Identification and molecular docking analysis alkaloids
Polyalthia longifolia leaves from Indonesia and the Philippines as anti-inflammatory

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Abstract. Polyalthia longifolia (family: Annonaceae) is widely planted to effectively reduces noise pollution. This plant spread in many countries including Indonesia and the Philippines. Alkaloids are the main active compounds other than terpenoids in P.longifolia and it has the potential to be anti-inflammatory. Each leaves considered have different active compounds because of the different geographic factor from each country. To confirm this, we investigated the differences alkaloid compounds from two different sources of plants and predicted their anti-inflammatory potential. Shade-dried leaves from Indonesia and the Philippines were extracted by ethanol 70%. Two extracts were analyzed with LC-MS to ensure alkaloid compounds. Ensured alkaloid compounds further take on molecular docking. The compounds were drawn with ChemDraw then convert to .pdb with Open Babel. The protein COX-2 obtained from .pdb then prepared with PyMol. The docking process held by PyRx and the interaction was visualized by LigPlot+. LC-MS analysis identified 5 alkaloids contained from the ethanol extract of P.longifolia leaves from Indonesia and the Philippines. O-methylbulbocapnine-N-oxide was found only in ethanol extract leaves from Indonesia, while N- methylnandingerine-β-N-oxide was only found in ethanol extract leaves from the Philippines. All compounds have the potential as an anti-inflammation. Liriiodenine as the most potent compound with binding energy -10.9kcal/mol. O-methylbulbocapnine-N-oxide has lower binding energy than N- methylmandingerine-β-N-oxide. In conclusion, there are differences between the alkaloid compounds and anti-inflammatory potential of the ethanol extract of P.longifolia leaves from Indonesia and the Philippines. Moreover, Indonesia's ethanol extract leaves showed more potential than Philippines's.

1. Introduction
Polyalthia longifolia is a plant commonly used to reduce noise pollution [1]. P.longifolia can be found in tropical and sub-tropical countries [2] such as Indonesia and the Philippines. This plant has been used as a traditional medicine to treat fever, skin disease, hypertension, diabetes. P.longifolia has pharmacological and biological activities such as anti-oxidant, anti-bacterial, anti-fungal, anti-cancer and anti-inflammatory [3].

Previous studies have reported that this plant contains flavonoids, alkaloids, sesquiterpenes, diterpenes, saponins, quercetin, bulbocapnine [2]. Geographical differences will cause differences in...
the content of chemical compounds in plants [4]. *P. longifolia* has the main secondary metabolites of alkaloids and diterpenoids. Various derivatives of the identified alkaloid compounds can be used as an anti-inflammatory [5]. For example, alkaloids are anti-inflammatory for IBD [6].

The anti-inflammatory mechanism can inhibit the enzyme cyclooxygenase (COX) [7]. The cyclooxygenase enzyme has two isoforms, namely COX-1 and COX-2. COX-2 is inducible and responsible for inflammatory [8]. Selective inhibition of COX-2 will reduce inflammation with a low risk of gastrointestinal [9]

In silico methods such as molecular docking are used to predict the ability of active compounds (ligands) to cause a biological effect computationally [7]. This study aims to determine the differences in the active compounds of *P. longifolia* plants from the regions of Indonesia and the Philippines. In addition, molecular docking is used to determine the ability of active compounds as anti-inflammatory compounds.

2. Materials and Methods

2.1. Plant Material

*P. longifolia* leaves were collected from two different countries. *P. longifolia* leaves from Indonesia were collected from Malang, East Java. While *P. longifolia* leaves from the Philippines were collected from Camiling, Tarlac. The Plants identified in plant taxonomy laboratories, Brawijaya University.

2.2. Hardware and Software

The computer used had the specifications of the Inter® Core ™ i3-4005U CPU @ 1.70GHz 1.70 GHz, Random Access Memory (RAM) 6.00 gigabytes. The software used is ChemDraw Ultra 12, PyMol, PyRx 0.8 and LigPlot + v.2.

2.3. Extraction

*P. longifolia* leaves from Indonesia and the Philippines were shade-dried and powdered. The powder was macerated with 70% ethanol for 72h. The filtrate was evaporated to get the extract. The extracts obtained were stored at 4°C until further analysis.

2.4. Alkaloid Analysis with LC-MS

LC-MS (TSQ Quantum Access MAX Triple, Thermo-Scientific) with C18 column (1.7 μm 100Å 50x2.1 mm, Kinetex) was adjusted according to the desired conditions (7.50 min separation process and the flow rate of 300 μl/min). The mobile phase was in the form of two solvents (eluent A = water and eluent B = acetonitrile). The gradient elution was carried out as follows, 0-0.60 minutes 90%: 10% (A: B), 5.00-5.50 minutes 25%: 75% (A: B) and 6.00-7.50 minutes 90%: 10% (A: B).

