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The Potential of the *Spatholobus littoralis* Hassk Plant as an Antioxidant and Prediction of the Mechanism of Activity Against ROS1 Kinase Receptor in Silico

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Abstract: Various phenolic compounds that exhibit antioxidant, anticancer, and anti-inflammatory activities are found in *Spatholobus.* Therefore, this study aimed to determine the antioxidant potential of *Spatholobus littoralis* wood in vitro and predict the mechanism of its activity against the ROS1 kinase receptor in silico. The ground-dried wood of *S. littoralis* was extracted with ethanol via maceration. The analysis of the total phenolic content (TPC) of extracts and fractions obtained from *S. littoralis* wood was determined by the Folin-Ciocalteu reagent. Similarly, the antioxidant activity was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (The ferric reducing antioxidant power) method. The human ROS1 kinase enzyme (4UXL and 3ZBF) was used to determine the molecular mechanism of the interaction from the genus *Spatholobus* in silico. The total ethanol extract, chloroform, and ethyl acetate fraction of *S. littoralis* showed a high content of phenolic compounds and antioxidant activity. Phenolic compounds in plants of the genus *Spatholubus* also showed good activity against ROS1 kinase receptors (3ZBF and 4UXL). In conclusion, the *S. littoralis* plant has the potential to be developed for the discovery of new drugs.

Keywords: *Spatholobus littoralis Hassk, antioxidant, ROS1 kinase receptors, In silico.*

1. Introduction

Spatholobus littoralis Hassk, known locally as Bajakah Tempala, is a plant that climbs on wood trees from the Phaseoleae tribe. This genus was discovered in 1842 by a German botanist and had 28 species that grow in the tropical forests of Indonesia (Ridder-Numan & Wiriadinata, 1985). Ethnopharmacologically, bajakah wood is used in traditional medicine in Kalimantan (Istiqomah & Safitri, 2021). Several studies have shown the pharmacological activities, such as anticancer (Aliviyanti et al., 2021), anti-inflammatory (Nastiti & Nugraha, 2022), antidiabetic (Arysanti et al., 2022) antioxidant (Fitriani et al., 2020; Iskandar et al., 2022), and antihepatotoxic (Adhityasmara & Ramonah, 2022). Furthermore, the chemical content of this genus *Spatholobus* generally contains flavonoids and tannins (Fitriani et al., 2020), phenolics (Iskandar et al., 2022), and steroids (Astuti et al., 2014). A previous study reported that the flavonoid compounds contained in *Spatholobus suberectus* included flavones, flavanones, chalcones, isoflavanes, and isoflavones (Liu et al., 2018; Peng et al., 2019). Some of these flavonoid compounds contain anti-inflammatory and inhibitory tyrosinase activity (Liu et al., 2018) and are cytotoxic against breast cancer cell lines (Peng et al., 2019), antioxidant and anticancer (Li et al., 2015). In silico studies on several compounds from the *Spatholobus* also showed anticancer (Tejasari et al., 2022) and anti-psoriasis (Prasetyorini et al., 2022) activity.

The search for new drugs from natural ingredients is not only based on empirical data but also computational methods to predict the pharmacokinetic and toxicological properties through an in silico study. This method is also beneficial to understanding the interaction between compounds and molecular targets and facilitates the testing of all interactions experimentally (Fang et al., 2018). The strength of the binding affinity can be determined by the Gibbs free energy value (ΔG kcal/mol) (Bajorath, 2015). Gibbs free energy minimizes the thermodynamic potential when a system reaches equilibrium at constant pressure and temperature. Furthermore, Gibbs energy value shows the spontaneity of a molecule or compound to bind to a receptor or target protein. A value of less than zero (0) shows that the bond between the compound and the target protein occurs spontaneously (Hill & Reilly, 2008).

Many studies are currently using ROS1 kinase as a target for new drug discovery. ROS1 kinase plays an essential role in several cellular processes, such as apoptosis, survival, cell migration, and transformation in various malignancies, including colorectal cancer, inflammatory myofibroblast tumors, ovarian cancer, and lung cancer cells (Vanajothi et al., 2022). The human ROS1 kinase receptors used were PDB 4UXL and 3ZBF. This study was conducted to determine the total phenolic content of extracts and wood fractions of *S. littoralis* plant stems and test the activity as antioxidants using the DPPH (1,1-diphenyl-2-picrylhydrazine) and the FRAP (The ferric reducing antioxidant power) methods. The mechanism activity against ROS1 receptor kinase in silico was also predicted.

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2. Method

Apparatus and Reagent

Glassware, analytical balance, evaporator Buchi Rotavapor R-114, and UV-Vis spectrophotometer were commonly used in this work. Powder and dryed of *S. littoralis*, ethanol, ascorbic acid, chloroform, *n*-hexan, ethyl acetic, aquadest, 1,1-diphenyl-2-picrylhydrazyl (DPPH, Aldrich), and gallic acid, were purchased and used without further purification. A hardware computer with Intel Xeon CPU specifications, 32 Gb RAM, 10 cores, and 500 Gb SSD was used. The software used AutoDock Tools 1.5.7, Pymol, Avogadro 2.0, LigPlot+2.2.8, and GIMP 2.0.

Preparation Extract and Fraction of *S. littoralis*

The wood of *S. littoralis* was obtained from the Pontianak, Indonesia market. The Faculty of Biology of Gadjah Mada University, Indonesia staff identified the plant, which confirmed that it is *Spatholobus littoralis* Hassk. The milled dried steam of *S. littoralis* (3 kg) was extracted exhaustively with ethanol by maceration for 24 hours. The extract was fractionated using solvents with increasing polarity, starting from *n*-hexane, chloroform, and ethyl acetate (Abubakar & Haque, 2020).

