Molecular docking study to reveal *Morinda citrifolia* fruits as a novel EGFR inhibitor for anticancer therapy

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**Abstract.** Lung cancer is the leading cause of cancer death in the world and represents a major public health burden. Epidermal Growth factor Receptor (EGFR) as targets for hormonal cancer therapy shown to decrease cell proliferation and tumor growth. Indonesian People usually consumed Health tonic from Morinda citrifolia fruits known as Noni juice. Morinda citrifolia fruits have been known for a therapeutic benefit such as antidiabetic, anticancer and pain. This study aims to investigate molecular interaction between Morinda citrifolia fruits compound with the active site of Erb antagonist and EGFR tyrosine kinase inhibitor for lung cancer therapy. Morinda citrifolia fruit compounds were retrieved from Dr. Duke's Phytochemical and Ethnobotanical Databases (https://phytochem.nal.usda.gov) meanwhile ligand was retrieved from PubChem and 3D protein from PDB. The first step was ligand and receptor preparation before docking process using VegaZZ. Molecular docking process was calculated using Autodock Vina in PyRx v.0.8. The Result of this study reveals 12 major compounds of Morinda citrifolia fruits Anthraquinone, Benzo acid, Beta carotene, Eugenol, Limonene, Quercetin, Rutin, Scopoletin. Based on the molecular binding analysis, Quercetin are best docking complex with EGFR. The result showed that Quercetin is the best antagonist for EGFR indicated by the highest affinity (-8.6 Kcal/mol). It can be concluded that Quercetin in *Morinda citrifolia* is main compound in noni fruits that act as anticancer agent.

Keyword: Anticancer, Bioinformatics, Lung Cancer, EGFR, Noni Fruits,

1. **INTRODUCTION**

Cancer is a disease in which there is a group of abnormal cells that experience uncontrolled division. Healthy cells usually respond to division signals and diverge to other cells or die. In cancer cells, cells ignore these signals so that the cells proliferate actively [1]. Lung cancer and cancer-related diseases have been widely treated with surgery, chemotherapy, radiotherapy or a combination of these three treatments. However, cancer remains one of the illnesses with high mortality [2].

Chemical prevention and treatment of cancer are defined as the use of natural or biological materials at the beginning of the pre-cancer stage to suppress tumor formation. At present some plant components have been successfully used in the treatment of cancer. Experimental studies show that phytochemical substances with antioxidant and anti-inflammatory properties can inhibit cancer formation and development. [3]
Natural plant extracts have been used for centuries by many cultures and civilizations as a basis for treating various types of diseases. More than 80% of the global population is currently dependent on natural plant extracts through traditional therapies. Researchers actively focus on research related to the development, treatment, and prevention of cancer with natural compounds [4]. Noni fruits containing multiple beneficial nutrients for human beings because the abundant of flavonoid and other compounds as secondary metabolites. Flavonoid is antioxidant that has been reportedly showed anticancer activity as chemo preventive and angiogenesis agent [5-6]. A substance that blocks the activity of a protein called epidermal growth factor receptor (EGFR). EGFR is found on the surface of some normal cells and is involved in cell growth. Blocking EGFR may keep cancer cells from growing [7]. Some EGFR inhibitors are used to treat cancer, one of them called Erlotinib. Erlotinib is a drug functionally act to block tumor cell growth by targeting a protein EGFR (epidermal growth factor) that is present on the surface of some cancer cells and some normal cells. Anti-angiogenesis inhibitors target the blood vessels that supply oxygen to the cancer cells, ultimately causing the cells to starve and die [8]. However, the use of Erlotinib for a long time causes a several side effect that harmful the body. So for minimizing the effect, Herb compound should be substitute as herb medicine for cancer therapy. Therefore, this study aimed to evaluate the active compound from noni fruits (M. citrifolia) to inhibit EGFR and reveal the potential for therapeutic computationally.

2. MATERIAL AND METHOD

Samples Preparation

Morinda citrifolia compound such as Anthraquinone, Benzo acid, Beta carotene, Eugenol, Limonene, Quercetin, Rutin, Scopoletin, which act as ligands, were retrieved in 3D format via PubChem (http://PubChem.ncbi.nlm.nih.gov) [9]. Preparation was done by minimizing all structures of active compounds using the steepest decent minimization in PyRx 0.8. The 3D structure of ERb and EGFR was downloaded from the Protein Data Bank (RCSB) PDB ID: 3ERT (http://www.rscb.org) [9]. Water molecules and drugs attached to the protein structure were eliminated, then the hydrogen atom was added to the structure of the protein since as a result of X-ray diffraction most the hydrogen atoms were incomplete.

PASS (Prediction of Activity Spectra for Substances) Test

The biological activity test was important to confirm before lab tests were conducted. The results would be shown by the probability activity score, which predicts the chance of success if tests were performed in the lab. The PASS test was performed using the PASS Online software (http://www.pharmaexpert.ru/passonline). First SMILES was searched for breadfruit derivative compounds using PubChem (http://pubchem.ncbi.nlm.nih.gov), then the ligand compounds were input into the PASS software and the activity prediction is performed (Get Prediction).

