# Interaction Prediction of Compounds Contained in Eleutherine Palmifolia with Serotonin, Norepinephrine, and BDNF Receptors by Computation

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**ABSTRACT**

This study aims: to know the active compounds of Eleutherine palmifolia, and to predict the interaction between those compounds with serotonin, norepinephrine, and BDNF receptors by computation (in silico). Eleutherine palmifolia ethanolic extract was analyzed using LCMS at State Polytechnic of Malang. Proteins and ligands were prepared by Discovery Studio Client 3.5 and PyRx 0.8. Dockings were done using HEX 8.0, then interactions of proteins and ligands and energy bindings were analysed by Discovery Studio Client 3.5. Eleutherine palmifolia extract contains three dominant compounds, which are eleutherine, eleutherole, dan quercetin. These three compounds have a tendency to bind to 5HT1A, 5HT1B, 5HT1D, α2, and trkB receptors. The smallest energy binding is between 5HT1B and quercetin (-283.91 kJ / mol). The smallest average energy binding is at 5HT1B (-261.58 kJ / mol), while the largest is 5HT1A (-78.4 kJ / mol).

**Keywords:** Eleutherine palmifolia, serotonin, norepinephrine, BDNF.

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## INTRODUCTION

Major depression disorder has the greatest lifetime prevalence compared to other psychiatric disorders, which is around 5-17% [1][2]. Given the high prevalence, comorbidity and losses due to depression, it is not surprising that Global Burden of Disease research by World Health Organization placed depression as the most burdensome disorder worldwide [2][3][4]. Serotonin and norepinephrine are the two neurotransmitters most implicated in the pathophysiology of depression [1].

Neuroplasticity aspect is one of the etiological factors of depression. Neuronal damages and structural changes in the hippocampus is one of the causes of depression [5]. Decreased brain-derived neurotrophic factor (BDNF) can contribute to neuronal damage and decreased hippocampal volume [5][6][7]. Serum BDNF levels decrease in depressed individuals [5][6][8][9].

St. John’s wort (Hypericum perforatum) is one of the plants that has been used as herbal therapy in depression [10][11]. One of the main components of St. John’s wort is quercetin, also found in onions [11]. Eleutherine palmifolia grow in Kalimantan forests and are used by local residents for generations as multifunctional medicinal plants [12][13][14][15]. Its bulbs contain napthoquinones (elecanamine, eleutherine, eleutherenone), are known as antioxidants [13]. Eleutherine palmifolia also contains triterpenoids, quinones, alkaloids, steroids, tannins, saponins, glycosides, phenolics and flavonoids [12][13][15][16]. Flavonoids have neuroprotective and therapeutic effects on cell culture and animal model neurodegenerative diseases [11]. Research needs to be done about the interaction of compounds contained in E. palmifolia, especially quercetin, with serotonin, norepinephrine, and BDNF receptors.

Eleutherine palmifolia were obtained from Palangkaraya, Central Kalimantan, Indonesia. Plant identification was carried out at Plant Conservation Center of Purwodadi Botanical Garden – LIPI. Bulbs extraction is based on the protocol in Pharmacology Laboratory of Faculty of Medicine, Brawijaya University. Eleutherine palmifolia extract was dissolved in methanol, then analyzed using TSQ Quantum Access Max Liquid Chromatography Mass Spectrometry (LCMS) from Thermo Fisher Scientific at the Chemical Engineering Laboratory, State Polytechnic of Malang, Indonesia.

The protein structure of receptors were obtained from PDB and SWISS MODEL. The 5HT1B structure (PDB ID: 5V54) is obtained from PDB. The structures of 5HT1A (Uniprot IDP09327), 5HT1D (Uniprot IDQ61224), norepinephrin α2 receptor (Uniprot IDQ0138), and tropomyosin-related kinase B (trkB, Uniprot IDW8YV28) were obtained from the SWISS MODEL. Ligands were downloaded in 3D SDF format from PubChem. The compounds used were eleutherine (CID10166), eleutherele (CID120697), and quercetin (CID5280343). Protein preparation was carried out using Discovery Studio Client 3.5 to remove water molecules and ligands. Ligand preparation was done using PyRx 0.8 software by minimizing energy and changing the file format to .pdb [17].

