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Cytotoxic, Docking, and Pharmacokinetic Evaluation of Ethyl Acetate Fraction of Sungkai (*Peronema canescens* Jack) Leaves Against A549 Lung Cancer Cells

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Abstract

Sungkai (*Peronema canescens* Jack) is known to have various bioactive compounds as anticancer agents. This study aims to evaluate the anticancer potential of the active compound from the ethyl acetate fraction of sungkai leaves through an experimental and computational approach. The cytotoxic activity of the ethyl acetate fraction was carried out using the MTT assay method on A549 lung cancer cells. The computational study carried out using the molecular docking method and continued with pharmacokinetic analysis of the five main compounds in the ethyl acetate fraction of sungkai leaves. The MTT assay revealed that the ethyl acetate fraction exhibited moderate cytotoxic activity against A549 lung cancer cells, with an IC_{50} value of 33.41 µg/mL. The LC-MS/MS analysis identified five main compounds in this fraction: isorhamnetin, physcion, pilosin, (3R)-sophorol, and takakin. Molecular docking simulations of these compounds with the EGFR protein (PDB ID: 5UG9) showed bond energies ranging from -7.7 to -8.9 kcal/mol. Physcion has a more negative bond energy value compared to the positive control (quercetin) and four other compounds. The compounds interacted with the protein through hydrogen bonds and van der Waals interactions, predominantly involving amino acid residues ALA A:743, VAL A:726, LEU A:718, LEU A:844, and GLN A:791. Pharmacokinetic analysis revealed that all the compounds met Lipinski's rule of five, indicating good oral absorption and membrane permeation. However, toxicity predictions showed that isorhamnetin, takakin, and pilosin had a high risk of gene mutations, whereas physcion had a moderate risk. Despite potential toxicity concerns, the compounds had drug scores greater than 0, suggesting their potential as drug candidates. Therefore, experimental and computational approaches can be an efficient early screening method to identify potential bioactive compounds as drugs. However, further analysis must be carried out regarding the risk of toxicity and a good dose of the drug.

Keywords: Cytotoxicity, Lung cancer, Molecular docking, *Peronema canescens* Jack, Pharmacokinetics.

Introduction

Lung cancer is the second most common cancer worldwide, with a diagnosis rate of 11.4%, and is the leading cause of death[(1)]. In 2022, globally there were around 2.48 million new cases and 1.8 million deaths due to lung cancer[(2)]. Various factors can cause lung cancer related deaths. An important factor in the development of lung cancer, especially non-small cell lung cancer (NSCLC), is the Epidermal Growth Factor Receptor (EGFR) oncogene. EGFR is one of the genes that most often mutates in NSCLC. Approximately 10%-30% of NSCLC patients have active mutations in EGFR[(3)]. Mutations in the EGFR gene cause constitutive activation of this receptor[(4,5)]which affect various upstream and downstream signaling molecules in various pathways, including the PI3K/Akt/mTOR pathway[(6)]. Activation of this pathway can increase the growth, survival, migration, and invasion of tumor cells by affecting the tumor cell cycle, inhibiting tumor cell apoptosis and autophagy, and increasing tumor angiogenesis and chemotherapy resistance[(7)].

In recent years, EGFR-targeted therapy has become one of the main approaches for treating NSCLC. However, a major challenge is drug resistance to existing EGFR inhibitors such as gefitinib and erlotinib[(4)]. Therefore, it is necessary to identify new drugs that can inhibit cancer cells growth. Researchers continue to explore and develop effective drugs to improve therapeutic strategies, including the use of plant-derived natural compounds as therapeutic agents. Sungkai (*Peronema canescens* Jack) are typical Indonesian plants that grow and spread widely on the islands of Sumatera and Kalimantan[(8)]. It is a traditional

plant that is widely used as a medicine for hypertension, hypercholesterolemia, toothache, malaria, and others[9]. The bioactivities of sungkai leaves have been widely reported, including their anticancer properties[10]. Sungkai leaves contain various secondary metabolites that can be used as medicines[11]. The potential of a compound or molecule as a medicine can be determined by its toxicity, which is expressed by its IC_{50} value, pharmacokinetics, and molecular docking.

(a) (b)

Figure 1. Sungkai (*Peronema canescens* Jack) a) sungkai plants, b) sungkai leaves[9].

Experimental studies on the cytotoxic activity of sungkai plants have been widely conducted using both BSLT and MTT assays against several cancer cell types[10-13]. However, to date, no study has specifically explained the role of sungkai leaves as an anti-lung cancer drug. In addition, the toxicity and potential properties of the compounds contained in sungkai leaves as drug candidates have not been studied computationally. Computational methods for determining the toxicity of new drug compounds have several advantages, such as fast and efficient work and ease of reporting the interaction of drug compounds with cancer cells, which are difficult to identify experimentally[14]. Therefore, this study aimed to determine the cytotoxic activity of sungkai leaves experimentally and computationally using cytotoxic, pharmacokinetic, and molecular docking tests. The combination of these two methods can facilitate the discovery of new drugs that are effective as lung cancer therapeutic agents, simply, easily, and efficiently.