Pharmacokinetics Analysis

The ADME test was used to determine which active compounds of the herb could be well absorbed in the human digestive system. The ADME test was performed using SWISS ADME online software (http://www.swissadme.ch/) [10-11]

Molecular Docking Analysis

Molecular docking was done to know the best EGFR inhibitor from the active compound from M. citrifolia that has similar activity with Erlotinib. The docking process was initiated by selecting the ligand and the prepared receptor. Thus, the docking process was started by setting the gird box on the
active site receptor. The docking results were saved in PDB format and the value of the binding affinity was saved in Microsoft Excel format [12]. The docking result visualization was performed using LigPlot v.1.4.5 software, while the interaction visualization in 3D form was done using Discovery Studio software.

3. RESULT AND DISCUSSION

Biological Activity Prediction of M. citrifolia

*Morinda citrifolia* activity was analyzed using PASS (Prediction of Activity Spectra for Substance) test resuling flavonoid compounds of noni fruits Pteryxin, Coniferaldehyde, Vanillin, Limonene, Scopoletin, Kaempferol, Rubiadin, Rutin, Eugenol and Quercetin has has Pa value more than 0.7 (Pa > 0.7) as Apoptosis agonist. Pteryxin, Scopoletin and Quercetin are active compound from *M.citrifolia* that has Pa value more than 0.7 (Pa > 0.7) All 3 Parameter, Anticancer, Apoptosis Agonist and Antioxidant activity. All the result can be seen in Figure 1.

![Figure 1](image)

**Figure 1.** Anticancer, Apoptosis agonist, Antioxidant activity of flavonoid compound from *M.citrifolia*

The data above show that Pteryxin, Scopoletin and Quercetin have an anticancer, Apoptosis agonist and Antioxidant activity which is provable either computationally or through laboratory scale, because they have Pa values more than 0.7 (Pa > 0.7). Meanwhile, for other compound that have Pa values 0.5 <Pa <0.7 showed that those compounds have a good anticancer activity but they have not been proven in the laboratory yet.

Molecular Docking EGFR inhibitor Activity of *Morinda citrifolia* Compounds

Molecular docking was done to know the best compound that can be inhibitor for EGFR, by comparing binding affinity of noni fruit compound that bind in similar binding site with EGFR inhibitor (Erlotinib). This study showed that the active compounds of *M.citrifolia* can be EGFR inhibitor. The results revealed that the compound is Quercetin is the most effective compound to be used as a Lung cancer therapy by prevent metastases of cancer cell through EGFR pathway, because it has the highest binding affinity value compared to the other compounds (Figure 2). More negative the
free energy binding, the better the bond stability level between the ligand and the receptor (stable) since the bond formed is also stronger.

![Molecular Docking of EGFR Inhibitor Receptor to Active Compound of Noni Fruits](image)

**Figure 2.** Molecular Docking of EGFR Inhibitor Receptor to Active Compound of Noni Fruits

| Table 1. Binding Affinity Value of Active Compound of Noni Fruits |
|---------------------------------|------------------|------------------|
| **Compound**                   | **Reseptor**     | **Binding Affinity** |
| Pteryxin                       | EGFR             | -7,6             |
| Coniferaldehyde                | EGFR             | -5,6             |
| Vanillin                       | EGFR             | -4,9             |
| Limonene                       | EGFR             | -5,1             |
| Scopoletin                     | EGFR             | -6,1             |
| Kaempferol                     | EGFR             | -7,9             |
| Rubiadin                       | EGFR             | -8,1             |
| Rutin                          | EGFR             | -8,1             |
| Eugenol                        | EGFR             | -5,4             |
| Anthraquinone                  | EGFR             | -7,6             |
| Benzyl-alcohol                 | EGFR             | -5,2             |
| Quercetin                      | EGFR             | -8,3             |
| **Erlotinib (Control)**        | EGFR             | -6,8             |

Differences in affinity binding values of each compound are influenced by the type of interaction. The hydrogen bond has an important role in determining the size of the affinity binding value generated from the docking process because it has a stronger energy than the hydrophobic bond.
Hydrogen bonds have higher energy than hydrophobic interactions with values of 1-7 kcal/mol versus 1 kcal/mol [13]. Hydrogen supplements which predicted important role in determining the strength of ligand interactions with estrogen receptor alpha are when the hydrogen bond binding to the amino acid residues Glu 353, Arg 394, Thr 347 and Asp 351 [14].

