and male fertility enhancement (Wijayanti and Ardigurnita 2019). Ethnomedical uses of Medinilla related to several pharmacological benefits. M. speciosa, M. magnifica, and M. myriantha are known as parijoto in the valley of Colo village, Muria Mount, Kudus district, Central Java, Indonesia, as endemic plants. Parijoto is closely associated with the ethnic-majority area of Muria Mount. M. speciosa is a tropical plant used as traditional medicine by the community. It is well-known as an herbal medicine in this valley to overcome infertility (Sugiarti and Pujiastuti 2017). Generally, pregnant women consume parijoto after the gestational age enters five months and above. However, it can also be consumed from two to three months during pregnancy. The fruit and leaves have the potential to treat male fertility (Wijayanti and Ardigurnita 2019). Parijoto is also consumed to treat mouth sores (local term: sariawan) and antiinflammation (Sugiarti and Pujiastuti 2017), diarrhea, mouth sores, antiinflammation, anticancer, and anti-bacteria.

The aerial parts (fruits and leaves) of M. speciosa are supposed to have the potential to treat infertility. To predict this potential, in silico analysis can be applied. The detected bioactive compounds of M. speciosa extracts should be first identified. After identification, docking analysis can prove the characteristic of their interaction with protein phosphatase 1 (PP1) (Balakrishnan et al. 2019). This enzyme catalyzes dephosphorylation of eIF2α (eukaryotic translation initiation factor 2α). By inhibiting PP1, the phosphorylated eIF2α may continue its translation initiation. Balakrishnan, et al. (2019) reported that salubrinal, an eIF2α phosphatase inhibitor, can enhance eIF2α phosphorylation and improve fertility (Balakrishnan et al. 2019). Salubrinal can reduce ovarian immunoglobulin heavy chain binding protein (BiP) expression, rescue Pi3k/Akt signaling, and a doubling of primordial follicles. Salubrinal treatment can also normalize estrus cycle stage lengths. Salubrinal protects against primordial follicle loss, including suppressing the dephosphorylation of eIF2α, and that intervention significantly improves and extends ovarian function, fertility, and fecundity (Balakrishnan et al. 2019) (Choy et al. 2015).

Study on the bioactive compounds of M. speciosa is limited. Many bioactivity studies were conducted using plant extracts that were not followed with LC-MS/MS phytochemical analysis. This study aims first to identify major bioactive compounds of aqueous and ethanol extract of Medinilla speciosa with the help of LC-MS/MS. After identification, related articles were searched for their pharmacological benefits. Finally, molecular docking analysis was done to understand their interaction profiles with the protein phosphatase 1, an enzyme that is associated with female fertility.

RESEARCH METHODS

Research Design

This research is an experimental study, using natural materials to explore the content of active compounds and their benefits. LCMS was used for analyzing the content of active compounds. Molecular docking was conducted using Pyrx 0.8 and discovery studio v21.1. The data analysis was conducted descriptively.

Population and Samples

Medinilla speciosa (local name parijoto) was taken from Muria Mount and grown in the garden of the university (Figure 1); the plant was identified by one of the authors according to the
determination keys of (Maxwell 1978). *M. speciosa* thrives and grows wild on mountain slopes or in forests on high humid and humid soils on mountain slopes starting at an altitude of 700 to 2,300 meters above sea level. One of the most common locations of the plant is found on the slopes of the Muria Mount, Colo Village, Dawe District, Kudus Regency, Central Java.

![Image of Medinilla speciosa](image)

**Figure 1.** *Medinilla speciosa*

**Instruments**

The LC-MS/MS analysis was done using the Waters LCMS/MS-QTOF system. The Tof MSE operation mode was employed, featuring an Electrospray Ionization (ESI) source capable of both positive and negative ion modes. The column utilized for this purpose was a C18 column (specifically, the ACE HPLC Column with dimensions 25cm x 4.6mm).