Materials and Methods

Materials

The sample utilized in this study comprised sungkai leaves (*Peronema canescens* Jack) collected from the Tanah Datar, West Sumatra, Indonesia. The solvents employed for extraction included hexane, ethyl acetate, and methanol (A distilled technical-grade solvent was obtained from PT. Novalindo Jaya Utama, Padang, Indonesia). Additionally, MTT reagents (3-(4,5-di-methylthiazol- 2-yl)-2,5 2,5-diphenyltetrazolium bromide) and RPMI-1640 media were utilized for the MTT assays. For molecular docking analysis, the Autodock Vina free version program was used, the PyMol education version and Discovery Studio 2024 free version, whereas the pharmacokinetic analysis was used the OSIRIS Property software free version.

Sample Preparation

Two kilograms of dried sungkai leaves were ground using a grinder. From this material, 500 g of the finely ground sample underwent maceration with 1.5 L of methanol for 72 h. Following maceration, the mixture was filtered to obtain the filtrate, which was concentrated using a rotary evaporator to remove the solvent. The maceration process was repeated under identical condition until the filtrate appeared clear, indicating that the soluble compounds had been thoroughly extracted. All concentrated filtrates were weighed[15].

Fractionation of Methanol Extract

A total of 20 g of the concentrated methanol extract was resuspended in a sufficient volume of distilled water to obtain a homogeneous aqueous phase suitable for liquid-liquid fractionation. The resulting suspension was transferred into a separatory funnel and fractionated using hexane as the first organic solvent. The mixture was gently agitated to facilitate the distribution of non-polar constituents into the hexane phase, followed by a resting period to allow complete phase separation. The hexane layer was collected, and the fractionation process was repeated several times under identical conditions until the hexane fraction appeared clear, indicating

exhaustive extraction of non-polar compounds. The remaining aqueous phase was separated with ethyl acetate to isolate semi-polar constituents. The mixture was shaken and allowed to settle until two distinct layers were formed. The ethyl acetate phase was separated, and the fractionation was repeated multiple times until a clear ethyl acetate layer was obtained, indicating that the semi-polar metabolites had been efficiently extracted. All collected fractions: the hexane fraction, ethyl acetate fraction, and the residual aqueous fraction, were concentrated under reduced pressure using a rotary evaporator to remove the solvents. The dried fractions were then analyzed to cytotoxicity assays and secondary metabolite profiling using LC-MS/MS[16].

Characterization of Ethyl Acetate Fraction of Sungkai Leaves Using Liquid Chromatography Tandem-Mass Spectrometry (LC-MS/MS)

The ethyl acetate fraction of the sungkai leaves was analyzed by LC-MS/MS to identify the secondary metabolite content. Samples were separated using UPLC (ACQUITY UPLC® H-Class System, Waters, USA) and analyzed using high-resolution mass spectrometry (Xevo G2-S QTof, Waters, USA). A C18 column was used with mobile phase A consisting of water containing 5 mM ammonium formate (phase A) and acetonitrile with 0.05% formic acid (Phase B). Gradient elution was performed at a flow rate of 0.2 mL/min for 23 min with a 5 µL injection. The standart used in analysis was methanol p.a. The mass spectrometer was operated in the positive ESI mode, scanning in the range of 50–1200 m/z with source and desolation temperatures set at 100°C and 350°C respectively. In addition, the cone gas flow rate and desolation are 0 L/h and 793 L/h, respectively. The collision energy varies between 4 to 60 eV to produce ion fragmentation. The analysis was controlled and processed using Masslynx software version 4.1[17]. The compounds were identified using the Massbank and Chemspider database.

Cytotoxicity Test of Ethyl Acetate Fraction Against A549 Lung Cancer Cells

Cytotoxicity analysis using MTT assay was performed as previously described by Fadholly et al.. A549 cells (ATCC CL185) were obtained from the Microbiology and Immunology Laboratory of the Center for Primate Animal Studies, LPPM IPB Bogor. The A549 cells were grown at a concentration of 5000 cells in 100 µL of growth medium (D-MEM or RPMI-1640) supplemented with 10% Fetal Bovine Serum (FBS) and 100U/mL penicillin, 100 ug/mg Streptomycin. The ethyl acetate fraction was added after cells reached 50% confluence within 24 h. The MTT assay was performed on day 3 by adding 10 µL of MTT (5 mg/mL) to each well and incubating for 4 h at 37°C. Formazan crystals were then dissolved in ethanol. The absorbance was measured at 595 nm using an ELISA reader and the work was repeated three times[18,19]. Persentase viabilitas sel dihitung sesuai dengan rumus berikut:

$$\text{Cell Viability (\%)} = \frac{\text{Absorbance of treated cells -Absorbance of blank}}{\text{Absorbance of controlles cells - Absorbance of blank}} \times 100 \quad (1)$$

Absorbance of treated cells = cells + media + ethyl acetate fraction + Solvent (ethanol)

Absorbance of controled cells = cells + media + DMSO

Absorbance of blank = media[20]

The half-maximal inhibitory concentration (IC_{50}) of ethyl acetate fraction of sungkai leaves was determined using GraphPad Prism trial software version 9.0[19].

Determining the Toxicity and Pharmacokinetic Properties of Molecules Computationally

The toxic properties of the main compound structure of sungkai can be determined based on toxicity risk values, such as irritation, mutagenicity, tumorigenicity, and reproductive problems, as well as physicochemical properties such as log P (coefficient of partition octanol-water), solubility (log S), molecular weight, drug similarity, and drug score, which can be calculated using the OSIRIS Property software free version[21–23].

