Original Research Article

Molecular Docking of Mangrove Plant as Therapeutic Agent to Treat Non-Small Cell Lung Carcinoma

ABSTRACT

Background: Cancer is one of the biggest health problems worldwide, with lung cancer as the first rank in the number of new cases and deaths. Non-small cell lung carcinoma (NSCLC) is a type of lung cancer that accounts for about 85% of all lung cancer cases. Previous research identified the role of epidermal growth factor receptor (EGFR) as the most suitable target to treat NSCLC. This study aims to identify the potential compounds derived from mangrove plants as agents to treat NSCLC using a molecular docking study.
Methodology: Six natural compounds, which include taraxasterol, stigmasterol, tretinoin, heritonin, ascochitine, and tricin, along with gefitinib as a drug comparative were used. Docking was carried out on EGFR as a receptor target by Autodock Tools. The visualizations of molecular interactions were carried out by BIOVIA Discovery Studio 2020.
Results: The results showed that all six compounds were compiled from several criteria as drugs based on Lipinski analysis and had an affinity to EGFR receptors. The docking results were found in the order of stigmasterol (-11.84 kcal/mol), taraxasterol (10.80 kcal/mol), tretinoin (-10.60 kcal/mol), tricin (-9.24 kcal/mol), ascochitine (-7.85 kcal/mol), heritonin (-7.81 kcal/mol), and gefitinib (-8.62 kcal/mol). Among these natural compounds, stigmasterol exhibited the highest binding affinity. ADME profile showed that these natural compounds are safe and drug-like compounds.
Conclusion: In this study, all compounds have the potential for development into drugs for the treatment of NSCLC. Further in vivo and in vitro investigations are needed to bring these compounds to the clinical setting.

Keywords: Epidermal growth factor; molecular docking; non-small cell lung carcinoma; taraxasterol.

1. INTRODUCTION

Cancer is one of the biggest health problems that lead to the leading causes of death worldwide [1]. This disease also shows an increasing trend in recent years and is predicted to increase every year [2]. In 2018, according to WHO, the most frequently diagnosed cancer was lung cancer with 2,094 million cases (11.6% of all cases). In 2022, American Cancer Society estimated 236,740 new cases of lung cancer will be diagnosed and 130,180

will die, approximately 350 deaths per day caused by lung cancer, the leading cause of death [3].

Lung cancer has been linked to several factors including smoking, genetic predisposition, and environmental factors [4]. Lung cancer can be classified into two types which are small cell lung cancer (SCLC) and non-small cell lung carcinoma (NSCLC), with NSCLC responsible for approximately 85% of lung cancer cases [5]. Lung cancer can develop because of genetic and epigenetic changes of the cellular genome, which is critical to the disease progression. The comprehensive molecular dissection of NSCLC found the mutation in epidermal growth factor receptor (EGFR) genes for about 10-40% cases with 14-19% of western patients and 40-48% of Asian patients. EGFR is a kind of tyrosine kinase receptor located at the cell surface [6]. EGFR can generate differentiation and proliferation of cells upon activation through the binding of one of its ligands [7]. Based on the fact that EGFR mutation leads to NSCLC, research has shown that targeting EGFR is currently considered the most suitable way to treat it [6].

The current treatments which are usually used to treat NSCLC are surgery, chemotherapy, and targeted therapy [8]. NSCLC patients whose tumors activate kinase domain mutations in EGFR often respond to EGFR tyrosine kinase inhibitors (TKI) such as erlotinib, gefitinib, and afatinib [9]. However, some studies have found TKI drug resistance in some NSCLC patients with EGFR mutation [10]. NSCLC treatment with surgery is invasive and limited to stage I-II and IIIA [11] Moreover, chemotherapy treatment which is often used in several types of cancer, had serious side effects [12] Therefore, the development of novel and treatment for treating NSCLC patients is needed [13]. In the last few decades, research on herbs as an alternative treatment with minimal side effects has been developed. Natural compounds are widely used in various therapeutic interventions due to their benefits as anti-cancer and minimum side effects [14].