Phytochemical Screening

Phytochemical qualitative analysis of extracts and fractions obtained from the wood of the *S. littoralis* plant was carried out using reagents for the terpenoid, alkaloid, phenolic, and saponin tests. Terpenoid and steroid tests were carried out with the Salkowski reagent. Meanwhile, alkaloid, phenolic, and foam tests were carried out with Wagner reagent, iron (III) chloride, and saponins, respectively (Harborne, 1998).

Analysis of Total Phenolic Content (TPC)

Analysis of the TPC of extracts and fractions from *S. littoralis* wood was determined using the Folin-Ciocalteu procedure (Hagerman et al., 2000). The sample was reacted with Folin-Ciocalteu reagent and sodium carbonate solution. The tube was vortexed and heated at 50°C for 10 minutes, and the absorbance of the resulting blue mixture was recorded at 725 nm against a blank containing only solvent. Each sample and blank were replicated three times (n=3). Gallic acid was dissolved in ethanol at various concentrations, and absorbance was measured at the same wavelength. TPC was calculated as gallic acid equivalent (GAE) from a calibration curve of the relationship between absorbance and concentration.

Determination of Antioxidant Activity Using the DPPH Method

The antioxidant activity of each extract and wood fraction of *S. littoralis* was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method (Atun et al., 2018). In this method, DPPH is the source of free radicals and each sample was dissolved in ethanol at various concentrations. Each solution was then added with DPPH reagent and stored in a dark place at room temperature for 30 minutes. Three replications (n=3) of the data were collected for the absorbance which was measured at a wavelength of 516 nm using a spectrophotometer. Antioxidant activity was calculated as the percentage of DPPH that decreased compared to the control. In this study, antioxidant activity was expressed as IC_{50}.

Determination of Antioxidant Activity using FRAP (The Ferric Reducing Antioxidant Power)

The FRAP test was based on the ability to reduce yellow iron (containing Fe^{3+}) to blue iron complexes (containing Fe^{2+}) due to its reaction with electron-donating antioxidants in an acidic medium. The antioxidant test method with FRAP free radicals used is a modification of the procedure reported by Kumar (2012). The sample was dissolved in ethanol at a certain concentration, then 1 mL was taken, 1 mL of 0.2 M phosphate buffer (pH 6.6), and 1 mL of 1% $K_3Fe(CN)_6$ were added, then incubated for 20 minutes at 50° C. Furthermore, 1 mL of TCA was added after incubation and centrifuged at 3000 rpm for 10 minutes. After centrifugation, pipette 1 mL of the top layer into a test tube, and add 1 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$. The solution was left for 10 minutes and the absorbance was measured at 720 nm. A mixture of solutions without samples was used as a blank. Calibration curves were prepared using ascorbic acid solutions with various concentrations. FRAP values were expressed in mg ascorbic acid equivalent/g sample.

Prediction of the Mechanism of Activity Against ROS1 Kinase Receptors in Silico

Phenolic compounds from plants of the genus *Spathollobus* were obtained from data on the KNApSAcK website (http://www.knapsackfamily.com/knapsack_core/top.php) and information on the composition of flavonoid compounds from the *S. littoralis* plant (Sianipar et al., 2023). However, only 14 phenolic compounds most commonly found in the *Spathollobus* were selected, namely dihydroquercetin, butin, (-)-epicatechin, eriodictyol, liquiritigenin, prunasin, afromosin, cajanin, formononetin, 3',4',7-trihydroxyflavone, licochalcone A, (+)-dihydrokaempferol, plathymenin, and 6-methoxyeriodictyol. The 3D structure was obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and stabilized using Avogadro 2.0. Subsequently, the 3D structure of each compound was created in a pdb file using the Pymol software and converted into a pdbqt file using AutodockTools1.5.7 software.

The human ROS1 kinase enzyme protein structures (PDB IDs: 4UXL and 3ZBF) were retrieved from the Protein Data Bank (www.pdb.org) to predict the molecular interaction mechanisms between phenolic compounds from the genus *Spatholobus* in silico. Proteins were separated from solvents and ligands or residues using Pymol software and saved in the pdb extension. The docking process was carried out using AutoDockTools-1.5.7 software. Re-docking between receptors and natural ligands was carried out on a grid box, which can produce an RMSD (Root Mean Square Deviation) value < 2 Å, suggesting that the method used is valid (Balgaria et al, 2016). The binding energy was determined by the Gibbs free energy value (ΔG kcal/mol) (Bajorath, 2015). A Gibbs free energy value of less than zero (0) shows that the bond between the ligand and the target protein occurs spontaneously

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and is stable (Hill & Reilly, 2008). Furthermore, the binding energy (ΔG kcal/mol) of each ligand is compared with the native ligand and positive control (ascorbic acid). LigPlot+ 2.2.8 software was used to determine the interaction between the ligand and receptor. The result was visualized using the GIMP 2.0 software and saved in jpg format (Forli et al, 2016).

3. Results and Discussion

Several studies reported that the wood or roots of plants from species of the genus *Spathollobus* contain high levels of phenolic compounds and antioxidant activity (Fitriani et al., 2020; Iskandar et al., 2022). This study used samples of *S. littoralis* wood obtained from traditional markets in Pontianak, Kalimantan, Indonesia. A total of 3 kg of *S. littoralis* plant wood was dried and ground. Dry wood powder was extracted by maceration using technical ethanol (95%). The extract was concentrated (around 420 g) and fractionated using successive solvents *n*-hexane, chloroform, and ethyl acetate. After being concentrated, the results of extraction and fractionation resulted in the *n*-hexane fraction (13.63 g), chloroform (104.26 g), ethyl acetate (153.57 g), and 65 g of ethanol extract residue. Each extract and fraction were subjected to phytochemical tests, phenolic content analysis, and antioxidant activity using the DPPH and FRAP methods. Phytochemical test data shows that each *S. littoralis* wood extract and fraction contains phenolic compounds, flavonoids, and triterpenoids, except for the *n*-hexane fraction, which shows negative results for phenolic compounds and flavonoids.

