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POTENTIAL BIOACTIVE COMPOUND OF *Curcuma Longa* AND *Phyllanthus Niruri* AS CANDIDAT NANO HERBAL FOR CHEMOPREVENTION AGENT TROUGH INHIBITING EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR)

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ABSTRACT

Chemopreventive agents derived from herbs are increasingly being explored as potential adjuncts or alternatives to cancer therapies, including Curcuma longa and Phyllanthus niruri. This study evaluates the potential efficacy of bioactive compounds as anti-proliferative to prevent the growth of cancer cells by binding to the epidermal growth factor receptor (EGFR), which has potential as a chemopreventive agent using a bioinformatics approach. The data was collected from a previous study. Next, the selected compounds' 3D structures and canonical smiles were obtained from the PubChem database, and EGFR 7aei protein data from RSCD PDB databases as protein targets for further analysis. The data were analyzed based on probable activity, molecular docking, and molecular dynamics using a PASS Online web server, PyRx Software, YASARA software, and the AMBER14 force field, respectively. The Discovery Studio 2019 software was used to generate 3D visualizations of the results obtained from the molecular docking analysis. The bioactive compounds C. longa and P. niruri have the potential to target EGFR. The potential compounds showed a strong bond with EGFR. The strongest bond are lupeol, isoquercitrin, and quercetin, with binding affinity values of -9.3, -8.6, and -8.6, respectively. The result of RMSD showed that potential compounds fluctuate more stability when interacting with the EGFR compared with the control, as seen from the RMSD value of the ligand-complex simulation 5.03 Å, 3.29 Å, 3.9 Å, and 4.57 Å for gefitinib (control), quercetin, isoquercetin, and lupeol, respectively. In summary, bioactive compounds from C. longa and P. niruri have the potential to prevent malignancy of cancer cells by targeting EGFR, which indicates the potential ability for chemopreventive activity..

Keywords: Anti-cancer; Bioactive compound; C. longa; Epidermal Growth Factor Receptor (EGFR); and P. niruri.

INTRODUCTION

Cancer is a significant global health with primary treatments like chemotherapy, radiation therapy, and surgery. Several essential proteins were identified that play a critical role in the development and progression of cancer. One such protein is Epidermal Growth Factor Receptor (EGFR), which has been the subject of extensive study due to its potential as a therapeutic target for cancer treatment. EGFR is involved in several signaling pathways that regulate cell growth and differentiation cells [1]. EGFR is a member of the HER family, which includes HER2 (ErbB2), HER3 (ErbB3), and HER4 (ErbB4). Abnormal increase in EGFR activity due to overexpression or mutation can contribute to the progression and malignancy of cancer cells [2]. The EGFR mechanism in cancer cells involves coordinated events starting with the binding of specific ligands such as EGF or TGF-α to the EGFR [3]. This interaction

between the ligand and receptor triggers the formation of receptor dimers by interacting with other EGFR molecules and HER family members. After dimerization, autophosphorylation occurs at well-defined tyrosine residues within the receptor structure, the autophosphorylation process is a crucial stimulus for initiating a series of intracellular signaling cascades [4]. Furthermore, the protein kinase B (AKT/PKB) and mitogen-activated protein kinases (MAPK) pathways are active and propagate signals within the cellular environment [5].

Overexpression EGFR triggers for activating downstream AKT/MAPK signaling pathways. This signaling convergence is implicated in the upregulation of anti-apoptotic proteins within cancer cells [6]. Cancer cells often exhibit a heightened anti-apoptotic activity, which makes them resistant to programmed cell death. This ability to resist apoptosis helps them survive even under unfavorable conditions that normally trigger cell death [7]. Heightened anti-apoptotic signaling may contribute to immune escape [5]. Cancer cells, fortified with increased anti-apoptotic defenses, can evade destruction by immune effectors such as cytotoxic T cells or natural killer (NK) cells. This evasion mechanism allows cancer cells to persist and increase, avoiding immune surveillance [8,9]. Furthermore, cancer cells may enhance their resilience against immune-mediated cell death by upregulating anti-apoptotic proteins.

This adaptive response makes the cells more resistant to the cytotoxic effects induced by immune effector cells, including cytotoxic T cells [10]. The orchestrated modulation of these factors collectively enables cancer cells to manipulate the immune microenvironment, fostering an immunosuppressive milieu that supports their survival and proliferation [11]. In order to effectively treat cancer, developing targeted therapies to disrupt cancer cells and enhance the immune system's ability to identify and destroy cancerous cells is needed [12]. One mechanism involves downregulating major histocompatibility complex (MHC) molecules, diminishing the presentation of cancer-specific antigens to immune cells [13,14]. Cancer cells can take advantage of immune checkpoint pathways like PD-1/PD-L1 and CTLA-4 to hinder the activity of cytotoxic T cells, which are responsible for identifying and destroying cancer cells. This process allows cancer cells to evade the immune system's response and grow unchecked [15].

Chemoprevention refers to using specific drugs or other substances that disrupt various stages of tumor initiation, promotion, and progression [16]. One way to prevent cancer is through chemopreventive action, which involves inhibiting EGFR [17]. Based on previous studies, several herbal medicines showed the potential ability to inhibit EGFR. *Curcuma longa* commonly referred to as turmeric, possesses various properties such as anti-inflammatory, anti-cancer, anti-bacterial, anti-viral, antioxidant, antiseptic, cardioprotective, hepatoprotective, and digestive properties [18,19]. In other, *Phyllanthus niruri* is a plant widely used as an herbal medicine to treat various daily health problems and can potentially enhance the immune system and antioxidant activity [20,21].