Mangrove plants are commonly found in Indonesia [15]. Mangroves grow and develop in an extreme environment with changing habitats due to environmental effects such high temperatures, tides, silt deposition, and an abundance of microorganisms [16]. Mangrove plants have also shown several other activities which economically and medicinally useful [17]. Mangrove have abundant bioactive compounds and can serve as reservoir for novel bioactive compounds such as amides, alkaloids, tannins, flavonoids, saponin, glycosides, terpenoids, and phenolics [16]. The pharmacological activities of terpenoids, phenolics, and limonoids indicate that they can serve as preventative supplements and pharmaceutical agents as anti-cancer, antifungal, antibacterial, antiviral, antioxidant, anti-inflammatory, and other activities [18]. Bioactive compounds derived from mangrove plants have anti-cancer properties, such as triterpenoid, which is cytotoxic against many cancer cell lines, and limonoid, which is cytotoxic against P-388 leukemia cells [17]. Taraxasterol is one of triterpene known as anti-cancer for a few types of cancer. Chen et al. revealed that taraxasterol cut the growth of gastric cancer by inhibition of EGFR signaling [19]. Besides, Song et al. reported that mangrove compounds can be a multi-target inhibitor such as inhibited activities of HER2, HER3, HER4, RET, and EGFR in treating NSCLC [20].

This study aims to identify the potential compounds derived from plants as therapeutic agents to treat NSCLC.

2. MATERIALS AND METHODS

This study was conducted utilizing molecular docking computational method. The materials used in this study were protein target, namely EGFR (PDB ID: 3G5Z) downloaded from

http://www.rcsb.org and mangrove compounds were downloaded from https://pubchem.ncbi.nlm.nih.gov. The drug used as comparative was Gefitinib (Compound CID: 123631) which downloaded from https://pubchem.ncbi.nlm.nih.gov.

2.1 Selection of Mangrove Compounds

The mangrove compounds were retrieved from previous studies. Six mangrove compounds were used in this study including taraxasterol (Compound CID:115250), stigmasterol (Compound CID: 5280794), tretinoin (Compound CID: 444795), heritonin (Compound CID: 130118), ascochitine (Compound CID: 73486), and tricin (Compound CID: 5281702).

2.2 Geometry Optimization

After the compounds from Pubchem were downloaded, the compounds were saved in pdb format using Discovery studio. Then, geometry optimization was carried out in Argus Lab 4.0.1. software using PM3 semi-empirical parameterization based on Hartree-Fock calculation method. Argus lab software computed the energy convergence (stopping point of the compound's molecule [21]. Furthermore, the compound's format was converted to pdb with OpenBabel software to make it readable with Autodock Tools program [22].

2.3 Preparation of Target Protein and Compounds

The target protein was used in this study was EGFR (PDB ID: 5UG8). The preparation of the target protein was performed by removing water molecules (H2O) contained in the target protein, adding polar hydrogen atoms, cleaning the target protein structure from natural ligands then saved its file in the pdbqt format [23]. The preparation of the compounds were carried out by changing sdf format to pdbqt format using Discovery Studio and AutoDock software.

2.4 Validation

Validation of the molecular docking method was done by redocking the native ligand (N-[(3R,4R)-4-fluoro-1-{6-[(1-methyl-1H-pyrazol-4-yl)amino]-9-(propan-2-yl)-9H-purin-2 yl}pyrrolidin-3-yl]propenamide) to the selected macromolecule (EGFR) using Autodock Tools software. The binding site and the parameters used in this study are considered valid the RMSD value is ≤ 2Å [24].