**Pharmacokinetic Analysis of Morinda citrifolia as Potential Drug Candidate**

Pharmacokinetic is one factor that used to be considered aspect to develop new drug. Pharmacokinetics is a branch of pharmacology that studies about drug circulation the body. Based on pharmacokinetic is make sure the new drug candidates is well penetrate and reach main target bioactivity. Some examples of pharmacokinetic aspects that are often calculated in the early stages of the process of finding and discovering new drugs are related to the level of absorption in the body (absorption), distribution, metabolism and excretion or more commonly known and abbreviated with the term "ADME".[9] It is very important to consider these factors in the pharmacological world because the drug interactions with targets are unlikely to occur if they are not based toward their targets [10].

According to SWISS ADME prediction, 12 active compounds of noni fruit is qualify to Lipinski Rule of Five predictions (Table 2).

**Table 2. Lipinski Rule of Five (Ro5) of Morinda citrifolia compounds**

| Compound        | Molecular Weight | Water Solubility      | Log Kp (skin permeation) | GI absorption |
|-----------------|------------------|-----------------------|--------------------------|---------------|
| Pteryxin        | 386.40 g/mol     | Moderately soluble    | -6.42 cm/s               | High          |
| Coniferaldehyde | 178.18 g/mol     | Soluble               | -6.31 cm/s               | High          |
| Vanillin        | 152.15 g/mol     | Soluble               | -6.37 cm/s               | High          |
| Limonene        | 136.23 g/mol     | Soluble               | -3.89 cm/s               | Low           |
| Scopoletin      | 192.17 g/mol     | Soluble               | -6.39 cm/s               | High          |
| Kaempferol      | 286.24 g/mol     | Soluble               | -6.70 cm/s               | High          |
| Rubiadin        | 254.24 g/mol     | Moderately soluble    | -5.67 cm/s               | High          |
| Rutin           | 610.52 g/mol     | Soluble               | -10.26 cm/s              | High          |
| Eugenol         | 164.20 g/mol     | Soluble               | -5.69 cm/s               | High          |
| Anthraquinone   | 208.21 g/mol     | Soluble               | -5.16 cm/s               | High          |
| Benzyl-alcohol  | 108.14 g/mol     | Soluble               | -6.18 cm/s               | High          |
| Quercetin       | 302.24 g/mol     | Soluble               | -7.05 cm/s               | High          |

Table 2 shows that the noni fruit compound that have been tested can pass through the cell membrane because they have a molecular weight smaller than 500 Da. The higher of molecular weight of a drug is more difficult to penetrate the cell membrane because it may interfere with the diffusion process. The smaller the molecular weight of the drug, the easier it will be to diffuse in the cell membrane [15]. The flavonoid compound tested also has an H-donor and H-acceptor value of no more than 5 that can penetrate the cell membrane.

**CONCLUSION**

It can be concluded that from twelve compounds of the Morinda citrifolia Quercetin is the best compound to be a candidate as EGFR inhibitor. Active compound from Noni fruit has high potential for anticancer treatment. This study could be used for basic information for drug discovery.
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REFERENCES
[1] Hejmadi M 2010 Introduction to Cancer Biology Momna Hejmadi and Ventus Publishing ApS: London
[2] Alamode TT 2013 Journal of Biochemistry and Bioinformatics 3(1) 7–14
[3] Hamizah S, Roslida A H, Fezah O, Tan KL, Tor YS, Tan C I 2012 Asian Pacific Journal of Cancer Prevention 2012 13(6) 2533-2539.
[4] Elujoba A A, Odeleye O M, Ogunyemi C M 2005 African Journal of Traditional, Complementary and Alternative Medicines 2(1) 46-61.
[5] Liwen W, Jinhan W, Lianying F, Zuliang Z, Dexian Z, Suying W, Shiming L, Chi-Tang H, Hui Z 2014 BioMed Research International 2014 453972
[6] Dallakyan S, Olson and Arthur J 2015 Bioinformatics 2015 1-9
[7] Sara S, Daniele A, Letizia L 2018 Mol. Oncol 12(1): 3–20.
[8] Lee S K, Lee I H, Kim H J, Chang G S and Chunf J E 2010 Drug Absorption and Drug-Like Properties Blackweel Publishing Massachusetts: USA
[9] Lipinsk C A 2004 Drug Discovery Today Technologies 1(4) 20-24
[10] Nerkar S A, Kudale P P, Joshi and Hikhale H U 2012 International Journal of Pharmacy and Pharmaceutical Sciences 4(3) 12-20
[11] Lazzeroni M, Serrano D, Dunn B K, Stoddard B M H, Lee O, Khan S and Decensi A 2012 Cancer Research 14(2) 214-220
[12] Jadhav P B, Yadav A R and Gore M G 2015 International Journal of Pharma and Bio Science 6(4) 142-154
[13] Chillistone S and Hardman J 2008 Anaesthesia and Intensive Care Medicine 9(4) 8 -12
[14] Patrick G 2001 Instant Notes in Medicinal Chemistry Oxford BIOS Scientific Publisher: England
[15] Etkins S, Mestres J, Testa B 2007 British Journal Pharmacology 152 21-37