**Procedures**

1. **Plant extraction**

   Aerial parts of the plant were cut and air-dried. Then, 50 grams of the air-dried materials were boiled with 500 mL of hot distilled water at 90°C for 15 min (aqueous extract). After boiling, the water was separated from the solid material by filtering. The filtrate was then used for further analysis. Another 50 grams of the dried sample were macerated with 500 mL absolute ethanol for the ethanolic extract for 24 hours. The filtered macerate was evaporated with a Rotary Evaporator. Then, the extract was filtered. The filtrate was used for further analysis.

2. **LC-MS/MS analysis**

   The LC-MS/MS analysis was done according to our previous method (Rahayu and Timotius 2022). LC-MS analysis was conducted using the Waters LCMS/MS-QTOF system. The mobile phases consisted of a solution containing 0.1% formic acid in acetonitrile and another with 0.1% formic acid in distilled water. These mobile phases were delivered at a 0.6 mL/min combined flow rate. To prepare the sample, 0.5 grams of the substance were dissolved in 10 mL of methanol and subjected to a 30-minute homogenization process in an ultrasonicator. The resulting suspension was then filtered through a 0.22 µm GHP/PTFE membrane filter. For injection into the LC-MS system, 10 µL of the filtered sample was utilized. The identification of active compounds within the samples via LC-MS/MS-QTOF was carried out using the UNIFI software. This software is equipped with a mass spectrum library containing data on natural active substances from the Waters database. By comparing the mass spectrum of compounds within the sample to those in the library, UNIFI software determined the presence of specific compounds. The identification criteria included a mass error of analyte less than or equal to 5 ppm, isotope matches MZ RMS 6, analyte intensity of 300, and one fraction with a break value less than 4 in the fragment elucidation system.

3. **Literature study**

   The searching step was carried out with the use of PubMed. The keywords were the names of the bioactive compounds detected by LC-MS/MS.
4. **In silico analysis**

We constructed models of the main predicted anti-targets PP1 using homologous modeling. Molecular docking studies were carried out with the obtained models. We performed molecular docking for all targets using AutoDock Vina, implemented in the PyRx 0.8 software package.

5. **Ligands preparation**

The bioactive compounds' structures was searched in the PubChem (https://pubchem.ncbi.nlm.nih.gov/compound). The compounds' basic structure (aglycone) was used in this docking. The chemical structures of Fisetin, Robinetin, Luteolin, Kaempferol, and Ellagic acid in 3D were acquired in sdf file format. Ligands were converted into the most stable structure energetically using energy minimization and then converted to pdbqt format using Open Babel in Pyrx.

6. **Protein preparation**

The three-dimensional crystal structure of the PP1 enzyme (4XPN) was loaded in the PDB format from the protein molecules data bank (http://www.rcsb.org). Prior to docking, the molecules of all the non-proteinaceous components were removed. Hydrogen atoms were added throughout the protein structure before molecular docking using Discovery Studio v21.1.

7. **Molecular docking procedure**

The prepared protein was converted to pdbqt file in Pyrx 0.8. The positioning of the active site was determined with Discovery Studio to ascertain the X, Y, and Z coordinates, which were subsequently employed to generate grid boxes during the docking procedure facilitated by Pyrx. The dimensions of were set manually and amounted along the X-axis – 40 Å, the Y-axis – 40 Å, and the Z-axis – 40 Å. These grid boxes effectively encompassed the entire active site of the protein structure, exploring potential protein-ligand interactions. All docking simulations were executed through Pyrx 0.8. Discovery Studio Visualizer v21.1 was employed to visualize the anchored complex structure fully (Chen et al. 2015; Le Donne et al. 2017).

8. **Molsoft and Swiss ADME**

Molsoft and Swiss ADME were used to find necessary data for Lipinski rule of five, drug likeness, drug solubility, and binding affinity of the bioactive compounds.