2.5 Docking Protocol

The molecular docking was carried out to predict the binding energies of the compounds toward target protein using the Autodock Tools, Autogrid4, and Autodock4 software [25,26]. The docking simulation was done by arranging the docking parameters, which are the grid box size (x = 40, y = 40, z = 40), the grid box coordinate (x = -13.156, y = 14.7, z = -25.718), 0.375Å spacing, 100 runs, medium number of evals, and Lamarckian Genetic Algorithm 4.2 The docking output is in dlg format. The lowest binding affinity was selected from a set of 100 conformation poses.The interactions which exhibit the strong binding energy were analyzed using Discovery Studio software.

2.7 Drug-likeness and Toxicity Analysis

The Lipinski rule of five was used in this study assess the drug-like properties of compounds [27]. The molecular weight, number of hydrogen donor and acceptor, solubility, permeability,

level of GI absorption, and number of Lipinski violations were screened by employing the Swiss ADME web tools http://www.swissadme.ch/index.php [28] AdmetSAR 2.0 online tool (http://lmmd.ecust.edu.cn/admetsar2) was used to predict the toxicological profile of selected compounds [29]. The finalized ligands' SMILES were submitted in the admetSAR website to check for toxicity [30].

3. RESULTS

3.1 Geometry Optimization

Geometry optimization was used to obtain a molecular conformation that is stable and has a low potential energy which is adapted to human body conditions [31]. The active conformation of taraxasterol, stigmasterol, tretinoin, heritonin, ascochitine, and tricin were found to have -169.879384 au (-106601.012254 kcal/mol), -164.38055 au (-103150.43893 kcal/mol), -124.535337 au (-78147.1693209 kcal/mol), -112.242081 au (70433.0282483 kcal/mol), -128.320098 au (-80522.144696 kcal/mol), and -157,434363 au (98791.6371261 kcal/mol), respectively. These active conformations were the minimum potential energy calculated by geometry convergence calculated by PM3 method in Argus Lab 4.0.1. software. This energy is considered as self-consistent field (SCF) energy which is required for drug-receptor interactions.

3.2 Validation

The redocking procedure of the native ligand to EGFR, using the determined parameters, showed the RMSD value of 1.91 Å. Since the value is less than 2 Å, the docking method can be used to dock the test compounds. The difference between the native ligand before and after the redocking procedure (Fig. 1).

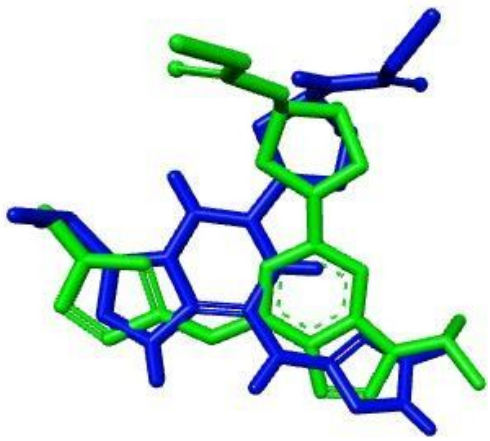


Fig. 1. Validation of molecular docking, Blue: re-docking results; Green: native ligand. RSMD: 1.91 Å

3.3 Molecular Docking Study

The docking results are represented in Table 1. Stigmasterol, taraxasterol, and tretinoin were found to have the highest binding energy of -11.84, -10.80, and -10.60 kcal/mol, respectively, than the comparative drug, gefitinib which was found to have binding energy of -8.62 kcal/mol. Besides, tricin, ascochitine, and heritonin were found to have lower binding energy than gefitinib with the binding energy of -9.24, -7.85, and -7.81 kcal/mol, respectively. The top three compounds and gefitinib were selected to visualize their interaction.