Quantitative analysis of TPC using a spectrophotometer followed the Folin-Ciocalteu method (Hagerman et al., 2000). The sample was reacted with Folin-Ciocalteau reagent, and sodium carbonate was heated at 50°C for 10 minutes. The absorbance of each sample was measured using a spectrophotometer at a wavelength of 760 nm. Gallic acid was used as a standard for phenolic compounds. The calibration curve results obtained have a linear regression equation $y = 0.0043x + 0.0339$, with an R^2 value of 0.993. The TPC of each sample was expressed in mg Gallic Acid Equivalents (GAE) per gram sample.

Inhibitory activity (%I) was calculated as the percentage decrease in absorbance of the sample solution compared to the DPPH solution without a sample. The inhibitory activity obtained from each sample concentration was represented in a graph, and the regression equation was used to calculate the antioxidant activity, expressed as IC_{50} (inhibitory activity at a concentration of 50%). Antioxidant activity was measured using the FRAP method with ascorbic acid solution as a standard. The purpose of adding TCA is for the potassium ferrocyanide complex to precipitate. The addition of $FeCl_3$ also aimed to form a green to blue complex (Berlin blue). Antioxidant ability was measured by the ability of a sample to convert Fe^{3+} to Fe^{2+}. The FRAP value was expressed in mg ascorbic acid equivalent/g extract (Kumar, 2012). Table 1 shows the analysis results of each extract's TPC and antioxidant activity of *S. littoralis*.

The results of the analysis of TPC expressed as Gallic Acid Equivalent (mg/g sample GAE) showed that the ethyl acetate fraction had the highest content. The ethanol extract also showed a high content of phenolic compounds. Due to the mixture of ethanol extract with non-phenolic compounds, the TPC was lower than ethyl acetate. Phenolic compounds generally dissolve in relatively polar and semi-polar solvents. The high content of phenolic compounds is closely related to antioxidant activity. Antioxidant potential is usually associated with the molecular structure of phenolic compounds due to the presence of a conjugated π electron system that facilitates the donation of electrons from the hydroxyl group to radical oxidation.

Table 1. Total phenolic content (TPC) and antioxidant activity of each extract and fraction of *S. littoralis*

No	Sample extract and fraction wood of *S. littoralis*	TPC (mg/g sample GAE)	Antioxidant activity test against DPPH (IC_{50} (µg/mL)	Antioxidant activity using FRAP (mg Ascorbic Acid Equivalent/g sample)
1	Total ethanol extract	545.10 ± 9.81	2.12 ± 0.51	313.25 ± 8.83
2	*n*-Hexane fraction	78.88 ± 1.31	173.47 ± 14.06	6.54 ± 1.80
3	Chloroform fraction	308.67 ± 2.52	1.46 ± 0.98	126.53 ± 19.16
4	Ethyl acetate fraction	909.91 ± 1.00	2.61 ± 0.31	740.24 ± 13.24
5	Ascorbic acid (Positive control)		1.45 ± 0.02	-

The antioxidant activity test using the DPPH method was based on the radical capture reaction formed by releasing hydrogen radicals from phenolic compounds, which will produce DPPH-H molecules in non-radical form (Molyneux, 2003). Total ethanol extract, chloroform fraction, and ethyl acetate fraction from *S. littoralis* wood showed IC_{50} values below 10 µg/mL, suggesting a very high activity. Ascorbic acid, a widely utilized natural antioxidant in various food, pharmaceutical, and cosmetic products, was employed as a positive control. The results of the analysis are consistent with the report of previous studies that plants from the genus *Spatholobus* are rich in phenolic compounds, such as isoflavones, flavanones, flavans, isoflavanols, chalcones, lignans, and others (Huang et al., 2023; Nguyen-Ngoc et al., 2022). The occurrence of cell damage in organisms due to free radicals requires chemical compounds that function as antioxidants in the body. Phenolic compounds from plants generally have strong antioxidant properties and a natural effect in preventing various diseases related to oxidative stress, such as cancer (Dai & Mumper, 2010). Furthermore, the antioxidant activity capacity using the FRAP method was based on the ability of the compounds to reduce Fe^{3+} ions to Fe^{2+}. The antioxidant capacity was calculated from a linear calibration curve and expressed as ascorbic acid equivalent per gram of sample. A weakness of the FRAP method is that not all Fe^{3+} reductants are antioxidants, and some are not able to reduce Fe^{3+} (Hidalgo, 2017). However, the results of this study showed that the data are

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similar. The total ethanol extract, chloroform fraction, and ethyl acetate fraction of *S. littoralis* showed excellent antioxidant activity, while the *n*-hexane fraction was weak. This is related to the phenolic compound content of each fraction.

Molecular mechanism of interaction from the genus *Spatholobus* on the ROS1 kinase receptor in silico using PDB code proteins include 3ZBF and 4UXL, downloaded from Protein Data Bank (www.pdb.org). Validation of the redocking method for the 3ZBF receptor produced a grid box with center x: 42.521; y: 19.649; z: 3.987, with box size x=y =z =30 Å, with an RMSD (root-mean-square deviation) value of 0.687 Å. The 4UXL receptor produced a grid box with center x: 42.587; y: -19.777; z: -5.719, with box size x=y =z =30 Å, and an RMSD value of 0.143 Å. This value meets the valid criteria for a docking method because the RMSD is <2 Å. Table 2 shows the results of the docking analysis of 14 phenols, redocking of native ligand, and positive control (ascorbic acid) against the 3ZBF receptor, while the 4UXL receptor is in Table 3. The data include binding energy (ΔG kcal/mol), hydrogen bonds, and hydrophobic interactions.