According to previous studies, both these plants contain various active compounds that act as antidiabetics, antioxidants, immunomodulators, and anticancers [22]. The combination of *Curcuma longa* and *Phyllanthus niruri* is a potential herbal medicine to treat several diseases, including cancer. Therefore, this study aimed to identify bioactive compounds combined with *Curcuma longa* and *Phyllanthus niruri*, which can potentially be chemoprevention agents through to reduce or delay the occurrence of malignancy by blocking EGFR using the in silico molecular docking method.

MATERIAL AND METODS

Compound data mining

The compounds contained in the herbal *C. longa* and *P. niruri* were obtained from earlier studies. The selected bioactive compounds were collected in 3D-SDF file format and canonical smiles from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) for further analysis [23].

Druglikeness and probablity activity screening

According to Lipinski's Guidelines, the SWISS ADME web server was used to screen herbal combinations of *C. longa* and *P. niruri* for drug-likeness to find valuable and safe compounds (https://www.swissadme.ch/). A pass online web server was used for probability activity screening (https://www.way2drug.com/passonline/). The prediction value of biological function was obtained by looking at the score of probability activity (Pa). The Pa value measures the accuracy of the prediction function obtained [24].

Protein-ligan preparation

This study used the RSCB protein databank (PDB ID: 7AEI /https://www.rcsb.org/structure/7AEI) to get the EGFR target protein. Once the EGFR target protein had been obtained, it was then prepared using PyRx software to remove residual ligands. At the same time, the preparation of the ligand was carried out on the open babel menu on PyRx software. This was followed by options of minimized all and converted all for autodock ligand in the form of pdb [25].

Molecular docking and molecular dynamic simulation

Each bioactive compound from the selected *C. longa* and *P. niruri* herbal combination using PyRx software was docked with the target protein that had been repaired using Autodock Vina. The following step is determining the active site on the toggle selection spheres menu in the PyRx software. The selected active sites were Leu718, Cys797, Val726, Ala743, Leu844, Met793 [26], Gln791, Phe723 [27], Glu804 [28], Pro790 [29]. The macromolecules and ligands were then docked with a grid setting of centers (-0.8978, 52.6145, -17.7912) with dimensions (17.8929, 23.4080, 26.5854). The docking visualization was performed with the Discovery Studio software. Additionally, molecular dynamics simulation was done using YASARA (Yet Another Scientific Artificial Reality Application) software and AMBER14 force field [30]. The macro-MD analysis tool was utilized to analyze the root-mean-square deviation (RMSD). The system parameters were adjusted to correspond with the physiological conditions of the cells, including a temperature of 37 °C, a pH level of 7.4, atmospheric pressure of 1 atm, a salt concentration of 0.9%, and duration of 20 nanoseconds. The parameters used in this simulation are ligand movement RMSD, ligand conformation RMSD, and protein-ligand complex RMSD.

RESULTS AND DISCUSSION

Bioactive compounds

Herbal plants such as *C. longa* and *P. niruri* are known to have pharmacological effects because they contain active compounds such as lignans, tannins, flavonoids, and other secondary metabolites. These two plants possess pharmacological properties, including hepatoprotective, antibacterial, antihypertensive, anti-inflammatory, and anticancer effects [31,32]. *C. longa* belongs to the ginger family (Zingiberaceae), commonly known as turmeric [33]. The content of bioactive compounds in *Curcuma longa* include bis-demethoxycurcumin, curcumin, demethoxycurcumin, ferulic acid, vanillin, and vanillic acid [34], ar-curcumene, ar-turmerone, germacrone, α-zingiberene, β-elemenene, β-sesquifelandren, β-turmerone [35]. A total of 13 bioactive compounds were selected for further analysis (Table 1). *Phyllanthus niruri* contains many bioactive compounds including hypophyllanthin, niranthin, nir tetralin, phyltetralin, astragalin, quercetin, isoquercitrin [36], catechin (+), (-)-epicatechin, catechin-3-o-gallate, cymene, estradiol, fraternusterol, gallocatechin, limonene, and lupeol [37]. Furthermore, 17 bioactive compounds from *P. niruri* were selected for further analysis (Table 1).

Table 1. Identification bioactive compound of *C. longa* and *P. niruri*.