2.5. Preparation of Ligand Structures

The ligands used for molecular docking were alkaloid compounds present in the ethanol extract of leaves of *P. longifolia* from LC-MS. The structure of the ligands was drawn using ChemDraw Ultra 12 and Open Babel is used to convert to .pdb. Alkaloid compounds included in the study were Polylogine, noroliveroline, liriodenine, oliveroline-β-N-oxide, N-methylnandingerine-β-N-Oxide and O-methylbulbocapnine-N-oxide (Results from LC-MS).

2.6. Protein Preparation

Preparation of COX-2 protein begins by selecting an active form protein that binds native ligand (GDP code: 3LN1). Water molecules, ligands and B, C and D chains were removed from proteins using PyMol.

2.7. Molecular docking

Molecular docking proteins with ligands was carried out using AutoDock Vina in PyRx. Docking was carried out on a grid with a center of 31,2552; -23,4834; -16,1872 and Dimensions 17,4839; 17,3785;
21,3620 (Angstrom). Docking results in the form of bond strength interactions between ligands and receptors. Analysis of interactions with amino acids residues was used by the LigPlot + v.2 program.

3. Result and Discussion

3.1. Alkaloids by LC-MS

Alkaloid compounds were identified using LC-MS with 7 targets molecular weight of compounds which included polylongine, polyfothine, liriodenine, noroliveroline, oliveroline β-N-oxide N-methylnandingerine-β-N-oxide and O-methylbulbocapnine-N-oxide. Identification of compounds from the target molecular weight using m/z [M+H]+. Five alkaloid compounds identified on P. longifolia leaves from Indonesia and the Philippines. The alkaloid compounds detected are shown in table 1. The structure of the alkaloid compounds is shown in figure 1. There are differences in the compounds found, namely N-methylnandingerine-β-N-oxide in Philippine extracts and O-methylbulbocapnine-N-oxide in Indonesian extracts. Differences in compounds were due to different geographies between Indonesia and the Philippines. This condition would affect environmental factors such as temperature, altitude and sun duration [4].

![Figure 1. Structure of alkaloid compounds. A) Polylongine, B) Liriodenine, C) Noroliveroline, D) Oliveroline β-N-oxide, E) N-methylnandingerine-β-N-oxide, F) O-methylbulbocapnine-N-oxide.](image)

Table 1. Alkaloid compounds identified in the ethanol extract of P. longifolia leaves from Indonesia and the Philippines.

| Compounds                          | MW (g/mol) | From     | RT(min) |
|-----------------------------------|------------|----------|---------|
| Polylongine                       | 243.261    | Indonesia| 0.68    |
|                                   |            | Filipina | 0.71    |
| Liriodenine                       | 275.263    | Indonesia| 3.09    |
|                                   |            | Filipina | 3.09    |
| Noroliveroline                    | 281.31     | Indonesia| 2.75    |
|                                   |            | Filipina | 2.75    |
| Oliveroline-β-N-Oxide             | 311.337    | Indonesia| 2.99    |
|                                   |            | Filipina | 2.99    |
| N-methylnandingerine β-N-oxide    | 341.363    | Indonesia| -       |
|                                   |            | Filipina | 4.87    |
| O-methylbulbocapnine-N-oxide      | 355.39     | Indonesia| 4.92    |
|                                   |            | Filipina | -       |

The polarity of alkaloid compounds was indicated by the retention time (RT) resulted. Table 1 showed the polarity of the alkaloid compounds found in P. longifolia leaves. Polylongine had the
highest polarity then noroliveroline, oliveroline-β-N-oxide, liriodenine, N-methylnandingerine-β-N-oxide and O-methylbulbocapnine-N-oxide. The greater retention time indicates that the compound was retained by non-polar column. While compounds with the smallest retention time such as polylongine were not held up in column because they were polar in contrast to the column. Liriodenine in previous studies had retention time (RT) 3.51 [10]. Retention time differences were possible due to differences in flow speed and type of column used. Liriodenine was a compound that had been shown antiradical activity [11] and antioxidants [12].

3.2. Docking Results
Alkaloid compounds from *P. longifolia* leaves are thought to have anti-inflammatory effects. The cyclooxygenase-2 enzyme is an inducible enzyme caused by inflammation[8]. Inhibition of COX-2 is clinically effective as an anti-inflammatory agent that does not adversely affect the gastrointestinal tract. Molecular docking was done to determine the potential of alkaloid compounds as an anti-inflammatory by looking at the interaction of compounds with the active site of COX-2. Molecular docking was done with the PyRx program to assess the potential of compounds to be anti-inflammatory.

The 3D structures of alkaloid compounds detected from the ethanol extract of *P. longifolia* leaves were created using ChemDraw (Figure 2).

![Figure 2. The 3D structure of Alkaloids. A) Polylongine, B) Liriodenine, C) Noroliveroline, D) Oliveroline β-N-oxide, E) N-methylnandingerine-β-N-oxide, F) O-methylbulbocapnine-N-oxide.](image)

The 3D structure of COX-2 proteins that binds to the original inhibitor is obtained at the RCSB GDP online (Figure 3A). Water molecules, other ligands and chain B, C and D removed by PyMol software (Figure 3B).