Molecular Docking

The interaction of compounds from the ethyl acetate fraction of sungkai leaves with proteins was determined using the AutoDock Vina program[24]. The optimization results were in the form of optimal molecular structure, bond length, and activation energy, which were then analyzed to determine the stability of the interaction between the compound and the protein[25]. The positive control used was quercetin and the protein used was EGFR (PDB ID: 5UG9)[4], which can be downloaded in www.rcsb.org. This protein file was downloaded in the PDB format. After the protein was downloaded, it was saved in PDB format, the protein was prepared using the AutoDock Vina program free version, the water molecules were removed, and the test protein ligand was released[26].

Results and Discussion

Secondary Metabolic Analysis of Ethyl Acetate Fraction by LC-MS/MS

Fractionation of approximately 20 g of methanol extract from Sungkai leaves yielded about 5.93 g of the hexane fraction, 5.08 g of the ethyl acetate fraction, and 1 g of the residual aqueous fraction. Secondary metabolite profiling was conducted on the ethyl acetate fraction using LC-MS/MS analysis. The LC-MS/MS results are presented as chromatograms depicting peak height and retention time (figure 2.), enabling the identification of compound molecular weights

within the extract and accurate quantification in each sample[27]. Based on retention time data, m/z values, and MS/MS fragmentation patterns, a total of thirty-seven chemical constituents were identified in the ethyl acetate fraction of Sungkai leaves after the comparison with reference databases. These compounds belong to several classes of secondary metabolites, including flavonoids and anthraquinones. Among the thirty-seven identified compounds, five with the highest peak area percentages were selected, as shown in Table 1. These include four flavonoids (isorhamnetin, pilosin, (3R)-sophorol, and takakin) with isorhamnetin exhibiting the highest area percentage at 12.71%. The anthraquinone compound, physcion was also detected, contributing an area percentage of 8.03%. Previous studies have similarly reported the presence of flavonoids and anthraquinones in sungkai leaves extracts, supporting the consistency of the metabolite profile identified in this study.

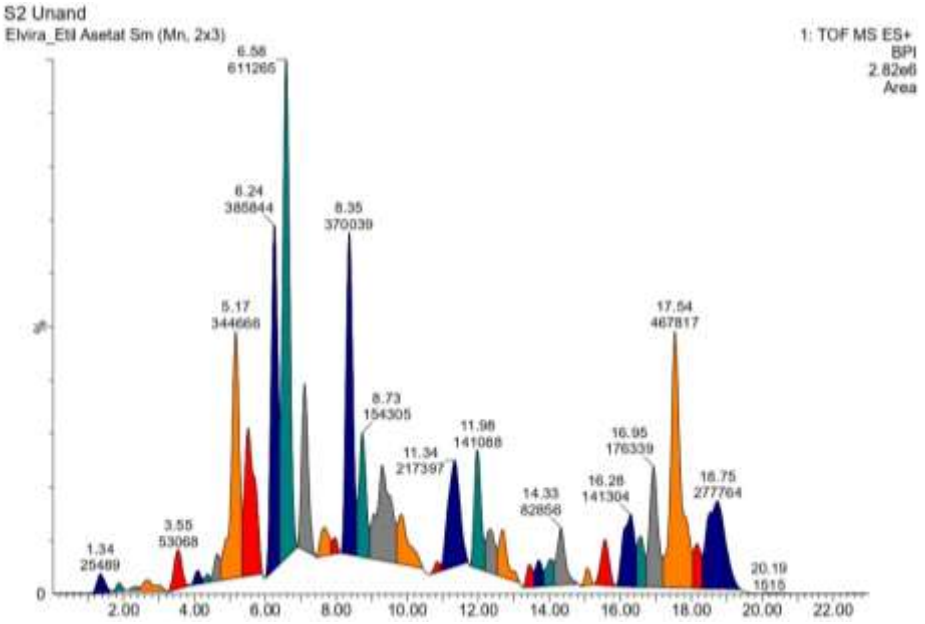
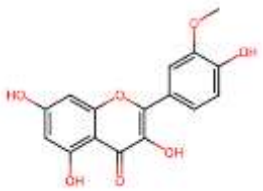
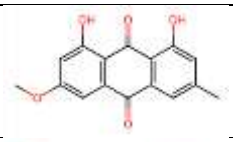
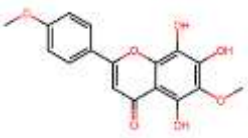
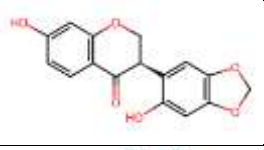
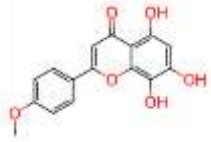


Figure 2. LC-MS/MS chromatogram result of ethyl acetate fraction

Table 1. Retention time, area percentage, and molecular weight of the main compounds in the ethyl acetate fraction of sungkai leaves.

No	Retention time (min)	Area Percentage (%)	Molecular weight	Structural Formula	Compound
1	6.58	12.71	316.265		Isorhamnetin
2	6.24	8.03	284.267		Physicon
3	8.35	7.7	330.292		Pilosin

Continued table 1.

