Secondary metabolites of Mirabilis jalapa structurally inhibit Lactate Dehydrogenase A in silico: a potential cancer treatment

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Abstract: Altered energy metabolism from phosphorylated oxidation to aerobic glycolysis is one of the cancer hallmarks. Lactate dehydrogenase A (LDHA) is a major enzyme that catalyses pyruvate to lactate in such condition. The aim of this study was to explore LDHA inhibitors derived from Indonesian herbal plants. In this study, LDHA and oxamate molecular structures were obtained from protein data bank. As a standard ligand inhibitor, oxamate was molecularly re-validated using Autodock Vina 1.1.2 software and showed binding energy -4.26 ± 0.006 kcal/mol and interacted with LDHA at Gln99, Arg105, Asn137, Arg168, His192, and Thr247 residues. Molecular docking was used to visualize interaction between Indonesian phytochemicals and LDHA. Indonesian phytochemicals with the lowest binding energy and similar residues with standard ligand was Miraxanthin-III (-8.53 ± 0.006 kcal/mol), Vulgaxanthin- I (-8.46 ± 0.006 kcal/mol), Miraxanthin-II (-7.9 ± 0.2 kcal/mol) and Miraxanthin-V (-7.96 ± kcal/mol). Lower energy binding to LDHA and binding site at these residues was predicted to inhibit LDHA activity better than standard ligand. All phytochemicals were found in Mirabilis jalapa plant. Secondary metabolites in Mirabilis jalapa have LDHA inhibitor property in silico. Further in vitro study should be performed to confirm this result.

1. Introduction
Cancer is the first leading cause of death in developed countries while in developing countries, cancer becomes the second cause of mortality after cardiovascular diseases [1]. In 2012, approximately 32.6 million cancer cases were reported globally and 8.2 millions of death was due to cancer [2]. Data from epidemiological studies shows that up to 14.1 millions of new cancer cases are found every year [3]. WHO estimates that in the next two decades, the number of new cancer cases will increase by around 70% worldwide [1].
Cancel cells use glycolysis pathway as the main pathway for energy metabolism even when the oxygen supply is sufficient. Glucose and energy metabolism in cancer cells are mainly through aerobic glycolysis mechanism [4,5]. The shift in energy metabolism from oxidative phosphorylation to glycolysis causes the cancer cells to compensate for increased energy demand by up-regulating glucose transport [6,7]. This glycolysis process creates nucleoside and amino acids which facilitate biosynthesis of macromolecule and cell organelles leading to rapid growth of the cancer cells [8].

Glycolysis process is initiated by glucose phosphorylation resulting in the formation of pyruvate molecule and ATP. The pyruvate is reduced to lactate by an enzyme catalyst called lactate dehydrogenase (LDH) [9]. LDH plays an important role in ATP formation and in maintaining the sustainability of glycolysis process. LDH has two subunits with distinct function. LDHA subunit is responsible to convert pyruvate into lactate while LDHB subunit has a reverse function [10]. It is reported that in invasive cancer cells, the expression of LDHA is up-regulated. Increased expression of LDHA is associated with several factors such as tissue damage, hypoxia and necrosis. These conditions increase glycolysis process giving rise to excessive lactate production and reduced cell dependency toward oxygen [11,12].

Most of the currently available anti-cancer drugs not only affect cancer cells but also normal cells. The most common side effect of anti-cancer drugs is the toxicity to kidney and gastrointestinal organs, bone marrow suppression and immune system suppression [13]. The development of LDHA inhibitor as anti-cancer drug is aimed to get new anti-cancer with less severe side effects. Previous studies showed that LDHA deficiency was not life threatening and selective LDHA inhibitor caused minimal side effects [14]. Reduced LDHA activity will induce mitochondrial respiration and intracellular oxidative stress. This condition will affect the ability of cancer cells to proliferate, lower the likelihood of metastasis, and increase the sensitivity to chemotherapy agent [15,16]. Today, bio-computational program has been widely used for drug development. This method requires less time and funding as compared to in vitro or in vivo research directly. One of the methods is molecular docking used to see interaction between drug and its target receptor [17,18]. This study aimed to explore potential LDHA inhibitor from Indonesian herbal plants.

2. Experimental

Virtual data of all phytochemicals from Indonesian herbal plants were obtained from Herbal Indonesia database (HerbalDB). Approximately 6,776 active compounds from 3,825 plant species in Indonesia can be found in this database [17]. Three dimensional structure of the phytochemicals were downloaded from PubChem NCBI (http://pubchem.ncbi.nlm.nih.gov). The standard ligand used in this study was Oxamate. Oxamate is a potent LDHA inhibitor [19]. The structure of oxamate resembles pyruvate’s structure so that it can compete with pyruvate [20]. Three dimensional structure of LDHA which binds with standard ligand’s inhibitor (oxamate) was downloaded from Protein Data Bank in http://www.rcsb.org/pdb/ with PDB IDI code : 1I10. Subsequently, structure of oxamate was separated from LDHA structure by using Chimera 1.9. LDHA in *.pdf format file was prepared using AutoDock Tools by removing water molecule and adding hydrogen atom in the polar site. Then they were saved in *.mol2 and *.sdf format. Interaction between oxamate and LDHA was validated five times using AutoDockVina program to measure the binding energy. AutoDock Vina software is the development of AutoDock program which can predict conformation and binding energy better [21].