### Data Analysis

Data analysis is conducted descriptively with the aim of providing a clear overview of the data without delving into deeper statistical inferences. We collected data from LCMS, which consisted of active compounds, and then grouped these active compounds based on their active compound groups. The analysis results are succinctly summarized in a table. Similarly, with molecular docking results, the categorization of molecular interaction types is performed to conclude the data processing outcomes.

### RESULTS

Five bioactive compounds were identified in the aqueous and ethanolic extracts of the aerial part of Medinilla speciosa. Four compounds (aglycon) were fisetin, robinetin, luteolin, and kaempferol except for robinetin, which was found in their glucosidic forms. In addition, a polyphenolic compound was found, namely ellagic acid (Figure 2, Table 1). Aqueous extract has a different composition compared with the ethanolic extract. Fisetin, robinetin, and luteolin exist in aqueous extract. Meanwhile, kaempferol and ellagic acid exist in ethanolic extract.

Information on the health benefits of the major bioactive compounds was obtained from various relevant published articles. Based on previous studies on the bioactive compounds from plant species, most of the compounds have antioxidant, antiinflammation, anticancer,
antimicrobial and antivirus, antidiabetic, and protective effects on cardiac, neuro, gastro, and liver. However, no information is available on their ability to enhance fertility (Table 1).

Table 1. Major components in aqueous and ethanol extracts of Medinilla speciosa

<table>
<thead>
<tr>
<th>Compound</th>
<th>A.E.</th>
<th>EtOH</th>
<th>Reported health benefits from other studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luteolin-7-O-glucoside</td>
<td>+</td>
<td>-</td>
<td>Antioxidant (Sarikurkcu et al. 2020) and antiinflammation (Aziz, Kim, and Cho 2018) (Francisco et al. 2014) (Nabavi et al. 2015) Neuroprotection (Nabavi et al. 2015), protects dopaminergic neurons (Qin et al. 2019)</td>
</tr>
<tr>
<td>Robinetin</td>
<td>+</td>
<td>-</td>
<td>Antinflammation (Germanò et al. 2015) Anthypertension (Asif et al. 2021) Antiangiogenic effects (Germanò et al. 2015) Antimicrobia (Cushnie and Lamb 2005)</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>-</td>
<td>+</td>
<td>Antioxidant (Bharathi and Jagadeesan 2014) (Hwang et al. 2014)</td>
</tr>
</tbody>
</table>

Note: A.E.: aqueous

**Table 2.** Pharmacokinetic properties (ADME) of the identified bioactive compounds in Medinilla speciosa

<table>
<thead>
<tr>
<th>Compound</th>
<th>TPSA</th>
<th>Water solubility</th>
<th>Drug likeness Score</th>
<th>Binding activity (kcal/mol)</th>
<th>Molecular Weight (g/mol)</th>
<th>NHBa ≤10</th>
<th>NHBd ≤ 5</th>
<th>Consensus LogP ≤ 5</th>
<th>MR 40–130</th>
<th>Lipinski’s Rules Violation ≤ 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fisetin: 5,7,8,2′-Tetrahydroxyflavone-7-O-β-D-glucoside</td>
<td>111.13</td>
<td>Soluble</td>
<td>Soluble</td>
<td>0.46</td>
<td>-10.1</td>
<td>286.24</td>
<td>6</td>
<td>4</td>
<td>1.5</td>
<td>76.01</td>
</tr>
<tr>
<td>Luteolin: Luteolin-7-O-glucoside</td>
<td>111.13</td>
<td>Soluble</td>
<td>Moderate</td>
<td>0.38</td>
<td>-7.8</td>
<td>286.24</td>
<td>6</td>
<td>4</td>
<td>1.73</td>
<td>76.01</td>
</tr>
<tr>
<td>Robinetin</td>
<td>131.36</td>
<td>Soluble</td>
<td>Soluble</td>
<td>0.15</td>
<td>-7.9</td>
<td>302.24</td>
<td>7</td>
<td>5</td>
<td>1.12</td>
<td>78.03</td>
</tr>
<tr>
<td>Kaempferol: 7-O-α-L-Rhamnosyl-3-O-β-D-glucopyranoside</td>
<td>111.13</td>
<td>Soluble</td>
<td>Soluble</td>
<td>0.5</td>
<td>-7.7</td>
<td>286.24</td>
<td>6</td>
<td>4</td>
<td>1.58</td>
<td>76.01</td>
</tr>
</tbody>
</table>
**Table 3.** Bound amino acids residue at A and C chain catalytic subunit of PP1