Herbal	Compound	Formula	Pubchem ID	Molecular Weight
C. longa	Curcumin	$C_{21}H_{20}O_6$	969516	368.38 g/mol
	Demethoxycurcumin	$C_{20}H_{18}O_5$	5469424	338.35 g/mol
	Bisdemethoxycurcumin	$C_{19}H_{16}O_4$	5315472	308.33 g/mol
	Ferulic acid	$C_{10}H_{10}O_4$	445858	194.18 g/mol
	2-Methoxy-4-vinyl guaiacol	$C_9H_{10}O_2$	332	150.17 g/mol
	Vanillin	$C_8H_8O_3$	1183	152.15 g/mol
	Vanillic acid	$C_8H_8O_4$	8468	168.15 g/mol
	Ar-turmerone	$C_{15}H_{20}O$	160512	216.32 g/mol
	β -turmerone	$C_{15}H_{22}O$	196216	218.33 g/mol
	α-zingiberene	$C_{15}H_{24}$	11127403	204.35 g/mol
	Ar-curcumene	$C_{15}H_{22}$	3083834	202.34 g/mol
	β -sesquifelandrene	$C_{15}H_{24}$	12315492	204.35 g/mol
	Germacrone	$C_{15}H_{22}O$	6436348	218.33 g/mol
	β -elemenone	$C_{15}H_{22}O$	10955018	218.33 g/mol
P. niruri	Hypophyllanthin	$C_{24}H_{30}O_7$	182140	430.49 g/mol
	Phyltetralin	$C_{24}H_{32}O_6$	11223782	416.51 g/mol
	Nirtetralin	$C_{24}H_{30}O_7$	182644	430.49 g/mol
	Niranthin	$C_{24}H_{32}O_7$	13989915	432.51 g/mol
	Astragalin	$C_{21}H_{20}O_{11}$	5282102	448.38 g/mol
	Catechin (+)	$C_{15}H_{14}O_6$	9064	290.27 g/mol
	(-)-Epicatechin	$C_{15}H_{14}O_6$	72276	290.27 g/mol
	Catechin-3-o-gallate	$C_{22}H_{18}O_{10}$	44257105	442.37 g/mol
	Quercetin	$C_{15}H_{10}O_7$	5280343	302.24 g/mol
	Phyllnirurin	$C_{20}H_{22}O_5$	179963	342.39 g/mol
	Cymene	$C_{10}H_{14}$	7463	134.22 g/mol
	Estradiol	$C_{18}H_{24}O_2$	5757	272.38 g/mol
	Fraternusterol	$C_{28}H_{48}O_2$	100951228	416.68 g/mol
	Gallocatechin	$C_{15}H_{14}O_7$	65084	306.27 g/mol
	Limonene	$C_{10}H_{16}$	22311	136.23 g/mol
	Lupeol	$C_{30}H_{50}O$	259846	426.72 g/mol
	Isoquercitrin	$C_{21}H_{20}O_{12}$	5280804	464.38 g/mol
Control	Gefitinib	$C_{22}H_{24}ClFN_4O_3$	123631	446.90 g/mol

Molecular therapy with specific targets are often used for cancer treatments. One of them is gefitinib which targets epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKIs). Gefinitib has a mechanism of action that inhibits phosphorylation and tyrosine kinase activity by competing for binding to ATP EGFR [38]. Gefitinib is a cancer treatment which is chosen based on EGFR mutations in colorectal cancer cases [3]. However, erlotinib and gefitinib are predicted to inhibit breast cancer with EGFR mutations in exons 18–21. The research found paradoxical gefitinib sensitivity in triple-negative breast cancer [39]. Therefore, gefitinib is used as a control which acts as an EGFR inhibitor. EGFR plays an important role in defining basal-like carcinoma and several studies suggest that this receptor can be used as targeted therapy with specific inhibitors. Basal type breast cancer is one of the subtypes associated with

EGFR expression and has a poor prognosis. Most of the TNBC patients have basal subtype tumors with EGFR expression [2].

Drug-likeness and probable activity

Based on drug-likeness and probable activity, there are 31 bioactive compounds have been screened to become 18 compounds selected that have high potential. Selected bioactive compounds from the combination of *C. longa* and *P. niruri* were screened according to the Lipinski rule to estimate the solubility and permeability of the compounds and to predict their qualifications as drug candidates whether these compounds were active orally or not [27]. Selected bioactive compounds were carried out according to the rules of Lipinski, Ghose, Veber, Egan, and Muegge (Figure 1a). Overall, the content of the selected bioactive compounds from *C. longa* and *P. niruri* had a violation value of the Lipinski parameter showing a value of ≤ 1, however only the bioactive compounds astragalin and Isoquercitrin which had a violation value of 2. Thus, the selected bioactive compounds *C. longa* and *P. niruri* can be categorized as a drug molecule because it is predicted to be able to penetrate the digestive membrane and cell membranes and can reach the target protein.

In order to determine the Pa value of each compound in the study, the analysis utilized the PASS online web server prediction program. PASS online has accurately predicted the biological activities of chemical compounds. Some of the biological activities selected from PASS online analysis include activity for anti-cancer such as antineoplastic, apoptosis agonist, MMP9 expression inhibitor, chemopreventive, proliferative diseases treatment, TP53 expression inhibitor, JAK2 expression inhibitor, anticarcinogenic, antineoplastic (breast cancer), Caspase 3 stimulants, and antioxidants (Figure 1b). Furthermore, the bioactive compounds that had, the highest activity were Lupeol (antineoplastic Pa 0.95 and antineoplastic breast cancer 0.799, Caspase 3 stimulant 0.978), Quercetin (apoptosis agonist Pa 0.887), demethoxycurcumin (MMP9 expression inhibitor Pa 0.919, and JAK2 expression inhibitor 0.978), Isoquercitrin (chemopreventive Pa 0.956, proliferative diseases treatment 0.921, Anticarcinogenic 0.965, and antioxidant 0.913), gallocatechin (TP53 expression inhibitor 0.963).