Molecular docking is a bio-computational program which can predict the structure of a molecule complex. This method comprises of two steps including predicting the binding location of a molecule and predicting the score of binding affinity [22]. The phytochemicals predicted to act as LDHA inhibitor must fulfill the Lipinski Rule of Five criteria, molecular weight less than 500 Dalton, have lower binding energy, and show similar conformation as the standard ligand. Molecular docking between phytochemicals and LDHA enzyme was done at mobile loop of the enzyme’s active site (at amino acid residue 163-247 and 267-331) and at the site of LDHA-oxamate binding (at His 192, Asn 137, Arg 105, Arg 168 and thr 247) using software AutoDockVina 1.1.2. This molecular docking result between standard ligand-LDHA was used to analyze the docking between Indonesian
phytochemicals and LDHA. Interaction between standard ligand inhibitor-LDHA and phytochemicals-LDHA were visualized with Pymol 1.7. Pymol is a software used to visualize the binding site between receptor and its ligand [23].

3. Result and Discussion

3.1. Validation of Docking between Oxamate and LDHA

Before docking the active phytochemical of Indonesian herbal plants with LDHA, we firstly validated the docking between standard inhibitor (Oxamate) and LDHA using Autodock Vina and Pymol 1.7 software. Validation was conducted five times and the final average score for Oxamate-LDHA docking was -4.26 ±0.06 kcal/mol (Table 1). This score becomes the reference to screen for candidate phytochemical of LDHA inhibitor. The phytochemicals must have lower binding energy to LDHA than the standard ligand (Oxamate).

Table 1. Result of docking validation between Oxamate and LDHA

| Docking Score (kcal/mol) | Binding site                      |
|-------------------------|-----------------------------------|
| Validation 1            | -4.3                              |
|                         | Arg 105, Asn 137, Gln 99, Arg 168, Thr 247 |
| Validation 2            | -4.2                              |
|                         | Arg 105, Asn 137, Gln 99, Arg 168, His 192, Thr 247 |
| Validation 3            | -4.3                              |
|                         | Arg 105, Asn 137, Gln 99, Arg 168, Thr 247 |
| Validation 4            | -4.2                              |
|                         | Arg 105, Asn 137, Gln 99, Arg 168, His 192, Thr 247 |
| Validation 5            | -4.3                              |
|                         | Arg 105, Asn 137, Gln 99, Arg 168, Thr 247 |
| Average                 | -4.26 ±0.06                       |

Visualisation of Oxamate-LDHA interaction showed that Oxamate binds with LDHA using Hydrogen bond (Figure 1). Validation of standard inhibitor (Oxamate) and LDHA interaction was conducted to get the docking score and binding site. Previous study reported that Oxamate bound with LDHA at 5 residues including Arg 105, Asn 137, Arg 168, His 192, and Th2 247 [24]. Arg 105 and His 192 plays a crucial role in LDHA enzymatic reaction. His 192 has two main functions. In addition to being proton exchanger, His 192 also facilitates pyruvate orientation enabling it to interact with co-factor Nicotinamide Adenine Dinucleotide Hidrogen (NADH). Arg 105 is responsible to stabilize Hydrogen ion transfer reaction [25]. Binding at these two residues is predicted to inhibit LDHA activity.

Oxamate has an identical structure as pyruvate so that it can act as substrate-like inhibitor competitor [20]. During validation process between Oxamate and LDHA, we removed NADH due to the fact that NADH can block Oxamate-LDHA interaction and thus affect the docking process. Validation process revealed that there was one additional binding site at Gln 99 (Figure 1). Gln 99 is part of the mobile loop of the active site that directly influence the catalytic reaction of pyruvate to lactate conversion. Binding of substrate at these residues can inhibit Lactate Dehydrogenase [24,25].
3.2. Result of Docking between active phytochemicals of Indonesian herbal plant with LDHA

After screening 6,776 active phytochemicals listed in HerbalDB database, we found that 516 of them met the Lipinski Rule of Five criteria. Docking of the 516 phytochemicals with LDHA was done three times resulting in 501 phytochemicals with docking score less than or equal to the standard ligand (Oxamate). Visualisation of the 501 phytochemicals using Pymol software revealed that 23 active phytochemicals bound with at least 5 amino acid residues similar as that of Oxamate. Among these, 4 phytochemicals bound at 5 similar residues as oxamate with one additional binding at Arg 98 which serves as a mobile-loop of the active site (Table 2). Visualisation of these 4 phytochemicals showed that they generally had identical structure and formation (Figure 2). During glycolysis metabolism, NADH serves as a co-factor which induces LDHA enzymatic reaction. Initially, NADH binds with LADH and creates a binding site for the substrate (in this case, pyruvate). Subsequently, pyruvate will bind with LDHA resulting in closing of the mobile-loop of the active site [26,27].