Table 1. Molecular docking results

3.4 Visualization of the Interactions

The visualization of docking results are shown in 3D form (Fig. 2) and 2D form (Fig. 3). The summary of 2D and 3D visualization were represented in Table 2. Taraxasterol was found to have 19 hydrophobic interactions and a hydrogen bond with the amino acid residues of Ser720, Leu718(2), Val726(4), Ala743(3), Met793(3), Leu844(5), Cys775, and Phe856. Stigmasterol had 13 hydrophobic interactions and a hydrogen bond with amino acid residues of Ser720, Leu718(2), Val726(3), Ala743, Leu844(2), Met793, Met790(2), Lys745, and Phe856. Tretinoin was found to have 19 hydrophobic interactions and 2 hydrogen bonds with the amino acid residues Lys728, Pro794, Leu718(2), Val726(3), Ala743(3), Cys775(2), Met790(2), Leu844(5), Met793, and Phe856. Gefitinib had 12 hydrophobic interactions and 3 hydrogen bonds with the residues of Lys728(2), Leu718(2), Met793(2), Val726, Ala743(2), Lys745, Cys775, Met790, Leu844, Leu792, Pro794. Four amino acid residues, Leu718, Leu844, Ala743, and Val726 were found in all interactions between the compounds and EGFR.

Compounds	Binding Energy (kcal/mol)
Stigmasterol	-11.84
Taraxasterol	-10.80
Tretinoin	-10.60
Tricin	-9.24
Ascochitine	-7.85
Heritonin	-7.81
Gefitinib	-8.62
Native ligand	-7.78

Table 2. The summary of visualization results

Compounds	Amino Acid Residues	Molecular Interaction

		Hydrophobic Interaction	Hydrogen bond
Taraxasterol	Ser720, Leu718(2), Val726(4), Ala743(3), Met793(3), Leu844(5), Cys775, Phe856	19	1
Stigmasterol	Ser720, Leu718(2), Val726(3), Ala743, Leu844(2), Met793, Met790(2), Lys745, Phe856	13	1
Tretinoin	Lys728, Pro794, Leu718(2), Val726(3), Ala743(3), Cys775(2), Met790(2), Leu844(5), Met793, Phe856	19	2
Gefitinib	Lys728(2), Leu718(2), Met793(2), Val726, Ala743(2), Lys745, Cys775, Met790, Leu844, Leu792, Pro794	12	3