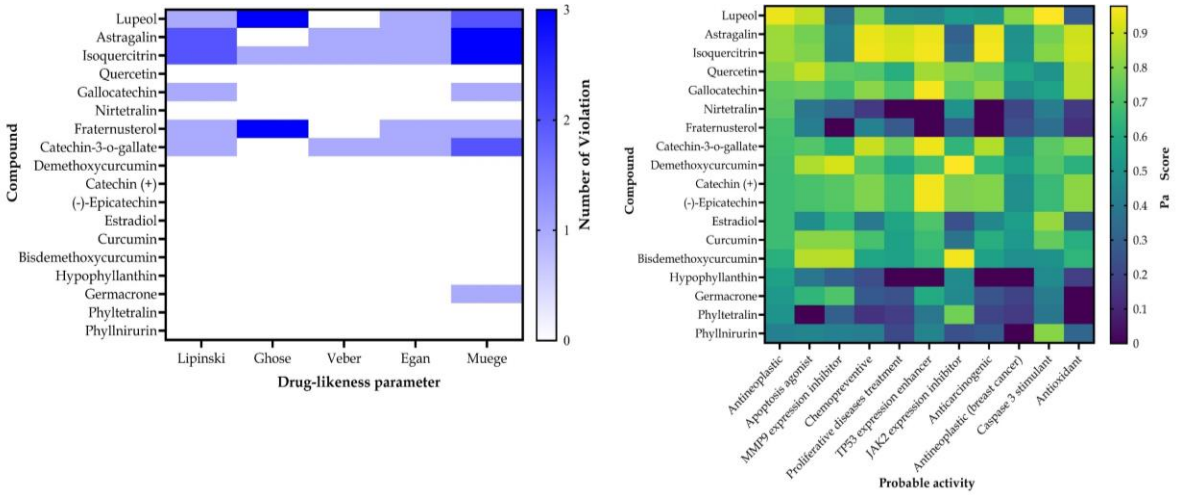
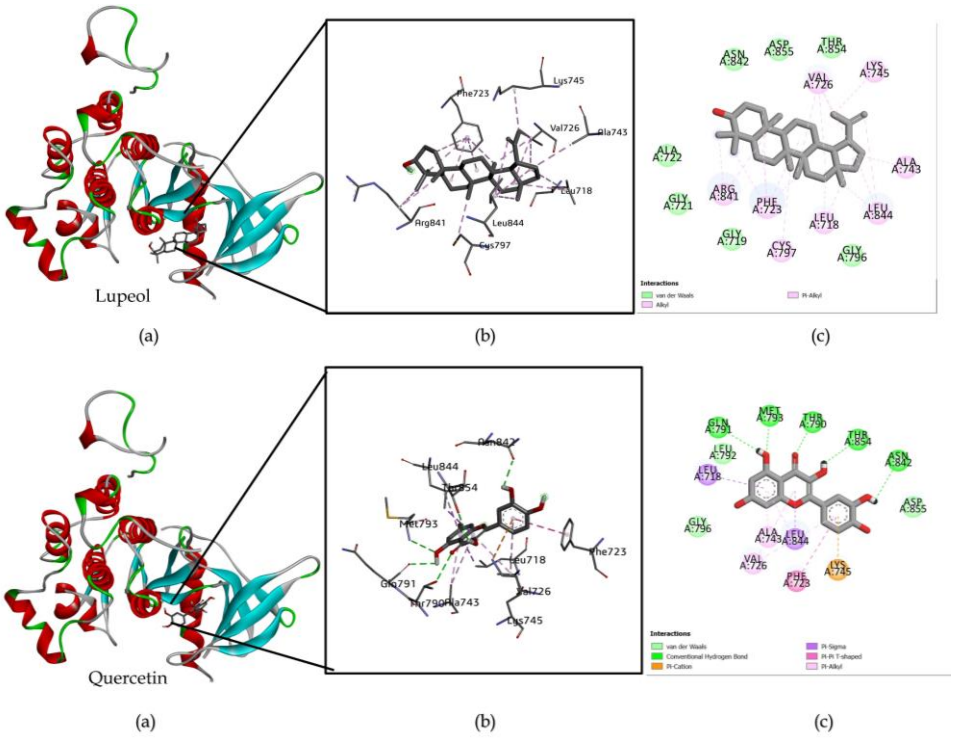


Figure 1. Bioactive compound of *C. longa* and *P. niruri* screening. (a) Drug-likeness parameter Screening based on five drug-likeness parameter; (b) PASS-Online Screening based on prediction of potential activity as anti-cancer.

Molecular docking

The results of the potential ligand interaction with EGFR compared to EGFR-gefitinib showed that the selected compounds could bind to the same active sites as the control. Therefore, the potential compounds from *C.- longa* and *P. niruri* can be used as alternative drugs to treat EGFR-targeted cancer. The results of the docking can also be seen by visualizing the 2D and 3D interactions between potential compounds with the EGFR receptor as shown in Figure 2. Therefore, the potential compounds from *C. longa* and *P. niruri* can be used as alternative drugs to treat EGFR-targeted cancer. The results of the docking can also be seen by visualizing the 2D and 3D interactions between potential compounds with the EGFR receptor as shown in Figure 2. Further, Table 2 showed docking results of the selected *C. longa* and *P. niruri* combination bioactive compounds for EGFR in the form of Gibbs free energy or binding affinity. It can be seen that 18 compounds have nearly the same binding affinity (ΔG) as the other comparator compounds (gefitinib). In the docking results of the *C. longa* and *P. niruri* bioactive compounds, 14 bioactive compounds which had a lower binding affinity value of -7.7 compared to the control (Table 2). The potential selected compound was found that Lupeol, Isoquercitrin, Quercetin had the lowest binding affinity values. The docking results indicate that lupeol binds to the ATP binding pocket of EGFR 7aei with the lowest binding affinity at -9.3 kcal/mol.