4	5.52	5.56	300.266		(3R)-Sophorol
5	7.1	3.86	300.266		Takakin

Cytotoxic Activity of Ethyl Acetate Fraction of Sungkai Leaves Using the MTT Assay Method

The cytotoxic potential of the ethyl acetate fraction was assessed through determination of the IC_{50} value using the MTT assay. This colorimetric method relies on the enzymatic reduction of tetrazolium salt [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) into insoluble formazan crystals by mitochondrial succinate dehydrogenase in metabolically active cells[28,29]. Viable cells convert MTT into a purple formazan product exhibiting maximum absorbance at approximately 550-595 nm[30]. whereas non-viable cells lose this capability[31]. Cell viability was quantified based on the amount of formazan formed, measured spectrophotometrically after treatment with varying sample concentrations against A549 lung cancer cells. The absorbance data, presented in Figure 3, demonstrated an inverse relationship between sample concentration and absorbance, indicating reduced formazan formation at higher concentrations due to increased cell death. Consequently, diminished succinate dehydrogenase activity at elevated concentrations limits tetrazolium reduction[28]. The negative control (0 µg/mL) exhibited the highest absorbance, confirming the absence of cytotoxic effects under untreated conditions.

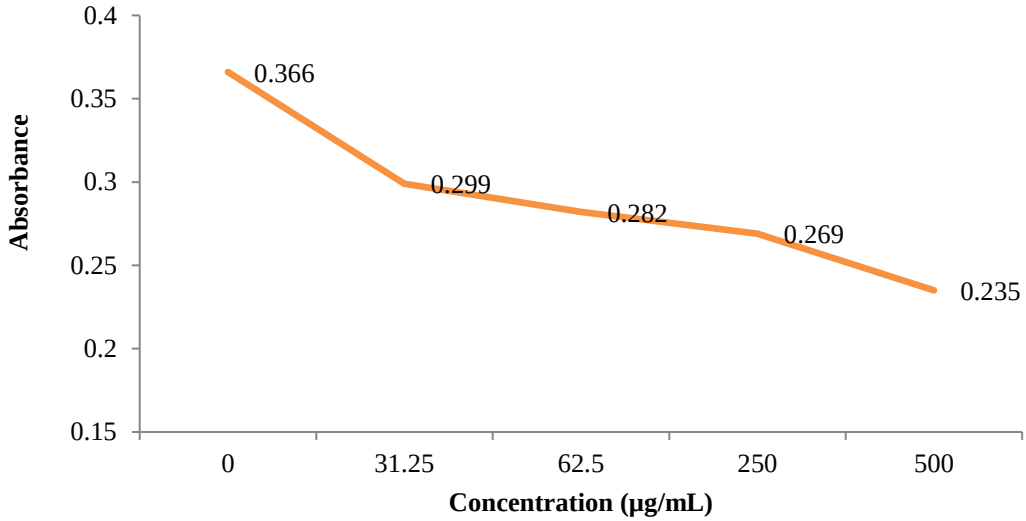


Figure 3. Graph of relationship between concentration of ethyl acetate fraction and absorbance

The morphologies of the cells before and after the treatment are shown in Figure 4. The figure shows that the higher the concentration of the test sample, the lower is the number of viable cells. In the control (Figure 4a) with a test sample concentration of 0 µg/mL, it can be seen that the cells have an oval shape, are clear, and are evenly distributed. The treatment resulted in a significant change in the viability of A549 cells. Cell viability decreased with increasing test sample concentrations. At a test sample concentration of 500 µg/mL (Figure 4e), almost all cells shriveled and clumped together, which was black, indicating cell death as evidenced by absorbance values and cell viability in each treatment in Figure 3 and Figure 5[32].

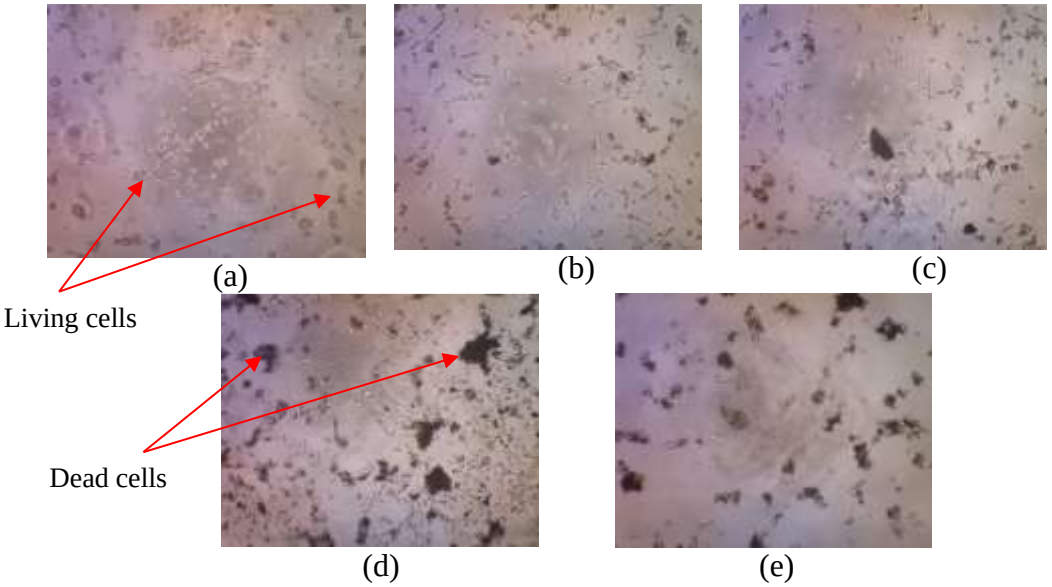


Figure 4. Morphology of A549 lung cancer cells a) control (0 µg/mL), b) 31.5 µg/mL, c) 62.5 µg/mL, d) 250 µg/mL, e) 500 µg/mL