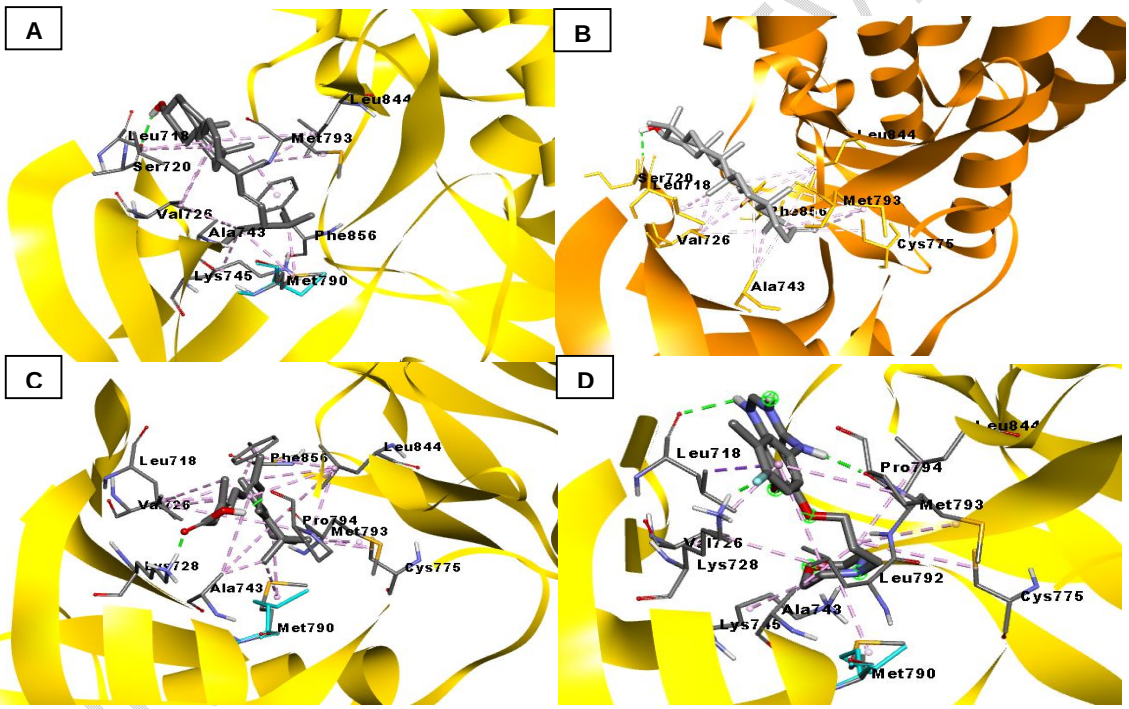
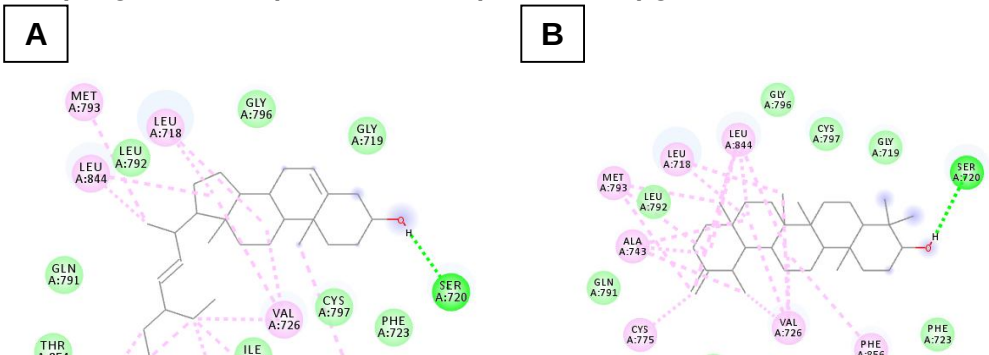


Fig. 2. 3D visualization of molecular docking results between EGFR and the compound A) stigmasterol; B) taraxasterol; C) tretinoin; D) gefitinib.



Compound	MW <500 (g/mol)	H-donor	H-acceptor	LogP	LogS	GI absorption	Violation

Fig. 3. 2D visualization of molecular docking results between EGFR and the compound A) stigmasterol; B) taraxasterol; C) tretinoin; D) gefitinib.

3.5 Drug-likeness and Toxicity Analysis

Taraxasterol	426.72	1	1	4.65	-8.24	Low	1
Stigmasterol	412.69	1	1	5.08	-7.46	Low	1
Tretinoin	300.44	1	2	4.28	-5.34	High	1
Heritonin	258.31	0	3	2.81	-3.41	High	0
Tricin	330.29	3	7	-0.07	-4.12	High	0
Ascochitine	276.28	2	5	1.84	-3.06	High	0

Drug-like properties analysis is shown in Table 3. All compounds were found to have no more than one violation. Specifically, taraxasterol, stigmasterol, and tretinoin had one violation, while heritonin, tricin, and ascochitine did not have violation. In Table 4, carcinogenicity and eye corrosion potencies of the compounds were not found. Tricin was the only compound that was predicted to cause eye irritation, whereas heritonin was predicted to cause Ames mutagenesis. Tricin, heritonin, ascochitine, and gefitinib were determined to be hepatotoxic. Most of the compounds belong to class III of acute oral toxicity.

Table 3. Lipinski results

Table 4. Toxicity prediction for taraxasterol, stigmasterol, tricin, heritonin, ascochitine, tretinoin, and gefitinib.