Table 2. Result of docking between phytochemical of Indonesian herbal plant with LDHA

| Phytochemical name | Molecular weight (g/mol) | Binding site | Average of Binding Energy (kcal/mol) | Docking Score (kcal/mol) |
|--------------------|--------------------------|--------------|--------------------------------------|--------------------------|
|                    |                          |              |                                      | I | II | III |
| Oxamate (standard ligand) | 89.05                   | Gln 99, Arg 105, Asn 137, Arg 168, His 192, Thr 247 | -4.26±0.006 | -4.3 | -4.2 | -4.3 |
| Miraxanthin-III | 330.34                   | Gln 99, Arg 98, Arg 105, Arg 168, His 192, Thr 247 | -8.53±0.006 | -8.6 | -8.5 | -8.5 |
| Vulgaxanthin-I | 339.304                  | Gln 99, Arg 98, Arg 105, Asn 137, Arg 168, Thr 247 | -8.46±0.006 | -8.5 | -8.5 | -8.4 |
| Miraxanthin-II | 326.261                  | Gln 99, Arg 98, Arg 105, Asn 137, Arg 168, Thr 247 | -7.9±0.2    | -8.1 | -7.7 | -7.9 |
| Miraxanthin-V | 346.339                  | Gln 99, Arg 98, Arg 105, Asn 137, His 192, Thr 247 | -7.96±0.06  | -7.9 | -8.0 | -8.0 |

Through molecular docking, we found that 4 phytochemicals potentially act as LDHA inhibitor including Miraxanthin-II, Miraxanthin-III, Miraxanthin-V, and Vulgaxanthin-I because they bound at the mobile loop of the enzyme’s active site (Figure 2). Oxamate has been proven to be LDHA inhibitor in cancer cell. Oxamate inhibited tumor growth both in vivo and in vitro, induced cancel cell
apoptosis, and reduced tumor size [28,29]. However, cell permeability toward Oxamate is low and non-specific due to its small molecular weight and intracellular penetration power [20]. The molecular weight of Miraxanthin-II, Miraxanthin-III, Miraxanthin-V, and Vulgaxanthin-I are larger than Oxamate (Table 2). It is estimated that with larger molecular weight, these phytochemicals will be more permeable and specific compared to Oxamate.

![Figure 2](image1.png)

**Figure 2.** Visualisation of phytochemicals-LDHA binding compare to Oxamate (red). (a) Miraxanthin-II (b) Miraxanthin-III (c) Miraxanthin-V (d) Vulgaxanthin-I.

A phytochemical tends to create stronger hydrogen bond to water than to lipophilic cell membrane as the number of H-bond acceptor increases [30]. Oxamate is a compound that has been proven to be LDHA inhibitor in some study [28, 29]. Of the four phytochemicals we found in this study, Miraxanthin-II has more H-bond acceptor than Oxamate. Thus, we predict that it will be more difficult for Miraxanthin-II to cross cell membrane as compared to Miraxanthin-III, Miraxanthin-V, and Vulgaxanthin-I. The molecular weight of the four phytochemicals we found are three times larger than Oxamate. It is estimated that the phytochemicals will bind NADH co-factor in hydrogen binding. This binding has impact to inhibit the glycolysis metabolism.

Based on HerbalDB database, Indonesian herbal plant which contains Miraxanthin-II, Miraxanthin-III, Miraxnathin-V, and Vulgaxanthin-I is *Mirabilis jalapa* (It is called *Bunga pukul empat* in Indonesia). Previous study reported that *Mirabilis jalapa* leaf extract has cytotoxic effect on cancer cells although the mechanism of its cytotoxicity is not clear yet [31]. It is important to isolate every single compound of *Mirabilis jalapa* to identify the phytochemicals with anti-cancer property.
4. Conclusion

Miraxanthin-II, Miraxanthin-III, Miraxanthin-V, and Vulgaxanthin-I can potentially act as LDHA inhibitor in silico. Docking result showed that all these four phytochemicals had lower binding energy than Oxamate and interacted at LDHA inhibitor binding site. Indonesian herbal plant, *Mirabilis jalapa* contains all these four phytochemicals thus raising the likelihood that secondary metabolites of this plant can serve as LDHA inhibitor. Further research is needed to confirm this finding.

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