<table>
<thead>
<tr>
<th>Bonding type</th>
<th>Ligands</th>
<th>Chain A</th>
<th>Chain C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fisetin</td>
<td>Luteolin</td>
<td>Robinetin</td>
</tr>
<tr>
<td>Conventional hydrogen bond</td>
<td>SerA177</td>
<td>AspA64</td>
<td>AspA179</td>
</tr>
<tr>
<td></td>
<td>LeuA180</td>
<td>AspA92</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GlnA181</td>
<td>AsnA124</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LysA234</td>
<td>HisA125</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ArgA221</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>HisA248</td>
<td></td>
</tr>
<tr>
<td>Van der Waals</td>
<td>LeuA176</td>
<td>HisA66</td>
<td>ArgA188</td>
</tr>
<tr>
<td></td>
<td>ProA178</td>
<td>ArgA96</td>
<td>GlnA185</td>
</tr>
<tr>
<td></td>
<td>AspA179</td>
<td>HisA173</td>
<td>GlnA198</td>
</tr>
<tr>
<td></td>
<td>ValA231</td>
<td>TrpA206</td>
<td>LeuA200</td>
</tr>
<tr>
<td></td>
<td>PheA235</td>
<td>GlyA249</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PheA267</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TyrA272</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbon hydrogen</td>
<td>None</td>
<td>None</td>
<td>none</td>
</tr>
<tr>
<td>Pi-PI Alkyl</td>
<td>None</td>
<td>PheA276</td>
<td>LysA98</td>
</tr>
</tbody>
</table>

10.31932/jpbio.v8i2.2800 Purba et al jurnaljpbio@gmail.com
<table>
<thead>
<tr>
<th>IleC130</th>
<th>TrpC206</th>
</tr>
</thead>
<tbody>
<tr>
<td>TyrC134</td>
<td>None</td>
</tr>
<tr>
<td>TyrC272</td>
<td>None</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Carbon hydrogen</th>
<th>TyrC134</th>
<th>None</th>
<th>None</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>TyrC272</td>
<td>None</td>
<td>None</td>
<td>LysC98</td>
</tr>
</tbody>
</table>

**Figure 2.** LC-MS/MS chromatograms of the aqueous and ethanolic extracts of *M.speciosa*

**Figure 3.** Chemical structure of the bioactive compounds from *M.speciosa*
DISCUSSION

Four flavonoids are major bioactive compounds of aqueous and ethanolic extracts from the aerial part of Medinilla speciosa. They are fisetin, robinetin, luteolin, kaempferol and ellagic acid, and for the first time reported in this study (Figure 2, Figure 3 and Table 2). The last three compounds, luteolin and kaempferol (Diaz Sanchez 2017; Han et al. 2019), and ellagic acids (Hanh Nguyen et al. 2020) are found in Medinilla septentrionalis. However, the use of flavonoids medicinal agents is limited due to their poor stability, apparently limiting their bioavailability. The sugar moiety or glycosidic form has an important role in determining the absorption and bioavailability of flavonoids. Glycosylation of flavonoids is considered an effective method to rise their solubility and stability in water. Therefore, the presence of flavonoid glucosides in the extract is very beneficial (Hanh Nguyen et al. 2020).