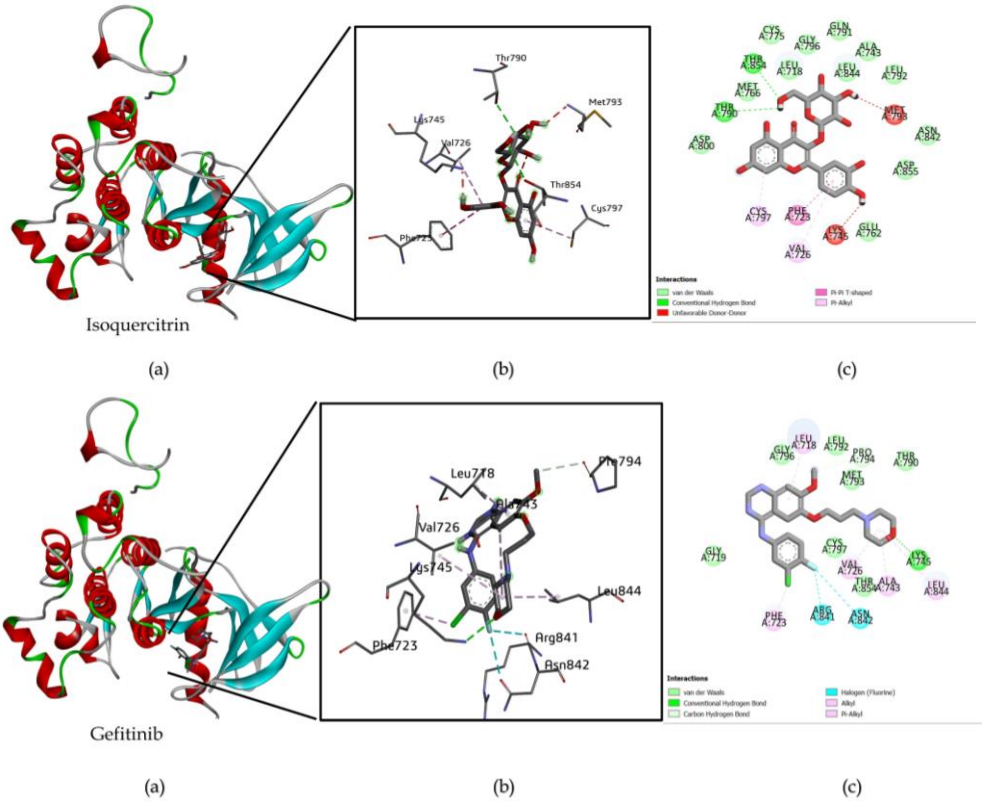


Figure 2. Visualization of the EGFR-Ligand interaction. (a) The binding interaction of ligand-protein complex, (b) The active site of ligand-protein complex, (c) 2d visualization of the binding interaction of ligan-protein complex

Table 2. Binding energy and residue of potential ligan interaction with EGFR

Ligan	*Interaction*	*Residue*	*Binding Afinity (Kcal/mol)*
Lupeol	Van der waals (7) Hydropobic (8)	Asn842, Asp855, Thr854, Ala722, Gly721, Gly719, Gly796 Val726, Lys745, Ala743, Leu844, Leu718, Cys797, Phe723, Arg841	-9.3
Quercetin	Van der waals (3) Hydrogen bond (5) Hydropobic (6)	Leu792, Gly796, Asp855 Gln791, Met793, Thr790, Thr854, Asn842 Leu718, Ala743, Val726, Leu844, Phe723, Lys745	-8.6
Isoquercitrin	Van der waals (12) Hydrogen bond (2) Hydropobic (3) Unfavorable (2)	Cys775, Leu718, Gly796, Gln791, Leu844, Ala743, Leu792, Asn842, Asp855, Glu762, Asp800, Met766 Thr790, Thr854 Cys797, Phe723, Val726 Lys745, Met793	-8.6
Gefitinib	Van der waals (8)	Gly796, Leu792, Met793, Pro794, Thr790,	-7.7

	Gly719, Cys797, Thr854
Hydrogen bond	Lys745
(1)	Leu718, Phe723, Val726, Ala743, Leu844
Hydropobic (5)	Arg841, Asn842
Halogen (2)	

Lupeol formed seven Van der waal bonds and eight hydropobic bonds in the five amino acid residues the same as ATP, involving Ala743, Leu844, Leu718, Cys797, and Phe723. Catechin-3-o-gallate forms van der Waals bonds (9), Hydrogen bonds (5), and Hydrophobics (3) with as many as seven amino acid residues, namely Cys797, Phe723, Met793, Gln791, Leu718, Val726, Leu844. Isoquercitrin forms bonds of van der Waals (12), Hydrogen bonds (2), Hydrophobics (3), and unfavorables (2) with as many as eight amino acid residues, namely Leu718, Gln791, Leu844, Ala743, Cys797, Phe723, Val726, Met793. While quercetin also forms bonds of van der Waals (3), Hydrogen bonds (5), Hydrophobic (6) with as many as seven amino acid residues, namely Gln791, Met793, Leu718, Ala743, Val726, Leu844, and Phe723. While the interaction of gefitinib with the target protein egfr shows that this compound can bind to EGFR 7aei with an affinity value of -7.7 and form bonds of van der Waals (8), Hydrogen (1), Hydrophobics (5), and halogens (2) by producing acid residues amino Met793, Pro794, Cys797, Leu718, Phe723, Val726, Ala743, and Leu844.