Compound	Carcino-genecity	Eye corrosion	Eye irritation	Ames muta-genesis	Hepato-toxicity	Acute oral toxicity
Taraxasterol	- (0.9571)	- (0.9834)	- (0.8878)	- (0.8400)	- (0.6250)	III (0.8879)
Stigmasterol	- (0.8571)	- (0.9886)	- (0.9673)	- (0.8300)	- (0.7750)	I (0.4287)
Tricin	- (1.0000)	- (0.9779)	+ (0.8092)	- (0.6800)	+ (0.7750)	III (0.5920)
Heritonin	- (0.9857)	- (0.9799)	- (0.6951)	+ (0.5200)	+ (0.6250)	III (0.4823)
Ascochitine	- (0.8714)	- (0.9852)	- (0.9051)	- (0.8700)	+ (0.6750)	III (0.4914)
Tretinoin	- (0.6714)	- (0.9886)	- (0.9569)	- (0.7800)	- (0.6000)	III (0.8050)
Gefitinib	- (0.9857)	- (0.9886)	- (0.9737)	- (0.5400)	+ (0.6750)	III (0.7006)

"+" means toxic; "-" means nontoxic. The numbers in brackets indicate the toxicity prediction

4. DISCUSSION

EGFR have shown various carcinogenic effects, including stimulation of DNA synthesis, the cell cycle, cell proliferation, cell metastasis, and invasion [32]. More than 60% of NSCLC produce EGFR and mostly overexpression [33]. A large number of experimental clinical research results have been observed that small molecule inhibitors targeting the EGFR have revealed excellent therapeutic effects in treating NSCLC. In addition, EGFR has become a promising target in the research and development of NSCLC drugs. EGFR inhibitor, tyrosine kinase inhibitors (TKIs) have been developed including gefitinib and erlotinib [33,34]. These drugs have been known to have efficacy in the treatment of NSCLC with EGFR mutation [34]. Nevertheless, acquired resistance and side effects have been found in most NSCLC patients when consuming the first to the third generation of TKIs [35,36]. Compound with clinically safe and effective need to be discovered to overcome the resistance and side effects of current drugs of NSCLC patients with EGFR mutation.

All compounds were docked to analyze their binding energy. Analysis of the molecular docking results were carried out by assessing the binding energy (ΔG). The binding energy is a parameter of conformational stability between the compounds and EGFR. Stigmasterol, taraxasterol, and tretinoin have been found to have a more negative binding energy value than gefitinib. Hence, the ability or affinity of the active compound to bind to EGFR was more exceptional. They showed that more robust and stable interactions that occur between the compounds and EGFR [37]. In addition, the binding energy value is directly linear with the constant inhibition value (Ki). So, the value of binding energy can be used to estimate the ability of a compound to inhibit protein target [38]. Based on the results, mangrove compounds have the ability to inhibit EGFR as the most suitable target to treat NSCLC.

The visualization showed in 3D and 2D form resulted from many amino acid residues of EGFR that bind with the compounds. Five amino acid residues have been found in all interactions between compounds and EGFR. Those amino acid residues were Leu718,

Leu844, Ala743, Met793, and Val726. Previous molecular docking study by Ibrahim et al. revealed that the active binding sites of EGFR were amino acid residues of Met793, Thr854, Leu718, Leu844, Met766, Val726, Ala743, Lys745, and Met790 [39]. Accordingly, those five amino acid residues were located in the active sites of EGFR. It means that all compounds bind in the active site of EGFR. The active site or binding pocket is the binding area of enzyme that involve amino acid residues that play a role in the binding. The interaction of amino acid residues at the active site with the compounds causes compounds to have the ability to inhibit EGFR as a competitive inhibitor. There is a correlation between binding energy and the active sites (binding pocket) of protein target [40].