Information obtained from molecular docking includes binding energy and interaction of the ligand with amino acid residues of the receptor (Du, X, 2016). The binding energy between the ligand and receptor was expressed in Gibbs free energy (ΔG kcal/mol). The result of redocking between the 3ZBF receptor and the native ligand VGH {(3-[(1R)-1-(2,6-dichloro-3-fluorophenyl)ethoxy]-5-(1-piperidin-4-yl-1H-pyrazol-4- yl) pyridine-2-amine)} shows the minor binding energy (-10,590 kcal/mol), which is the most stable. Among the 14 phenolic compounds that showed the highest activity, licochalcone-A had the most binding energy of -8.823 kcal/mol. This compound also showed a higher binding energy than ascorbic acid which had a binding energy of -5.028 kcal/mol.

Results from redocking of 4UXL receptor and native ligand 5P8 {(10R)-7-amino-12-fluoro-2,10,16-trimethyl-15-oxo-10,15,16,17-tetrahydro-2H-8,4-(methanol)-pyra-zolo[4,3-h]-[2,5,11] benzoxadiazacyclotetradecine-3-carbonitrile)} shows an energy affinity of -11,400 kcal/mol. This implies that the native binding of the ligand to the 4UXL receptor is relatively stable. The most stable energy affinity of the 14 phenolic compounds tested was licochalcone-A at -9,384 kcal/mol. The antioxidant activity test with the DPPH ascorbic acid reagent had the highest antioxidant activity. However, analysis using the docking method with 3ZBF and 4UXL receptors showed a lower binding energy compared to the phenolic compounds tested from *Spatholobus*. This result shows that the bond between ascorbic acid and the 3ZBF and 4UXL receptors is due to the relatively small size compared to other phenolic compounds. Licochalcone A, is a phenolic compound that has activity as an antibacterial (Tsukiyama et al, 2022), antioxidant, and anti-aging (Ara et al, 2023). The compound also showed anti-inflammatory activity through inhibition of COX-2 synthesis (Cui et al, 2007).

Various factors influence the binding energy between the ligand and the receptor, including electrostatic interactions, Van der Walls forces, hydrophobic bond interactions, hydrogen bonds, and flexibility of the receptor structure. However, the data obtained showed that the greater the number of hydrogen bonds and hydrophobic interactions formed between the ligand and receptor, the stronger the bond (Du et al., 2016). Observation of hydrophobic interaction data between the ligand and the 3ZBF and 4UXL receptors (Tables 2 and 3) showed that the amino acid residue often present in the interaction was Leu 2026, Leu 2028, and Leu 2086.

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Table 2. The Binding Energy of the Ligands, as Well as Their Interaction with the Amino Acid Residues of the Human ROS1 Kinase Receptor 3ZBF

No	Ligands/ Compound	Binding Energy (kcal/mol)	Hydrogen bond	Hydrophobic interaction
1	VGH (Native ligand)	-10.590	Glu2027	Leu2026, Val1959, Leu2028, Met2029, Ala1978, Gly2032, Leu1951, Leu2086, Leu2010, Arg2083, Lys1980
2	Ascorbic acid (positive control)	-5.028	Met2029	Leu1951, Leu2028, Leu2086, Leu2026, Ala1978, Val1959
3	Licochalcone-A	-8.823	Glu1961	Leu2026, Leu2086, Leu2010, Ala1978, Met2029, Glu2027, Glu2030, Gly2032, Leu2028, Leu1951, Val1959
4	Cajanin	-8.559	Lys1980; Met2029	Leu2026, Leu2086, Ala1978, Leu2028, Gly2032, Glu2030, Leu1951, Val1959, Gly2101, Asp2102
5	6-Methoxyeriodictyol	-8.244	Met2029; Gly2101	Leu2026, Leu2086, Glu2030, Gly2032, Leu1951, Val1959, Asp2102, Leu2010
6	Eriodictyol	-8.105	Met2029; Lys1980 Asp2102	Leu2086, Leu2028, Gly2032, Leu1951, Val1959
7	Dihydroquercetin	-8.036	Met2029; Asp2033	Leu1951, Gly2032, Val1959, Leu2086, Leu2026, Ala1978
8	Dihydrokaempferol	-8.007	Asp2102; Met2029 Lys1980	Leu2028, Ala1978, Leu1951, Gly2032, Leu2086, Val1959
9	Plathymenin	-7.928	Lys1980; Met2029 Asp2102	Val1959, Leu1951, Gly2032, Ala1978, Leu2028, Leu2086
10	Liquiritigenin	-7.924	Lys1980; Met2029 Asp2102	Leu2086, Leu2028, Ala1978, Leu1951, Gly2032, Val1959
11	Epicatechin	-7.870	Asp2102; Lys1980 Met2029	Val1959, Leu1952, Gly2032, Ala1978, Gly2101, Leu2086
12	3', 4', 7'-trihidroxyflavone	-7.779	Asp2102; Lys1980 Met2029	Leu2086, Ala1978, Gly2032, Leu1951, Leu2028, Leu2026
13	Butin	-7.760	Asp2102; Met2029 Lys1980	Leu2086, Leu2028, Ala1978, Gly2032, Leu1951, Val1959
14	Formononetin	-7.540	-	Gly2101, Leu2026, Leu2086, Ala1978, Met2029, Leu2028, Gly2032, Leu1951, Val1959, Lys1980, Asp2102
15	Prunasin	-7.356	Met2029; Glu2027	Ala1978, Arg2083, Asp2102, Asn2084, Gly2101, Leu2026, Lys1980, Leu2086, Leu2028, Gly2032, Leu1951
16	Afrormosin	-7.171	Lys1980	Leu2010, Gly2101, Leu2086, Asp2033, Gly2032, Leu1951, Met2029, Val1959, Leu2026

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Table 3. The Binding Energy of the Ligands, as Well as Their Interaction with the Amino Acid Residues of the Human ROS1 Kinase Receptor 4UXL