The ligand movement simulations between the protein and ligand showed more stable fluctuations after 5 ns for gefitinib, quercetin, and isoquercetin, while lupeol stabilised after 15 ns based on the results of a molecular dynamics analysis. The average RMSD values for ligand movement were 2.93 Å, 3.39 Å, 2.43 Å, and 4.43 Å for gefitinib, quercetin, isoquercetin, and lupeol, respectively. The results of ligand conformation analysis indicated that quercetin's fluctuations could stabilise after 10 ns with an average RMSD value of 1.04 Å, whereas isoquercetin and lupeol required more time with average RMSD values of 0.92 Å and 1.05 Å, respectively. In contrast, gefitinib exhibited sustained fluctuations between 5 and 15 ns with an RMSD value of 1.7Å. In addition, the ligand-complex simulation results of the three ligands and gefitinib demonstrated more stable fluctuations after 10 ns with RMSD values of 5.03 Å, 3.29 Å, 3.9 Å, and 4.57 Å for gefitinib, quercetin, isoquercetin, and lupeol, respectively. Molecular dynamic simulation between potential compounds with the EGFR receptor as shown in Figure 3.

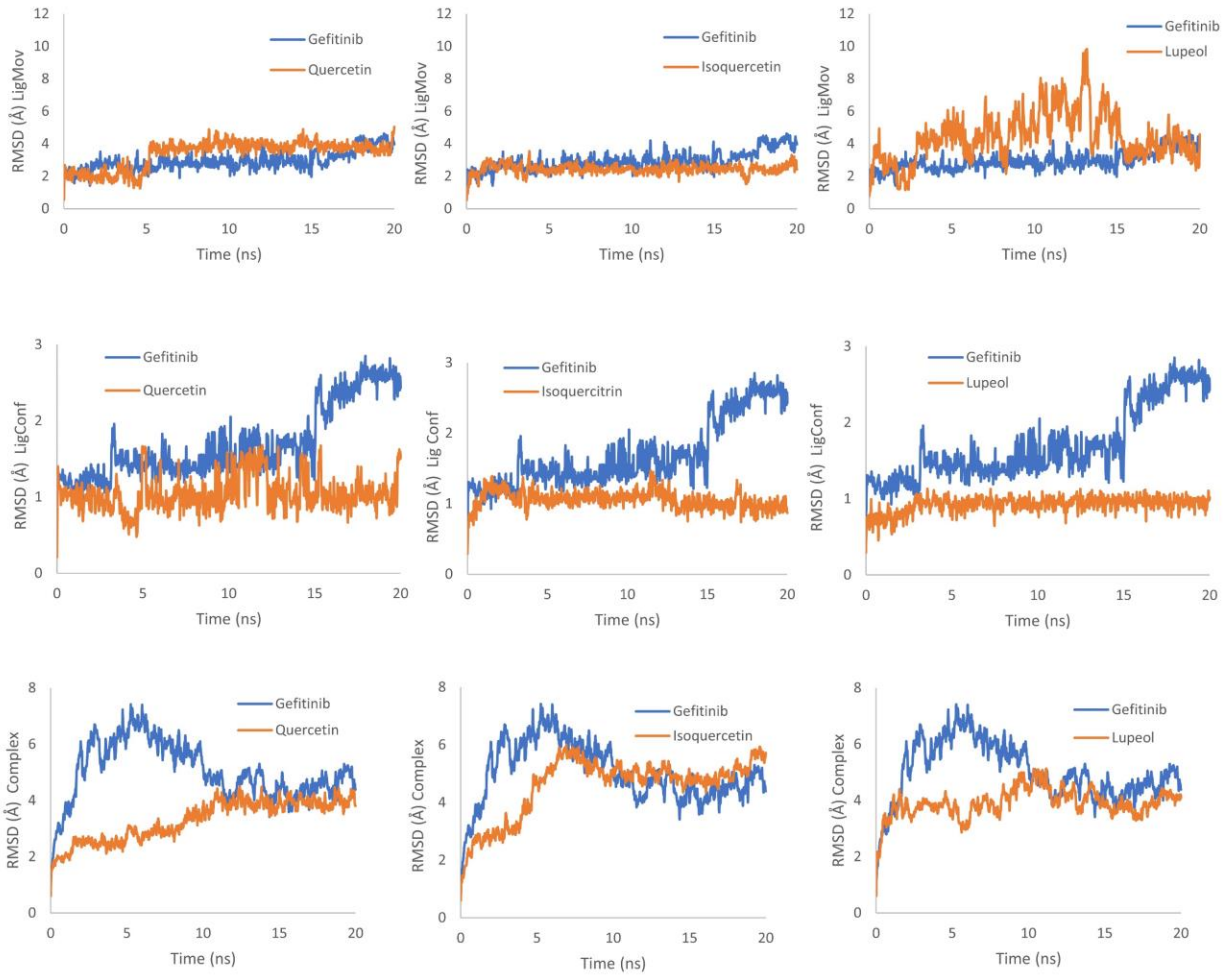


Figure 3. Molecular dynamic simulation of the protein-compound complex.