This study analyzed hydrogen bond and hydrophobic interaction and both of them can affect the binding energy value. The hydrogen bond is the interaction of hydrogen atoms with electronegative atoms such as fluorine (F), nitrogen (N), and oxygen (O), while hydrophobic interaction is an interaction that occurs between nonpolar molecules. Hydrophobic interactions are alkyl-alkyl, pi-alkyl, pi-pi stacked, and pi-pi T-shaped interactions [41,42].

A previous study stated that hydrogen bonds and hydrophobic interactions could stabilize the compound at the active site of the enzyme and change the ΔG value as well as enhance the efficacy of the compound when interacting with the enzyme [43]. However, other studies have shown that the bond strength of the molecules is obtained because of the optimal hydrophobic interaction compared to the presence of the hydrogen bond [44]. Increasing the number of hydrophobic interactions at the active site of an enzyme may enhance the biological activity or effect of the compound. Another study stated that hydrophobic interactions and hydrogen bonds both also make large contributions to compound stability [45]. Hydrogen bond and hydrophobic interaction have a key role in strengthening molecular bond or enhancing binding energy, although it is still debatable between both of them regarding which type has more potential role in increasing the binding energy.

Lipinski rules state that for any compound to be considered as a drug-like compound, a compound must obey these criteria: Molecular Weight (MW) <500 Dalton, number of H-bond acceptors <10, number of H-bond donors <5, Log P <5, and if more than one violation were found, then the compound cannot be considered as a drug-like compound [28]. This study yielded that all compounds followed the Lipinski criteria due to there was no more than one violation. So, they can be considered as a drug-like compound.

On toxicity analysis, most of the compounds are a class of III on acute oral toxicity, which means that based on US EPA classification, the LD 50 values are between 500 mg/kg and 5000 mg/kg, while stigmasterol belongs to class I with LD 50 values of less than 50 mg/kg [46]. This study showed that taraxasterol and tretinoin showed less toxicity compared to the other compounds using the given toxicity parameters. These outcomes give fundamental data in regards to the toxicological profile of the compounds and might be helpful in choosing the preferred dosage and the route of administration. A previous study analyzed the toxicological profile of the mangrove plant (Rhizophora mucronate) using the Brine Shrimp Lethality Test (BSLT) and stated that the plant is moderately toxic [47].

Previous studies reported that mangrove compounds were known to have anti-cancer activity. Taraxasterol was found to have strong antioxidant, anti-cancer, and anti-inflammation. In the latest studies, taraxasterol can inhibit colorectal, breast, cervical, ovarian cancer, and melanoma cell growth in vitro, along with spontaneous breast carcinogenesis and carcinogen-induced skin papilloma [48,49]. Bao, et al suggested that taraxasterol inhibited liver cancer cells' growth by inducing cell cycle arrest at G0/G1 phase and apoptosis in vitro and in vivo [50]. In gastric cancer, poor prognosis is associated with overexpression of EGFR. Recent study revealed that taraxasterol might play a role as anti-

gastric cancer by inactivation of EGFR/AKT1 signaling pathway. It is shown that taraxasterol significantly downregulated EGFR, p-EGFR, AKT1, and p-AKT1 level in the tumor tissues [51].

Stigmasterol prevents the development of cholangiocarcinoma by downregulating TNF-alpha and VEGFR-2 and suppresses skin cancer by increasing lipid peroxide levels and inducing DNA damage [52]. Tricin had proven as anti-inflammatory, antiviral, antihistamine, and anti-cancer [53,54]. Naoko Seki, et al reported that tricin inhibited proliferation of HSC (Hepatic Stellate Cells) in vitro. The mechanism was known by blocking tyrosine phosphorylation of PDGF receptor and signaling pathways [55].

4. CONCLUSION

Six compounds have the potential as a drug candidate. Stigmasterol exhibited the highest binding energy. All three compounds bind in the binding pocket of EGFR. All compounds have drug-likeness properties based on Lipinski rules. Moreover, further in vivo and in vitro investigation are needed to bring these compounds at the clinical setting.

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