![Figure 3. A) The 3D of COX-2 protein, B) The 3D chain A structure of COX-2 protein.](image)
Molecular docking results and interactions with residual amino acids were shown in Table 2. Table 2 showed the potential of each compound as anti-inflammatory seen from the binding energy and amino acid interactions. The total binding energy of the compounds that were docking had a value of RMSD (Root Mean Square Deviation) <2. RMSD was the difference between predictive and experimental observations, the smaller the RMSD value obtained, the predicted position of the compound was closer to native [7]. Therefore, it can be concluded that this molecular docking was valid for predicting COX-2 inhibition.

Table 2. The results of molecular docking between alkaloid compounds in *P. longifolia* leaves with COX-2 enzyme.

| Compounds/Ligands          | Binding energy (kcal/mol) | Hydrogen bonds distance (Å) | Amino acid residues from hydrogen binding | Amino acid residues from hydrophobic bonds |
|----------------------------|--------------------------|----------------------------|------------------------------------------|-------------------------------------------|
| Polylongine                | -8.7                     | 3.29, 2.83                 | Tyr341                                   | Leu517, Ala513*, Ser516, Val335, Phe504*, Leu338, Val509*, Val335*, Ala513*, Ser516, Leu338*, Val509*, Phe504*, Ser339*, Tyr341*, Val335*, Leu338, Phe504*, Ser339*, Val509*, Tyr341*, Leu517, Arg106 |
| Liriodenin                 | -10.9                    |                           |                                          | Phe504*, Val509*, Met508*, Leu338*, Tyr371, Trp373, Ser516, Leu517, Val335*, Ala513*, Tyr341*, Ser339*, Leu517, Val335*, Ala513*, Gly512*, Leu338*, Ser339*, Phe504*, Val509*, Tyr341*, Leu345, Tyr371, Trp373*, Phe504*, Leu338*, Val509*, Tyr341*, Ser516, Ala513*, Val335*, Arg106, Ser339*, Val335*, Leu517, Leu345, Val102 |
| Noroliveroline             | -10.7                    | 3.27, 3.12                | Ala513*, Ser516                          |                                          |
| Oliverine-β-N-Oxide        | -9.3                     |                           |                                          |                                          |
| N-Methylnandingerine-β-N-Oxide | -7.4                   | 3.05                      | Ser516                                   |                                          |
| O-Methylbulbocapnine-N-Oxide | -8.2                   |                           |                                          |                                          |

* same amino acid interactions with reference inhibitor

Binding energy was a conformational stability parameter between receptors (COX-2) and ligands (compounds). All alkaloid compounds that had been docking had low binding energy which ranges from -7.4 to -10.9 kcal/mol. Low binding energy causes the protein-ligand complex (compound) to become stable so that the ability to inhibit COX-2 was getting better [13]. This showed that these compounds had the potential as an anti-inflammatory. Based on the binding energy, it can be classified as anti-inflammatory compounds as follows: liriodenin > noroliveroline > oliverine-β-N-oxide > polylongine > O-methylbulbocapnine-N-Oxide > N-methylnandingerine-β-N-oxide.

The LigPlot+ program was used to visualize protein-ligand interactions. The results obtained illustrate the interaction of amino acid residues with ligands, which were mediated by hydrogen bonds with hydrophobic interactions. The interaction of ligands with amino acid residues of the O-methylbulbocapnine-β-N-oxide compound and N-methylnandingerine-N-oxide was shown in Figure 4.
In addition to binding energy, a comparison of amino acid residue interactions from reference inhibitors was carried out [14], [15]. This was done to see that the alkaloid compounds were on the same side (active side) as celecoxib. Redocking from celecoxib as a reference inhibitor obtained of hydrophobic interaction on the amino acid residues of Met508, Ala513, Gly512, Val509, Trp373, Leu370, Try341, Val335, His575, Phe504, Ile503 and Ala502 and hydrogen bonds acceptor with Ser339, Gln178, Leu338 and Arg499. Based on amino acids binding to the compounds in Table 2, it was found that all alkaloid compounds interacted on the active site of the receptor (COX-2). Hence, it could be concluded that the alkaloid compounds of ethanol extract of P.longifolia leaves had the potential as an anti-inflammatory by selectively inhibiting COX-2. Based on the potential of anti-inflammatory compounds O-methylbulbocapnine-N-Oxide was better than N-methylnandingerine-β-N-oxide. Thus, extract ethanol of P.longifolia leaves from Indonesia was better than the Philippines.

4. Conclusion
In conclusion, P.longifolia leaves from Indonesia and the Philippines contained different alkaloid compounds. N-methylnandingerine-β-N-oxide was only found in extracts from the Philippines, whereas O-methylbulbocapnine-N-oxide was only found in extracts originating from Indonesia. All alkaloid compounds found had anti-inflammatory abilities by inhibiting COX-2 in silico. Molecular docking results showed that compound from Indonesia had better anti-inflammatory potential than the Philippines.

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