No	Ligands/ Compound	Binding Energy (kcal/mol)	Hydrogen bond	Hydrophobic interaction
1	5P8 (native ligan)	-11.400	Met2029; Glu2027	Glu2030, Ala1978, Leu2026, Leu2010, Leu2086, Gly2101, Arg2083, Val1959, Gly2032, Leu1951, Leu2028
2	Ascorbic acid (positive control)	-5.244	Leu2028; Lys1976 Ser2088; Glu2030	Glu2027
3	Licochalcone-A	-9.384	Glu1961	Glu2030, Met2029, Leu2028, Ala1978, Glu2027, Leu2086, Leu2010, Leu2026, Val1959, Gly1952, Leu1951, Gly2032
4	Eriodictyol	-9.116	Lys1980; Leu1951 Met2029; Asp2102	Val1959, Gly2101, Leu2028, Leu2086
5	Plathymenin	-9.080	Asp2102; Met2029 Lys1980	Leu2086, Gly2101, Ala1978, Leu2028, Leu1951, Val1959
6	Dihydrokaempferol	-9.060	Lys1980; Met2029	Gly2101, Leu2026, Val1959, Leu1951, Leu2028, Ala1978, Leu2086
7	6-methoxyeriodictyol	-8.996	Gly2101; Leu1951 Met2029	Asp2102, Val1959, Gly2032, Glu2030, Leu2086, Leu2026
8	Liquiritigenin	-8.961	Lys1980; Met2029 Asp2102	Leu1951, Ala1978, Leu2028, Leu2086, Gly2101
9	Dihydroquercetin	-8.912	Gly2101; Met2029 Leu1951	Asp2102, Leu2086, Gly2032, Glu2030, Val1959, Leu2026
10	Butin	-8.908	Lys1980; Met2029 Asp2102	Val1959, Leu1951, Leu2028, Ala1978, Leu2086, Gly2101
11	Cajanin	-8.745	Lys1980; Asp2102	Val1959, Leu1951, Met2029, Leu2086, Gly2101
12	3'-4'-7'-Trihidroxyflavone	-8.608	Met2029; Lys1980	Leu2026, Gly2032, Leu1951, Leu2086, Leu2028, Ala1978
13	Epicatechin	-8.587	Leu1951; Met2029 Lys1980	Gly2101, Asp2102, Val1959, Gly2032, Glu2030, Leu2028, Ala1978, Leu2086
14	Formononetin	-8.250	Lys1980	Asp2102, Leu2026, Gly2101, Leu1951, Leu2028, Gly2032, Glu2030, Met2029, Leu2086, Val1959
15	Afrormosin	-7.962	-	Leu2086, Leu1951, Met2029, Glu2030, Val1959, Gly1954
16	Prunasin	-7.624	Met2029; Glu2027	Arg2083, Lys1980, Leu1951, Gly2032, Leu2028, Leu2086, Ala1978, Val1959, Leu2026

Phenolic compounds in plants of the *Spatholobus* genus generally have phenol groups, which allows for interaction with amino acids through hydrogen bonds and hydrophobic interactions. Figure 1 shows the visualization of the interaction of licochalcon-A against the 3ZBF receptor. Meanwhile, Figure 2 shows the visualization of the interaction of licochalcon-A against receptor 4UXL. Hydrogen bonds are between molecules with a hydrogen atom bonded to an atom with high electronegativity.

The partial positive charge in hydrogen bonds comes from the H atom of the ligand. However, the partial negative charge results from from highly electronegative atoms, such as oxygen, nitrogen, flour, and sulfur in the amino acid residues of the receptor. Hydrophobic interactions occur between non-polar groups and enhance protein stability by increasing the entropy of water molecules.

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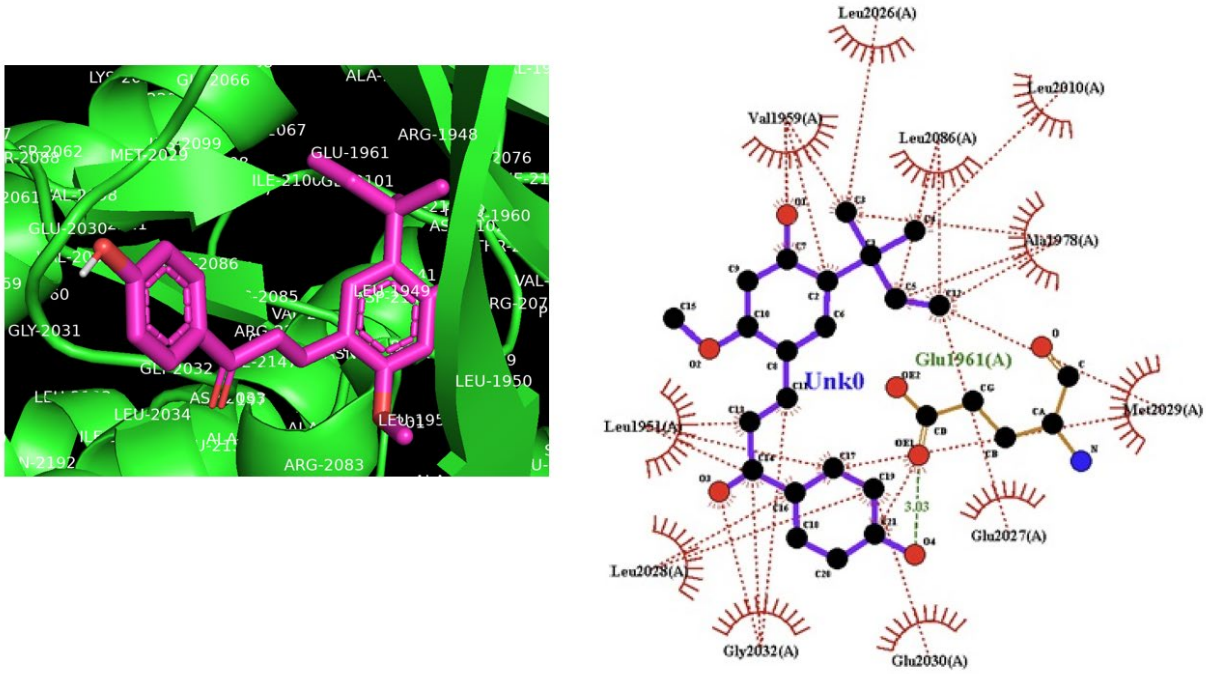