Figure 5 shows the percentage viability of the A549 lung cancer cells at various concentrations. At 0 µg/mL, 100% cell viability indicated no cell death. At 500 µg/mL, 100 percentage of cell death was observed. This indicated that the cell growth was completely inhibited at this concentration.

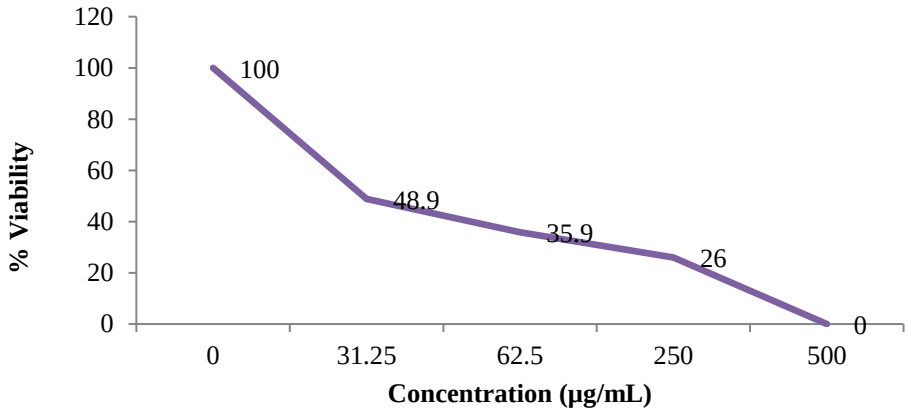


Figure 5. Graph of relationship between % viability of A549 lung cancer cells with concentration of ethyl acetate fraction

The cytotoxic activity of the ethyl acetate fraction of the sungkai leaves was calculated based on the IC_{50} value. The IC_{50} value is the concentration required to inhibit the growth of 50% cells. The results showed that the ethyl acetate fraction of sungkai leaves had an IC_{50} value of 33.41 µg/mL (Figure 6). Based on the National Cancer Institute (NCI) classification, this value was categorized as moderately cytotoxic. A compound can be considered highly cytotoxic if it has an IC_{50} value ≤ 20 µg/mL, moderately cytotoxic if it has an IC_{50} value of 21-200 µg/mL, weakly cytotoxic if it has an IC_{50} value of 201-500 µg/mL, and non-cytotoxic if it has an IC_{50} value > 501µg/mL[20]. A compound has the potential to be an anticancer drug if its IC_{50} value is < 100 µg/mL[28]. Thus, the ethyl acetate fraction of sungkai leaves has the potential to be used as an anticancer agent. Previous research conducted a cytotoxic test of crude extracts of sungkai leaves using different solvents, such as nano emulsion using ethanol extract of sungkai leaves on preputial cells showing an IC_{50} value of 82.2 µg/mL[33]. Research by Ibrahim et al. (2022) showed that the cytotoxic activity of ethyl acetate extract of sungkai leaves on HT-29 and HeLa cells had IC_{50} values of 48.635 µg/mL and 28.186 µg/mL, respectively[34].

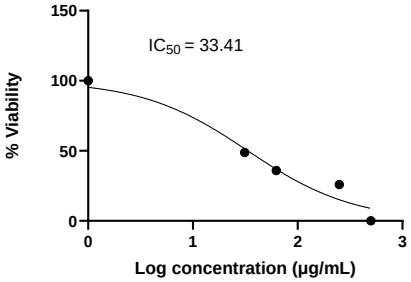


Figure 6. Relationship between log concentration of ethyl acetate fraction and % viability of A549 lung cancer cells

Molecular Docking of EGFR Protein

Five main components of the secondary metabolites from the flavonoid and anthraquinone groups were identified, and the cytotoxic activity of the ethyl acetate fraction against A549 lung cancer cells was moderate. Docking simulations were performed on five compounds against protein-ligand complexes with PDB ID: 5UG9, that is the crystal structure of the EGFR kinase domain using AutoDock Vina's software. Molecular docking was performed using a 3D crystal structure of the target protein, and the interaction between ligands and proteins was visualized using the Discovery Studio Visualizer software.

The first step in validating the docking results is the protein re-docking with native ligand. The protein is tethered with the native ligand: N-[(3R,4R)-4-fluoro-1-{6-[(3-methoxy-1-methyl-1H-pyrazol-4-yl) amino]-9-(propan-2-yl)-9H-purine-2-yl} pyrrolidin-3-yl]propanamide. Based on the results of redocking with grid box center x = -13.127, y = 14.012, and z = -25.57 and size x = 10.0, y = 12.0, and z = 12.0, the bond energy is -8.2 kcal/mol.