DISCUSSION:

The main bioactive compounds of *C. longa* are curcumin, bisdemethoxycurcumin, and demethoxycurcumin. Curcuminoid bioactive compounds are known to have potential activity as immunomodulators, antioxidant, anti-inflammatory, and antitumor [19]. *P. niruri* contains several anticancer chemicals, including phyllanthin and methyl brevifolincarboxylate [40], geraniin [32,41], galic acid, corilagen, rutin [32], quercetin [32,40], and ellagic acid [42]. Meanwhile, methyl brevifolincarboxylate plays a role in the immune system by inhibiting the production of IL-6 and TNF-a [40]. In addition, showed that phyllanthin, hypophyllanthin, and corilagin could act as immunomodulators [43]. Pharmaceutical plants are potential candidates for anticancer treatment. Moreover, good sources of bioactive compounds that have the potential for antioxidant and immunomodulatory functions [44]. In addition, natural compounds are also becoming prevalent cancer therapeutic agents due to their efficacy and safety [45].

This study investigates the potential biological activity of bioactive compounds to reduce or delay the occurrence of malignancy through a comprehensive activity using the PASS-online tool. The evaluation focuses on various biological activities associated with comprehensive activity, including antineoplastic, apoptosis agonist, MMP9 expression inhibitor, chemopreventive, proliferative diseases treatment, TP53

expression inhibitor, JAK2 expression inhibitor, anticarcinogenic, Caspase 3 stimulants, and antioxidants. PASS analysis identifies and characterizes the bioactive compounds demonstrating the highest activity across these biological parameters. Notably, compounds such as Lupeol, Quercetin, demethoxycurcumin, isoquercitrin, and gallocatechin exhibit significant anti-cancer potential, as evidenced by their respective probable pharmacological activity (Pa) values.

The findings reveal several bioactive compounds with high activity across multiple potentials for comprehensive, suggesting their potential efficacy in cancer treatment and prevention. Lupeol demonstrates significant antineoplastic and Caspase 3 stimulant activity, while Quercetin exhibits potent apoptosis agonist effects. Demethoxycurcumin notably inhibits MMP9 and JAK2 expression, indicating its potential as a molecular targeted therapy. Isoquercitrin emerges as a promising chemopreventive agent with antioxidant properties, and gallocatechin shows potent inhibition of TP53 expression. The study underscores the utility of computational approaches such as PASS in identifying bioactive compounds with promising chemoprevention for cancer treatment.

The ERBB family of receptor tyrosine kinases comprises five transmembrane signaling proteins, namely EGFR, ERBB1, ERBB2, ERBB3, and ERBB4. These proteins are responsible for regulating the processes of cellular proliferation and differentiation process. They act as essential mediators in transmitting signals from the extracellular environment to the intracellular environment, thereby ensuring the proper functioning of cells. Furthermore, the ERBB family's activation can activate cancer cell growth. Cancer malignancies have been associated with somatic mutations of the ERBB gene. Thus, ERBB is a crucial treatment target, as many ERBB-based treatments currently used to treat lung, breast, and colorectal cancer are clinically [29]. ErbB-targeted therapeutic strategies have sensitised growth tumour cells with conventional chemotherapy and radiotherapy. Targeting ERBB family has been helpful for breast cancer therapy [46].

The EGFR has an important role in cell signaling pathways that control cell division, including cell proliferation and migration, through the binding of extracellular ligands and the subsequent activation of the intracellular tyrosine kinase domain. EGFR migration causes the EGFR protein to be expressed in higher amounts than usual, which accelerates the division of cancer cells [4,6]. Clinical studies have reported that gefitinib can increase the growth inhibition effect on breast cancer by inhibiting EGFR, but the use of gefitinib did not show significant results. These results can be attributed to the molecular heterogeneity of TNBC, which is characterized by diverse genetic alterations in the EGFR signaling pathway [47].

Molecular docking studies offer valuable insights into the binding interactions between bioactive compounds and protein epidermal growth factor receptor (EGFR), aiding in discovering potential therapeutic agents. Results reveal bioactive compounds from Curcuma longa and Phyllanthus niruri exhibit comparable binding affinities (ΔG) to gefitinib. Lupeol mimicking the binding pattern of ATP gefitinib forms various bonds, including van der Waals, hydrogen bonds, and hydrophobic interactions, with EGFR residues, indicating their ability to interact with the receptor and potentially inhibit its activity. On the other hand, Isoquercitrin and Quercetin also have the potential to form various bonds, including van der Waals bonds, hydrogen bonds, and hydrophobic interactions, in inhibiting EGFR. The study found that Lupeol exhibits the lowest binding affinity to the ATP binding pocket of EGFR, suggesting its potential as a therapeutic agent. In addition, the findings from molecular docking experiments shed light on the specific binding interactions between three compounds from *Curcuma longa* and *Phyllanthus niruri* to EGFR. Among these, Lupeol, Isoquercitrin, and Quercetin show promise as EGFR inhibitors due to their low binding affinity values, indicating high potential to replace gefitinib for cancer treatment.