Figure 1. Visualization of interactions between licochalcone-A against receptor 3ZBF (Hydrogen bonds on the green line and hydrophobic interactions on the red line)

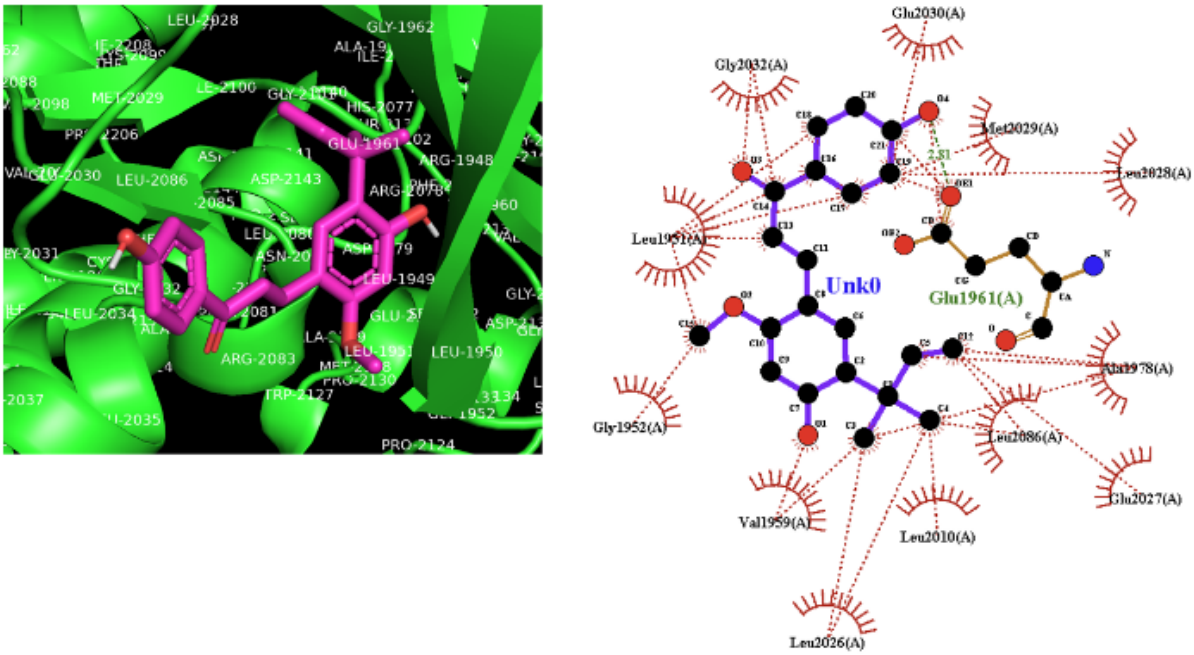

Figure 2. Visualization of interactions between licochalcone-A against receptor 4UXL (Hydrogen bonds on the green line and hydrophobic interactions on the red line)

Several plants of the genus *Spatholobus* have been widely used as part of crucial traditional medicine. For example, *Spatholobus suberectus* grape stems are an essential medicinal ingredient in traditional Chinese, Vietnamese, and Korean medicine (Huang et al., 2023; Nguyen-Ngoc et al., 2022). The plant was used to treat blood disorders, such as anemia, menstrual irregularities, and rheumatic diseases. Similarly, the wood of the *Spatholobus littoralis* plant was widely used in traditional medicine in Kalimantan, Indonesia. The main compounds found in plants of the genus *Spatholobus* are from the phenolic group, especially flavonoids. The results of this study are consistent with previous reports regarding the content of phenolic compounds and

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antioxidant activity in plants of the genus *Spatholobus*. Proof through molecular docking using ROS1 kinase receptors (3ZBL and 4UXL) showed that there are hydrogen bonds and hydrophobic interactions, and some compounds have relatively small energy affinities, despite being relatively more extensive when compared to the native ligands.

ROS1 is a receptor tyrosine kinase that has been shown to undergo genetic rearrangement in various types of human cancer, including glioblastoma, non-small cell lung cancer (NSCLC), ovarian cancer, and others. This rearrangement can produce fusion proteins, thereby promoting cell proliferation (Davies & Doebele, 2013). Previous studies showed that ROS1 played a significant role in several cellular processes, such as apoptosis, survival, cell migration, and transformation in various malignancies including colorectal, ovarian, and NSCLC, as well as inflammatory myofibroblast tumors. Therefore, ROS1 has become a potential drug target. Several studies showed a relationship between compounds that act as antioxidants and have anticancer activity (Grigalius & Petrikaite, 2017; Milella et al., 2023). Several compounds found in plants of the genus *Spatholobus* show antioxidant and anticancer activity (Huang et al., 2023; Li et al., 2015; Nguyen-Ngoc et al., 2022)

4. Conclusion

In conclusion, *S. littoralis* was a medicinal plant widely used in traditional medicine in Kalimantan, Indonesia, containing phenolic compounds and high levels of antioxidant activity. Phenolic compounds in plants of the genus *Spatholubus* also showed high binding energy to ROS1 kinase receptors (3ZBF and 4UXL). Observation of hydrophobic interaction data between the ligand and the 3ZBF and 4UXL receptors showed that the amino acid residues frequently included in the interaction were Leu2026, Leu2028, and Leu2086.