The five bioactive compounds that exist in the extracts of M. speciosa have many pharmacological benefits (Table 1). Most pharmacological studies of the experimental activities have been carried out in vitro and/or in vivo with experimental animals. However, limited report is available on the clinical studies. The in vitro and in vivo experiments are covering beneficial for human health, such as antioxidative, anticarcinogenic, antiallergenic, antiinflammatory, antibacterial, antidiabetes, antimutagenic, and antitumor activities (Pertiwi et al. 2019; Tusanti, Johan, and Kisdjamiatun 2014; Sa’adah, Nurhayati, and Purwani 2018). From the best of our knowledge, there is no information available on the role of M. speciosa on the enhancement of fertility. Further research activities are needed to confirm the above mentioned benefits.

It is necessary to know whether bioactive compounds of M.speciosa have role in the enhancement female fertility and pregnancy health (Wijayanti and Ardigurnita 2019). In this study, inhibition of protein phosphatase 1 (PP1) was used as a model for this in silico study may help us to predict the strength of bioactive compounds. Table 2 shows that Fisetin has the highest binding affinity energy compared to other active compounds (-10.1 kcal/mol). The compound appears to have the potential to inhibit PP1 activity. Table 3 shows that fisetin binds to amino acid residues in the domain C of PP1 through Conventional hydrogen, Van der Waals, and Pi-Pi alkyl interactions. Ribonetin and ellagic acid bind to amino acid residues in domain A of PP1 through Conventional hydrogen, van der waals and Pi-pi alkyl. Only luteolin is bound to the amino acid residue of the two domain (A and C).

ADME properties were assessed using swissADME to evaluate the molecules’ solubility, bioavailability, and their potential to function as effective drugs. The analysis indicated that fisetin and three other compounds met the criteria of Lipinski rule without any violation and fell within an acceptable range concerning its bioavailability (Table 2). This compound has the ability to be absorbed and distributed within the body based on Lipinski’s rule. The Lipinski rule sets specific limits for factors such as molecular weight (MW), the number of hydrogen bond acceptors and donors (HBA and HBD), and the water/octanol partition coefficient (log P). It was determined that compounds failing to meet two or more of these criteria are likely candidates for exclusion from further development. To achieve adequate drug absorption and distribution within the body, according to Lipinski’s rule, the following criteria must be met: a maximum of 5 HBD, a molecular weight not exceeding 500 Da, a log P value no greater than 5, and a maximum of 10 HBA (Lipinski et al. 1997).

Unfortunately, using salubrinal, the known PPI inhibitor(Balakrishnan et al. 2019), control in docking process was not workable due to its three-dimensional (3D) conformer of salubrinal can not be generated. Salubrinal does not follow the criteria of 3D generation. Overall, this study shows that aqueous and ethanolic extracts from aerial part of M.speciosa can be predicted as suppressors of the de-phosphorylation of eIF2α, and that intervention in this way significantly improves and extends ovarian function, fertility, and fecundity.
CONCLUSION

The bioactive compounds of the aqueous and ethanolic extracts of Medinilla speciosa are flavonoids (fisetin, robinetin, luteolin, and kaempferol) and polyphenol (ellagic acid). These bioactive compounds have various pharmacological benefits, like antioxidant, antiinflammation, anticancer, antimicrobial, antidiabetes, and organs-protection. An in silico study predicted these bioactive compounds have the potential to inhibit protein phosphatase (PP1), an ovarium-key enzyme in fertility that is responsible for the dephosphorylation of eIF2α. These findings illustrate that aqueous and ethanolic extracts from M. speciosa are a promising therapeutic agent for the treatment of infertility. Therefore, the ethanol extract of M.speciosa is suitable anti infertility agent.

ACKNOWLEDGMENT

No external financial support. The authors thank Universitas Kristen Krida Wacana (UKRIDA) for the support and facilitation.

REFERENCES


Medinilla speciosa.

etches anti-

on. Ellagic acid, a phenolic compound, ex-

(1),

(2),