Lupeol is a compound found in *P. niruri* and exhibited anti-cancer effects in nasopharyngeal carcinoma cells by inducing apoptosis, ferroptosis, oxidative stress, and suppressing inflammation via the AMPK/NF-B pathway [48]. Lupeol is potential therapeutic agent targeting ERK and MEK proteins effectively. In addition, lupeol substantially inhibited cell migration in A549 cells by reducing the expression of the pErk1/2 protein, N-cadherin, and vimentin genes [49]. Lupeol targets critical biochemical pathways in a variety of cells, including nuclear factor kappa B (NF-kB), cFLIP, Fas, Kras, PI3 K/Akt, and Wnt/β-catenin. At therapeutic levels, Lupeol is nontoxic to normal cells and tissues [50]. Based on the results of probable activity lupeol has greater activity as an anti-cancer, such as antineoplastic, antineoplastic breast cancer, and Caspase 3 stimulant compared to other selected small molecules. In addition, lupeol also has the most substantial bond value with the target protein by forming van der Waals and hydrophobic bonds and having no cytotoxic effects.

Previous studies have extensively investigated the chemopreventive potential of flavonols such as quercetin and isoquercitrin, focusing on their biological activity. The biological activity of Isoquecitrin is greatly affected by its anti-oxidant and anti-inflammatory properties. Isoquercitrin has demonstrated antioxidant, anti-angiogenesis, inhibition of melanogenesis, and anti-carcinogenic properties in vitro studies. [51]. According to research on the chemopreventive effect of isoquercitrin, this compound possesses broad anti-proliferative activity against various forms of cancer. Isoquercitrin exhibited potent antiproliferative activity in vitro and in vivo by inducing apoptosis and cell cycle arrest in cancer cells [52]. These findings suggest that isoquercitrin has the potential to be a therapeutic agent that prevents the growth of cancer cells [52].

Quercetin is one of the many naturally occurring flavonoid compounds. Previous studies have shown that quercetin possesses various benefits, including anti-inflammatory, anti-oxidative, and anti-cancer benefits. Quercetin has potential as a natural bioactive compound for cancer prevention and treatment [53]. Quercetin has been shown to benefit lipid metabolism by regulating the AMPK signaling pathway. The expression of AMPK promotes lipid oxidation and reduces the deposition of lipids in cells. Dysregulated lipid metabolism is often observed in cancer cells, contributing to their growth and survival [54]. Due to its role in the suppression of numerous tumor-related processes, quercetin is regarded as an essential flavonoid for cancer chemoprevention [55].

These potential compounds possess the same activity as gefitinib, which inhibits the EGFR receptor. The strength of the bond between the receptor complex and the compound depends on the ΔG value released during their interaction, with a lower value indicating a stronger bond. The bioactive compounds form a stable and strong non-covalent connection with the receptor, allowing them to easily penetrate the cell and disrupt DNA replication or metabolic processes, ultimately leading to the cell's death. Figure 2 shows that the bioactive compounds *C. longa* and *P. niruri*, when combined through the docking process, bind to the target compound area and are known to have properties as EGFR inhibitors. Furthermore, these bioactive compounds demonstrate a comprehensive activity that can reduce or inhibit the malignancy of cancer cells.

Molecular dynamics simulation provides static and dynamic information on the atomic scale, which can be transformed into information on the macroscopic scale, such as pressure and temperature. RMSD is the average displacement of atoms during the simulation towards a suitable structure. The RMSD value is used to determine if a structure is stable over a specified period of simulated change. If the RMSD value is low, it indicates that the ligand has a tendency to remain in a position close to the desired bonding structure, which may indicate a strong bond and stable interaction between the ligand and the target protein [56].

Quercetin and isoquercetin show better fluctuation compared with control. Control (Gefitinib) exhibited quicker fluctuations, but fluctuations were unstable after 15 ns. Lupeol show more stable fluctuations after 15 ns, indicating that a longer simulation time is required to reach this level of stability. This could indicate that the interactions between the ligand and the protein need some time to develop into a more stable and tightly bound form. Isoquercetin showed low ligand movement RMSD compared to the other. it show signifies a high degree of closeness to the target, indicating a more favorable binding of the ligand [57].

Based on the stability of the protein-ligand bond, this indicates that isoquercetin has the potential to maintain its binding to the target protein, with results most similar to the control. Low ligand mobile RMSD typically indicates that the ligand is closest to the target and therefore binds better [58]. A molecular dynamic simulation was carried out to analyze the stability of the interactions between the EGFR and potential compounds. The simulation showed that the EGFR conformation tended to be stable when interacting with the three compounds seen from the RMSD backbone which was always below 2 Å and had minimal fluctuations.

The RMSD analysis showed that all compounds had more stable fluctuations than the control, and all complexes had RMSD values below the control of 5.03 Å, indicating more excellent stability. Based on the results, the compounds analyzed appeared to maintain a configuration that closely aligns with their desired functional state. The molecular dynamics simulations conducted show that the interactions between the proteins and compounds found in *C. longa* and *P. niruri* are stable. As a result, these compounds have the potential to serve as highly effective inhibitors of EGFR proteins.

CONCLUSION

In conclusion, the bioactive compounds *C. longa* and *P. niruri* have potential chemopreventive activity as inhibitors of EGFR that have mechanisms for proliferation and differentiation in cancer cells. The potential compounds with the highest activity and the strongest bond are lupeol, isoquecitrin, and quercetin. The result of RMSD showed that potential compounds fluctuate more steadily when interacting with the EGFR than when interacting with the control, as seen from the RMSD value. These results provide new insights into the benefits of *C. longa* and *P. niruri* and their potential as promising cancer treatment agents.

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CONFLICT OF INTERESTS

The author affirms that have no conflicts of interest to declare.

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