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6. References

Abubakar, A.R. & Haque, M. (2020). Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. *J Pharm Bioallied Sci*. *12*(1):1-10. https://doi.org/ 10.4103/jpbs.JPBS_175_19.

Adhityasmara, D., & Ramonah, D. (2022). Efek hepatoprotektor ekstrak etanol batang bajakah tampala (*Spatholobus littoralis* hassk) pada tikus yang diinduksi isoniazid. *Jurnal Ilmiah Sains*, *22*(1), 40. https://doi.org/10.35799/jis.v22i1.36293

Aliviyanti, R. U. Y., Sudibyo, R. S., & Murwanti, R. (2021). Efek sitotoksik beberapa akar bajakah kalimantan terhadap sel kanker payudara T47D. *Jurnal Penelitian Saintek*, *26*(2). https://doi.org/10.21831/jps.v26i2.41211

Ara, I., Rita Turcio, R., Islam, T., Hossain S., Md., Hasan, K. Md. (2023). Anti-aging related activities and health benefits of Licochalcone A: A review, *Clinical Complementary Medicine and Pharmacology*, 100125:1-32. https://doi.org/10.1016/j.ccmp.2023.100125.

Arysanti, R. D., Wirjatmadi, B., & Winarni, D. (2022). Effect of Bajakah (*Spatholobus littoralis* Hassk.) extracts on malondialdehyde serum of Wistar rats induced by streptozotocin. *Jurnal Kesehatan Prima*, *16*(2), 143. https://doi.org/10.32807/jkp.v16i2.837

Astuti, M. D., Maulana, A., & Kuntowati, E. M. (2014). Isolasi steroid dari fraksi *n*-heksana batang kayu bajakah tempala (*Spatholobus littoralis* Hassk.). *Prosiding Seminar Nasional Kimia, Jurusan Kimia FMIPA Universitas Negeri Surabaya*.

Atun, S., Handayani, S., Rakhmawati, A., Aini Purnamaningsih, N., An Naila, B. I., & Lestari, A. (2018). Study of potential phenolic compounds from stems of *Dendrophthoe falcata* (Loranthaceae) plant as antioxidant and antimicrobial agents. *Oriental Journal of Chemistry*, *34*(5), 2342–2349. https://doi.org/10.13005/ojc/340515

Balgaria, A., Jaravine, V., Huang, Y.J., Montelino, G.T., Güntert, P. (2016). Protein structure validation by generalized linear model root-mean-square deviation prediction. *Protein Science*, *21*(2): 229–238. https://doi.org/10.1002/pro.2007

Bajorath, J. (2015). Computer-aided drug discovery. *F1000Research*, *4*, 630. https://doi.org/10.12688/f1000research.6653.1

Cui, Y., Mingzhang, A. M., Li, W., Hu, J., and Yu L. (2007). Anti-Inflammatory Activity of Licochalcone A Isolated from Glycyrrhiza inflata, *Z. Naturforsch*. *63*(c): 361-365.

Dai, J., & Mumper, R. J. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*, *15*(10), 7313–7352. https://doi.org/10.3390/molecules15107313

Davies, K. D., & Doebele, R. C. (2013). Molecular pathways: ROS1 fusion proteins in cancer. *Clinical Cancer Research*, *19*(15), 4040–4045. https://doi.org/10.1158/1078-0432.CCR-12-2851

Du, X., Li, Y., Xia, Y.L., Ai, S.M., Liang, J., Sang, P., Ji, X.L., & Liu, S.Q. (2016). Insights into protein-ligand interactions: Mechanisms, models, and methods. *International Journal of Molecular Sciences*, *17*(2), 144. https://doi.org/10.3390/ijms17020144

Fang, J., Liu, C., Wang, Q., Lin, P., Cheng, F. (2018) In silico polypharmacology of natural products. *Brief Bioinform*. *19*(6):1153-71. https://doi.org/10.1093/bib/bbx045.

DOI:https//doi.org/10.22452/mjs.vol44no2.4
Malaysian Journal of Science 44(2): 42-51 (June 2025)

Fitriani, F., Sampepana, E., & Saputra, S. H. (2020). Karakterisasi Tumbuhan akar bajakah (*Spatholobus littoralis* Hassk) dari Loa Kulu Kabupaten Kutai Kartanegara. *Jurnal Riset Teknologi Industri*, *14*(2), 365. https://doi.org/10.26578/jrti.v14i2.6590

Forli, S., Huey, R., Pique, M.E., Sanner, M.F., Goodsell, D. S., Olson, A.J. (2016). Computational protein–ligand docking and virtual drug screening with the AutoDock suite. *Nature Protocols 11*(5): 905–919. https://doi.org/10.1038/nprot.2016.051

Grigalius, I., & Petrikaite, V. (2017). Relationship between antioxidant and anticancer activity of Trihydroxyflavones. *Molecules*, *22*(12), 2169. https://doi.org/10.3390/molecules22122169

Hagerman, A., Harvey-Mueller, I., Makkar, A. H., Mueller, I. H., & Makar, H. P. S. (2000). *Quantification of tannins in tree and shrub foliage: A laboratory manual*. FAO/IAEA.

Harborne, A. J. (1998). *Phytochemical methods a guide to modern techniques of plant analysis* (3rd ed.). Springer.