Root Mean Square Deviation (RMSD) is used to visualize docking results. The RMSD value obtained from the validation results is 1.935 Å, which indicates that the validation results are acceptable because the RMSD ≤ 2.0 Å. An RMSD value of less than 2 Å indicates that the position of the docked ligand is still quite close to its original position on the reference structure. Thus, the docking results can be considered feasible and quite accurate[35,36]. Molecular docking simulations are used to identify amino acid residues, bond energy, and RMSD values between protein and ligands. The bond energy values and amino acid residues involved as shown in Table 2 and Figure 7. Based on the data in Table 2, the bond energy of the five compounds ranges from -7.7 to -8.9 kcal/mol. The more negative the bond energy, the greater the potential of ligands in inhibiting EGFR proteins that act as receptors that promote cancer cell growth. The most negative bond energy value is shown by the physcion compounds, which is -8.9 kcal/mol. Based on the results of the docking simulation on a positive control (quercetin) with the protein, a smaller bond energy was obtained compared to the bond energy of physcion, isorhamnetin, and pilosin, which was -7.9 kcal/mol. Based on these data, physcion compounds have greater potential than quercetin compounds and native ligand (-8.2 kcal/mol) as drug candidates in inhibiting EGFR protein.

Based on the data in Figure 7, the interactions that occur between ligands and proteins involve van der Waals interactions and hydrogen bonds, which are mediated by various amino acid residues. Quercetin, as a positive control, showed six van der Waals interactions with residues such as LEU A:718, ALA A:743, and VAL A:726, and one strong hydrogen bond with GLN A:791, which had a bond length of 2.69 Å. Isorhamnetin showed more hydrogen bonds, especially with residues MET A:793, CYS A:797, ARG A:841, and THR A:854. Physcion formed two hydrogen bonds with MET A:793 and ALA A:743, at bond lengths of 1.54 and 2.61 Å, respectively.

In addition, physcion also exhibited van der Waals interactions with hydrophobic groups such as LEU A:844, VAL A:726, and PHE A:856. Pilosin exhibited similar interactions to quercetin but had more hydrogen bonds. (3R)-Sophorol exhibited strong van der Waals interactions with residues CYS A:797, MET A:790, ALA A:743, VAL A:726, and LEU A:844. Meanwhile, takakin formed two hydrogen bonds with residues GLN A:791 and CYS A:797. In addition, takakin also formed many van der Waals interactions with residues MET A:790, CYS A:775, ALA A:743, VAL A:726, LEU A:844, and LEU A:718. Overall, ligand binding to protein active sites involved a combination of van der Waals interactions with residues ALA A:743, VAL A:726, LEU A:718, and LEU A:844, as well as hydrogen bonding at MET A:793 and GLN A:791. The van der Waals interaction of the hydrophobic groups plays an important role in the formation of a stable complex between the ligand and protein but is also driven by the presence of additional interactions, such as hydrogen bonds, ionic interactions, electrostatic interactions, and van der Waals interactions[37]. In addition, the interaction strength

between the ligand and protein is influenced by the bond length, with a shorter bond length (with a distance cutoff of 3.5 Å)[38] resulting in a stronger interaction. When a compound binds to the EGFR protein, it inhibits the auto polymerization process thereby inhibiting the activation of the downstream signal cascade that plays an important role in cell polymerization, survival, and regulation of angiogenesis[39].

Table 2. Bond energy and RMSD of ligands with EGFR protein

No	Ligand	Bond energy (kcal/mol)	RMSD (Å)
1	Quercetin (positive control)	-7.9	1.810
2	Isorhamnetin	-8.2	1.935
3	Physcion	-8.9	1.200
4	Pilosin	-8.1	1.956
5	(3R)-Sophorol	-7.7	1.749
6	Takakin	-7.8	1.452