Dockings were done using HEX 8.0, then interactions were analyzed by Discovery Studio Client 3.5. Data analyzed were energy binding, using HEX 8.0. Interaction data between proteins and ligands were analyzed using Discovery Studio Client 3.5 and PyRx 0.8. Dockings were done using HEX 8.0, then interactions of proteins and ligands and energy bindings were analyzed by Discovery Studio Client 3.5.

## MATERIAL AND METHODS

**RESULTS**
3.1 Examination of the active compounds of *E. palmifolia* extract

The analysis of the active compounds of *E. palmifolia* extract using LCMS find that there are 3 dominant compounds, which are eleutherine, eleutherole, and quercetin. Chromatograms of these compounds are showed in Figure 1. Reference data of those compounds are obtained from PubChem [19] and listed in Table 1.

### Table 1: Examination results of *E. palmifolia* extract using LCMS

<table>
<thead>
<tr>
<th>Peak</th>
<th>Retention time (minutes)</th>
<th>Molecular weight (g/mol)</th>
<th>Compound name</th>
<th>Molecular formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.14</td>
<td>272.29</td>
<td>Eleutherine</td>
<td>C_{16}H_{16}O_{4}</td>
</tr>
<tr>
<td>2</td>
<td>6.03</td>
<td>244.24</td>
<td>Eleutherole</td>
<td>C_{14}H_{12}O_{4}</td>
</tr>
<tr>
<td>3</td>
<td>6.01</td>
<td>302.3</td>
<td>Quercetin</td>
<td>C_{15}H_{16}O_{7}</td>
</tr>
<tr>
<td>Compound</td>
<td>Molecular Weight</td>
<td>Molecular Formula</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>------------------</td>
<td>-------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quercetin 3,4'-diglucoside</td>
<td>626.5</td>
<td>C_{27}H_{30}O_{17}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quercetin 7-O-beta-D-glucoside</td>
<td>464.4</td>
<td>C_{21}H_{20}O_{12}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quercetin 4'-galactoside</td>
<td>450.3</td>
<td>C_{20}H_{18}O_{12}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rutin (quercetin 3-rutinoside)</td>
<td>610.5</td>
<td>C_{27}H_{30}O_{16}</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig 1: Chromatogram of LCMS. Eleutherine at RT 6.14, m/z 272.5-273.5 (top figure). Eleutherole at RT 6.03, m/z 244.5-245.5 (middle figure). Quercetin at RT 5.96, m/z 302.5-303.5 (bottom figure)
3.2 Prediction of interaction between active compounds of *E. palmifolia* extract and serotonin, norepinephrine, and BDNF receptors

Receptor model validation was using Ramachandran plot analysis. Figure 2 shows the validation of 5HT1A, 5HT1, a2 receptors, and trkB before docking. The results show that the distribution of amino acid residues are most abundant in favored regions in the Ramachandran plot. These indicate that the receptors models have stereochemical qualities for structural analysis and validates the efficacy of protein-ligand bindings [18].

Fig 2: Ramachandran plot analysis. Residues in favored region: 5HT1A 94.8% (top left figure), 5HT1D 96.9% (top right figure), a2 92.7% (bottom left figure), trkB 94.5% (bottom right figure).

Energy bindings between serotonin, norepinephrine, and BDNF receptors and eleutherine, eleutherol and quercetin by computation are listed in Table 2. The smallest energy binding is 5HT1B-quercetin (-283.91 kJ/mol). Receptor that has the smallest average energy binding is 5HT1B (-261.58 kJ/mol), while the largest is 5HT1A (-78.4 kJ/mol).
In silico analysis of protein-ligand interactions find that eleutherine, eleutherol and quercetin have tendencies to bind to serotonin receptors 5HT1A, 5HT1B, 5HT1D, norepinephrine α2 receptors, and BDNF trkB receptors. Eleutherine, eleutherol and quercetin tend to bind more easily to 5HT1B, 5HT1D, α2, and trkB receptors than 5HT1A. Protein-ligand interactions between 5HT1B and these compounds are shown in Figure 3.

**Table 2:** Energy bindings between serotonin, norepinephrine, and BDNF receptors and eleutherine, eleutherol and quercetin by computation.