Hidalgo, G.-I., & Almajano, M.P. (2017). Red fruits: extraction of antioxidants, phenolic content, and radical scavenging determination: A Review. *Antioxidants* (Basel). 19, 6(1): 1-27. https://doi.org/10.3390/antiox6010007

Hill, A. D., & Reilly, P. J. (2008). A Gibbs free energy correlation for automated docking of carbohydrates. *Journal of Computational Chemistry*, *29*(7), 1131–1141. https://doi.org/10.1002/jcc.20873

Huang, X., Fei, Q., Yu, S., Liu, S., Zhang, L., Chen, X., Cao, L., Wang, Z., & Shan, M. (2023). A comprehensive review: Botany, phytochemistry, traditional uses, pharmacology, and toxicology of *Spatholobus suberectus* vine stems. *Journal of Ethnopharmacology*, *312*, 116500. https://doi.org/10.1016/j.jep.2023.116500

Iskandar, D., Widodo, N., Warsito, Masruri, Rollando, & Antang, Y. P. P. (2022). Phenolic content, antioxidant, cytotoxic of fractions of *Spatholobus littoralis* Hassk from Kalimantan, Indonesia. *Journal of Hunan University Natural Sciences*, *49*(3), 14–23. https://doi.org/10.55463/issn.1674-2974.49.3.2

Istiqomah, I., & Safitri, D. (2021). Pharmacological activities of *Spatholobus littoralis*. *Infokes: Info Kesehatan*, *11*(2), 463–469.

Kumar, S. (2012). Assay-guided comparison for enzymatic and non-enzymatic antioxidant activities with special reference to medicinal plants. InTech. Egypt. Mansoura University. http:/doi.org/ 10.5772/50782

Li, W., Liu, J., Guan, R., Chen, J., Yang, D., Zhao, Z., & Wang, D. (2015). Chemical characterization of procyanidins from *Spatholobus suberectus* and their antioxidative and anticancer activities. *Journal of Functional Foods*, *12*, 468–477. https://doi.org/10.1016/j.jff.2014.11.009

Liu, R.X., Xu, Y.L., Ma, L.F., Ying, Y.M., & Zhan, Z.J. (2018). A new flavanone from *Spatholobus suberectus* dunn. *Journal of Chemical Research*, *42*(10), 529–530. https://doi.org/10.3184/174751918X15386515371813

Milella, R. A., De Rosso, M., Gasparro, M., Gigante, I., Debiase, G., Forleo, L. R., Marsico, A. D., Perniola, R., Tutino, V., Notarnicola, M., Velasco, R., & Flamini, R. (2023). Correlation between antioxidant and anticancer activity and phenolic profile of new Apulian table grape genotypes (V. Vinifera L.). *Frontiers in Plant Science*, *13*. https://doi.org/10.3389/fpls.2022.1064023

Molyneux, P. (2003). The use of the stable radical Diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *J.Sci. Technol*, *26*(2), 211–219.

Nastiti, K., & Nugraha, D. F. (2022). Aktivitas antiinflamasi ekstrak kayu bajakah (*Spatholobus littoralis* Hask). *Jurnal Surya Medika*, *7*(2), 45–50. https://doi.org/10.33084/jsm.v7i2.3202

Nguyen-Ngoc, H., Vu-Van, T., Pham-Ha-Thanh, T., Le-Dang, Q., & Nguyen-Huu, T. (2022). Ethnopharmacology, phytochemistry, and pharmacological activities of *Spatholobus suberectus* vine stem. *Natural Product Communications*, *17*(12), 1934578X2211427. https://doi.org/10.1177/1934578X221142724

Peng, F., Zhu, H., Meng, C.-W., Ren, Y.-R., Dai, O., & Xiong, L. (2019). New isoflavanes from *Spatholobus suberectus* and their cytotoxicity against human breast cancer cell lines. *Molecules*, *24*(18), 3218. https://doi.org/10.3390/molecules24183218

Prasetyorini, B. E., Kusumawardani, A., Fitriani, F., Rachman, P. O., Amelinda, N., & Ramadhani, A. (2022). Analisis in silico senyawa aktif batang kayu bajakah (*Spatholobus littoralis* hassk) sebagai terapi psoriasis. *Herb-Medicine Journal*, *5*(1), 26. https://doi.org/10.30595/hmj.v5i2.12744

Ridder-Numan, J. W. A., & Wiriadinata, H. (1985). A revision of the genus *Spatholobus* (Leguminosae-Papilionoideae). *Journal on Taxonomic Botany*, *10*(2), 107–270.

Sianipar, R.N.R., Suryanegara, L., Fatriasari, W., Arung, E.T., Kusuma, I.W., Achmadi, S.S., Azelee, N.I.W., Hamid, Z.A.A. (2023). The role of selected flavonoids from bajakah tampala (*Spatholobus littoralis* Hassk.) stem on cosmetic properties: A review, *Saudi Pharmaceutical Journal, 31*(3):382-400. https://doi.org/10.1016/j.jsps.2023.01.006

Tejasari, M., Respati, T., & Yuniarti, L. (2022). Exploring anticancer potential in Bajakah tampala by in silico virtual screening. *KnE Life Sciences*. https://doi.org/10.18502/kls.v7i5.12517

DOI:https//doi.org/10.22452/mjs.vol44no2.4
Malaysian Journal of Science 44(2): 42-51 (June 2025)

Tsukiyama, R., Katsura, H., Tokuriki, N., Kobayashi, M. (2002). Antibacterial activity of licochalcone A against spore-forming bacteria. *Antimicrob Agents Chemother*. *46*(5):1226-30. https://doi.org/ 10.1128/AAC.46.5.1226-1230.2002.

Vanajothi, R., Vedagiri, H., Al-Ansari, M. M., Al-Humaid, L. A., & Kumpati, P. (2022). Pharmacophore-based virtual screening, molecular docking, and molecular dynamic simulation studies for finding ROS1 kinase inhibitors as potential drug molecules. *Journal of Biomolecular Structure and Dynamics*, *40*(8), 3385–3399. https://doi.org/10.1080/07391102.2020.1847195

DOI:https//doi.org/10.22452/mjs.vol44no2.4
Malaysian Journal of Science 44(2): 42-51 (June 2025)