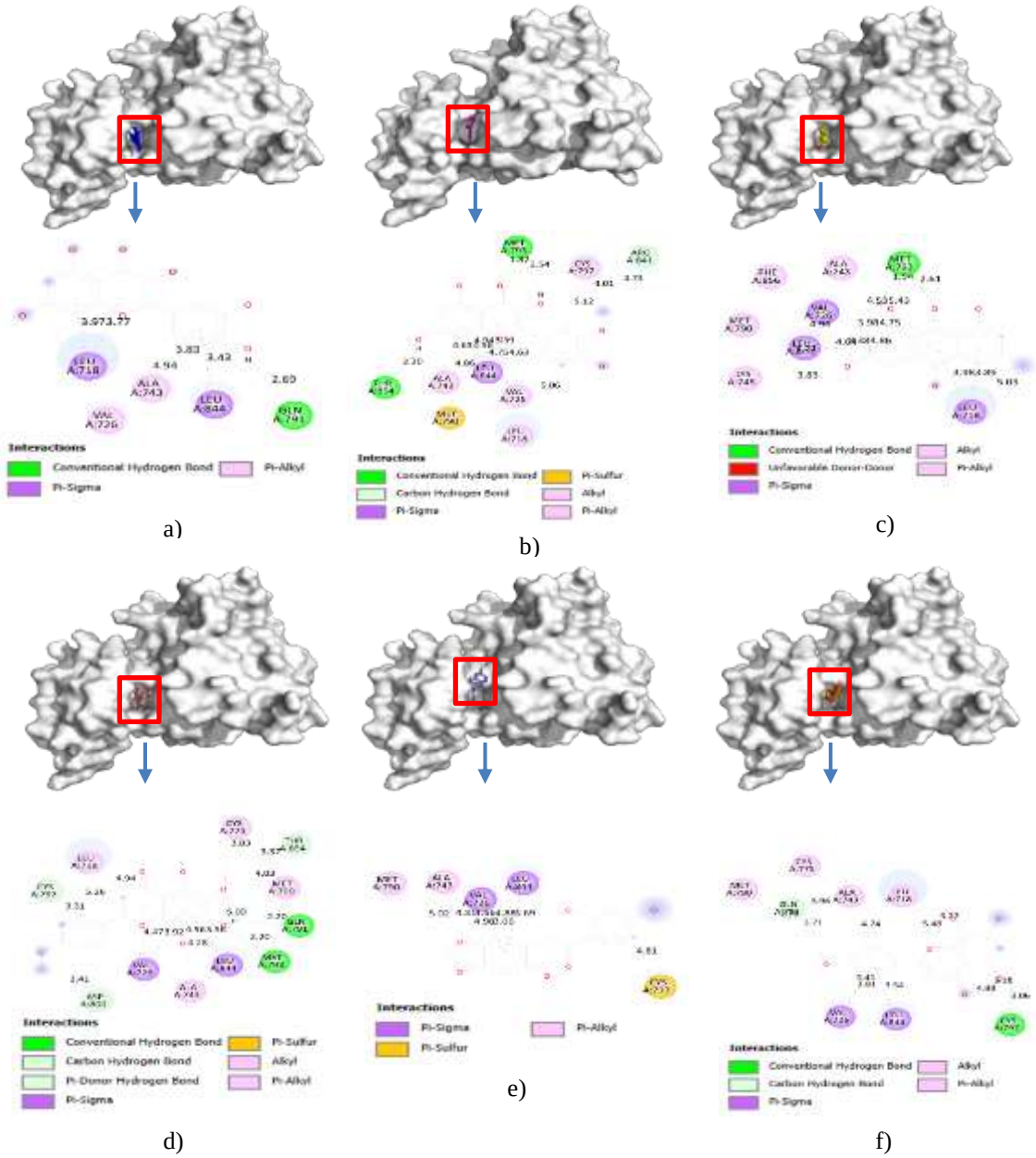


Figure 7. 3D and 2D interactions between EGFR protein and ligands: a) quercetin, b) isorhamnetin, c) physcion, d) pilosin, e) (3R)-sophorol, and f) takakin

Analysis of pharmacokinetic properties, toxicity, and drug scores of compounds

The relationship between the physicochemical properties and pharmacokinetics was determined using Lipinski's five rules: molecular weight < 500 Da, Hydrogen Bond Donor (HBD) < 5, Hydrogen Bond Acceptor (HBA) < 10, LogP < 5, and molar reactivity 40-130 Å[40–42]. Poor absorption or permeation occurs when there are more than 5 H-bond donors, 10 H-bond acceptors, molecular weight (MWT) greater than 500 Da and calculated Log P (CLogP) greater than 5 (or MlogP >4.15)[42]. The pharmacokinetic properties were analyzed using OSIRIS software, as presented in Tables 3 and 4. The solubility (log S) of a drug is an important parameter for understanding its dissolution in the digestive tract and absorption into the bloodstream. The solubility of a drug is influenced by surface area of its solid compound[21]. A drug is said to have potential if it has a solubility value of -6. Based on the data in Table 3, the solubility values of the five test compounds were -2.8 to -4.5, which means that they were still within the permissible range. A compound has good solubility if Log S is greater than -4, quite good with a Log S value between -4 and -6, and poor if the Log S value is less than -6[43].

Among the five test compounds, isohamnetin, takakin, and pilosin exhibited similar solubility values of -2.8, -2.87, and -2.89, respectively. Isorhamnetin had a greater solubility value, indicating that it is more soluble than takakin and pilosin in biological systems. The total surface area (TPSA) of the five compounds ranged from 83.83 to 116.4 Å, which was closely related to the absorption parameters. These results indicate that all compounds meet the Lipinski rule of five, namely, TPSA less than 150 Å, indicating high polarity with good oral absorption and membrane permeation[21].

Table 3. Pharmacokinetic values of the main compounds of the ethyl acetate fraction

Compound	Mark					
	Solubility (log S)	TPSA (Å)	Log P	HBA	HBD	Molecular Mass (g/mol)
(3R)-Sophorol	-3.79	85.22	2.84	6	2	300
Physicion	-4.5	83.83	2.62	5	2	284
isorhmanetin	-2.8	116.4	1.77	7	4	316
Takakin	-2.87	90.22	2.27	6	3	300
Pilosin	-2.89	104.4	2.2	7	3	330