<table>
<thead>
<tr>
<th>Protein – ligand</th>
<th>Energy binding (kJ/mol)</th>
<th>Means of energy binding (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5HT1A – eleutherine</td>
<td>-80.3</td>
<td></td>
</tr>
<tr>
<td>5HT1A – eleutherol</td>
<td>-67.5</td>
<td>-78.40</td>
</tr>
<tr>
<td>5HT1A – quercetin</td>
<td>-87.4</td>
<td></td>
</tr>
<tr>
<td>5HT1B – eleutherine</td>
<td>-264.06</td>
<td>-261.58</td>
</tr>
<tr>
<td>5HT1B – eleutherol</td>
<td>-236.78</td>
<td></td>
</tr>
<tr>
<td>5HT1B – quercetin</td>
<td>-283.91</td>
<td></td>
</tr>
<tr>
<td>5HT1D – eleutherine</td>
<td>-224.23</td>
<td>-222.42</td>
</tr>
<tr>
<td>5HT1D – eleutherol</td>
<td>-214.11</td>
<td></td>
</tr>
<tr>
<td>5HT1D – quercetin</td>
<td>-228.92</td>
<td></td>
</tr>
<tr>
<td>α2 – eleutherine</td>
<td>-215.02</td>
<td></td>
</tr>
<tr>
<td>α2 – eleutherol</td>
<td>-201.74</td>
<td>-213.75</td>
</tr>
<tr>
<td>α2 – quercetin</td>
<td>-224.50</td>
<td></td>
</tr>
<tr>
<td>trkB – quercetin</td>
<td>-247.21</td>
<td>-247.21</td>
</tr>
</tbody>
</table>
DISCUSSION

Eleutherine palmifolia contains naphtoquinones (eleutherine, eleutherole) which are known as antioxidants [13]. Eleutherine palmifolia also contains flavonoids [12][13][15][16]. Flavonoids are known as powerful antioxidants and can be turned into more active antioxidants [12][13]. Some studies have also found neuroprotective and therapeutic effects on cell cultures and animals model for neurodegenerative diseases [11].

Quercetin is one of the most studied polyphenolic flavonoids in onions. Low doses of quercetin reduce oxidative damage in neuron culture, but high doses of it damage neurons [11]. This research finds quercetin on the extract of E. palmifolia bulbs, both in a single form or bound to other compounds such as glucoside.

Protein-ligand interactions analysis in silico find that eleutherine, eleutherole and quercetin have tendencies to bind to serotonin receptors 5HT1A, 5HT1B, 5HT1D, norepinephrine α2 receptors, and BDNF receptors trkB. Activation of the trkB will induce dendrite formation, thereby increasing and maintaining the survival of serotonergic neurons [9].

Serotonin receptors 5HT1A, 5HT1B/D are postsynaptic receptors that regulate various neuronal pathways. The 5HT1A receptor inhibits pyramidal neurons in the cortex and regulates hormones, cognition, anxiety and depression. Norepinephrine α2 receptors are present in postsynaptic and presynaptic [20]. Binding with the postsynaptic receptor of monoamine neurotransmitter will trigger some processes in the cell membrane and intracellular [8]. Receptors in cell membranes interact with the intracellular environment via protein G. Protein G is associated with various intracellular enzymes, such as adenylate cyclase, phospholipase C, and phosphodiesterase. These enzymes regulate energy use and form 2nd messengers, such as cyclic nucleotides (e.g cyclic adenosine monophosphate, cyclic guanosine monophosphate), phosphatidilinositol (e.g inositol triphosphate, diacylglycerol) and calcium-calmodulin [1][8].

Phosphorylase enzymes, such as protein kinase A, protein kinase C, and calcium-calmodulin-dependent protein kinase are the 3rd messengers that regulate genetic expression and phosphorylation of transcription factors (4th messenger) for the synthesis of proenkephaline, BDNF, neurotransin, or tyrosine hydroxylase [8].

This study find that the 3 main compounds of E. palmifolia have tendencies to bind to serotonin, norepinephrine, and BDNF receptors. This shows the potency of E. palmifolia to be herbal adjunctive therapy in depression. The smallest energy binding between the protein-ligand is 5HT1B-quercetin (~83.91 kJ/mol). Quercetin also has smaller energies binding than eleutherine and eleutherole at the 5HT1A, 5HT1D, α2, and trkB receptors. This indicates the early potential of quercetin as an antidepressant. Further investigation is needed to compare the interactions between serotonin and/or these compounds and serotonin autoreceptors.

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772

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