Hydrogen bonds were formed between the lone pair of electrons on an electronegative atom in a compound interact with hydrogen atoms in other compounds. Compounds that provide electronegative atoms are called Hydrogen Bond Acceptors (HBA), whereas compounds that provide hydrogen atoms are called Hydrogen Bond Donors (HBD). Based on the analysis of the HBA and HBD properties of the five test compounds, all compounds met the Lipinski Rule of five criteria, namely, the number of HBA ≤ 10 and HBD ≤ 5[43]. Compounds with HBA < 10 or HBD < 5 tend to have low membrane permeability and decreased oral bioavailability because they are more likely to be in an aqueous environment than to penetrate lipophilic membranes[44]. This value is also closely related to the balance among solubility, permeability, and metabolic stability. The greater number of HBAs relative to HBDs can be attributed to the ability of HBAs to form hydrogen bonds with donor groups, including water molecules and protein receptors. This interaction tends to be weaker than that involving HBD. Increasing the number of HBA groups generally has a minimal impact on membrane permeability. In contrast, HBD involves a hydrogen atom bonded to an electronegative atom, resulting in stronger interactions. This interaction increases polarity and strengthens the interaction with water. However, this higher polarity may hinder the ability of a compound to pass through hydrophobic cell membranes, thereby affecting its permeability and biological activity[43]. It can be concluded that compounds with high HBA or HBD counts may have reduced membrane permeability due to increased polarity, which limits passive diffusion.

Lipophilicity, or Log P, describes the ability of a compound to dissolve in oil or nonpolar solvents. The ideal Log P value is in the range of 1-3, which reflects the balance between the water solubility and membrane permeability. Compounds with a Log P value ≤ 1 tended to be more soluble but had low permeability. If the Log P value is greater than 3, the opposite is true; that is, high permeability but low solubility[21,43]. Based on the data in Table 3, the Log P values of all test compounds were in the range of 1-3, indicating that the compound had good lipophilicity, with a good balance between solubility and permeability. In addition, molecular weight also plays an important role in pharmacokinetic analysis, as molecular weight increases and oral absorption decreases[44]. Molecular weight also determines a

compound's ability to penetrate physiological membranes, reach and interact with targets, and eliminate the compounds from the body. Therefore, based on the Lipinski rule of five, the maximum recommended molecular weight is less than 500 Da, because it is easier to absorb, distribute, and eliminate. A molecular weight of the range 250-350 Da is considered optimal for better absorption, ease of interaction with the target, and uncomplicated elimination[43]. All compounds had an optimal molecular weight of 284-330 Da. All these molecules are easily absorbed, interact with the target, and are eliminated.

Table 4. Toxicity risk parameter values and drug scores of the main compounds of the ethyl acetate fraction

Compound	Risk of Toxicity				Drug Score
	Gene Mutation	Tumorigenic	Irritation	Production	
(3R)-Sophorol	1.0	1.0	1.0	0.6	0.25
Physcion	0.8	1.0	0.6	1.0	0.2
isorhamnetin	0.6	1.0	1.0	1.0	0.47
Takakin	0.6	1.0	1.0	1.0	0.31
pilosin	0.6	0.6	1.0	1.0	0.18

A molecule can be used as a drug if it has a positive bioactivity score, moderately active if the score is between 0 and -5, and inactive if the score is less than -5[45]. Based on the data in Table 4, it can be said that all compounds tested were potential drug candidates, because the drug score value was greater than 0. However, several compounds have a toxic risks in the toxicity test results. The toxicity risk parameters include genetic mutations, tumorigenic irritation, and reproduction[45]. There are three types of risks based on toxicity analysis: no risk (1), moderate risk (0.8), and high risk (0.6)[21]. Of all the compounds, three of them, namely, isorhamnetin, takakin, and pilosin, had a high risk of gene mutation, whereas physcion had a moderate risk. The risk of toxicity of a compound depends on the amount of medication administered. Therefore, smaller amounts will always act as a drug. To overcome the risk of toxicity from these compounds, further analysis is needed regarding the dosage and target of therapy[21].

Conclusion

Based on the experiments conducted on the cytotoxic activity of the ethyl acetate fraction of sungkai leaves and molecular docking, it was found that the ethyl acetate fraction of sungkai leaves had moderate cytotoxic activity against A549 lung cancer with an IC_{50} value of 33.41 µg/mL. Molecular docking analysis of the EGFR protein with isorhamnetin, physcion, pilosin, (3R)-Sophorol, and Takakin showed that all compounds had strong potential as an EGFR protein inhibitor, as indicated by negative bond energy, the resulting interaction between the ligand and protein and also value of RMSD < 2 Å. Furthermore, the pharmacokinetic analysis of all compounds met the Lipinski rule of five, and the toxicity properties of three compounds, namely, isorhamnetin, takakin, and pilosin, had a high risk of gene mutation, whereas physcion had a moderate risk. Therefore, it is recommended for further research to conduct further analysis for a more accurate risk assessment and analysis related to the dosage and target of therapy so that it can become an orally active drug for humans.

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Conflicts of Interest

The authors declare that the authors have financial interests/personal relationships that could be considered as potential conflicts of interest as follows: The author reports the financial support provided by the Faculty of Mathematics and Natural Sciences, Universitas Andalas (No. 06/UN.16.03.D/PP/FMIPA/2024). For the support and financial assistance that has been provided, so that this research can be carried out properly.

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Ethics Statements

This research does not require an ethics committee as it does not include studies on human subjects, human data or tissues, or animals.

Author Contribution

The authors confirm their contribution to this paper as follows: study conception and design: Suryati and Imelda; data collection: Elvira Deswita; analysis and interpretation of results: Suryati, Imelda, and Elvira Deswita; manuscript preparation:

Suryati. All authors reviewed the results and approved the final version of the manuscript.

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