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Lagerstroemin from *Lagerstroemia speciosa* as Antibreast Cancer Candidate Targeting AURKA, EGFR and SRC Protein: A Comprehensive Computational Study

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Abstract

Breast cancer is a type of cancer that has a high rate of diagnosis and mortality in the world. *Lagerestroemia speciosa* is a herb that has anticancer activity. This study aims to analyze the compounds in *L. speciosa* that most act as anticancer of the breast. The compounds in *L. speciosa* were selected based on drug-likeness, physicochemical properties, ability to penetrate the lipid bilayer and toxicity. The Lagerstroemin target related to Lagestroemin breast cancer was predicted using DisGeNET, SWISS Target Prediction and cBioportal. Molecular docking between Lagerstroemin and AURKA, EGFR and SRC was performed using AutoDock Vina. The interaction stability of each complex was analyzed by molecular dynamic simulation using YASARA with parameters of RMSD protein, RMSD ligand, number of hydrogen bonds and molecular dynamic binding energy. Of the 22 compounds, Quercetin, Caffeic acid, Lagerstroemin and Dypirone were predicted to have good ADME properties and can easily penetrate lipid membranes. Therefore, Quercetin, Caffeic acid and Lagerstroemin were predicted to have anti-breast cancer bioactivity. Of the 3 compounds, Lagerstroemin had the lowest toxicity. Lagerstroemin was predicted to interact with breast cancer-related proteins AURKA, EGFR and SRC. Molecular docking and dynamics showed that Lagerestroemin interacted stably at the ATP binding site of the 3 proteins, so it was very potential as an inhibitor of these 3 proteins. Therefore, Lagerstroemin was predicted to be the compound in *L. speciosa* with the most potential breast anticancer agent by targeting AURKA, EGFR and SRC.

Keywords: Apoptosis, AURKA, Breast cancer, EGFR, *L. speciosa*, SRC

Introduction

Based on Global Cancer Statistics 2022, breast cancer is the most prevalent type of cancer worldwide. The mortality rate for breast cancer is the 4th highest after lung, liver and stomach cancer [1]. Every year, around 2 million people are estimated to have breast cancer, predominantly in Asia and America. Several

factors can increase the risk of breast cancer, such as obesity, depression, vitamin D deficiency, lack of sun exposure, alcohol consumption and hormone therapy [2]. These factors trigger mutations in the genes related to tumour development. Breast cancer develops when the genes in epithelial cells of the mammary gland ducts undergo alterations which cause uncontrolled division.

Dysregulation of several pathways plays a role in breast cancer progression. This pathway dysregulation is caused by mutations or overexpression of various proteins. Aurora Kinase A (AURKA), Epidermal Growth Factor Receptor (EGFR) and proto-oncogene tyrosine-protein kinase Src (SRC) are 3 proteins that play a role in inducing breast cancer development. AURKA is a mitotic protein kinase that regulates the process of mitosis [3]. Previous studies reported that AURKA was overexpressed in approximately 73 % of breast cancer patients [4]. AURKA regulates spindle assembly, coordinates centrosomes and cytoskeleton, and regulates cytokinesis [5]. AURKA is also reported to have a role in cell adhesion and migration [6]. AURKA overexpression is reported to suppress Breast Cancer 1 (BRCA1) and Breast Cancer 2 (BRCA2) protein, which have a role in DNA damage response [7]. EGFR is a receptor tyrosine kinase which plays a role in activating various pathways related to cell growth [8]. Signalling pathways activated by EGFR is Rat sarcoma (RAS) signalling pathways that play a role in cell invasion, Phosphatidylinositol-3-kinase/Protein kinase B (PI3K/AKT) signalling pathways that play a role in tumour growth and survival, and Nuclear Factor Kappa-B (NF-κB) signalling pathways that play a role in inflammation and angiogenesis [9]. Approximately 70 % of breast cancer patients experience overexpression of EGFR protein, especially the triple-negative breast cancer subtype [10]. SRC is a non-receptor tyrosine kinases member which regulates various cellular responses to external stimuli [11]. Several mechanisms regulated by SRC include mitogenic signalling, angiogenesis, cell cycle, cytoskeleton organization, cell motility, survival and cell proliferation [12]. Previous studies have noted that SRC overexpression is associated with breast cancer progression [13]. Inhibition of AURKA, EGFR and SRC proteins is predicted to inhibit breast cancer progression. Various synthetic drugs have been developed to inhibit breast cancer progression, such as cisplatin, sonidegib and so on [14]. However, the use of synthetic drugs often results in adverse side effects, and it is even recorded that around 100,000 people are affected by the side effects of synthetic drugs [15]. Therefore, research on using natural ingredients for cancer therapy is growing rapidly.

L. speciosa is a plant of the Lythraceae family which is spread in tropical and subtropical regions [16]. This plant is traditionally used as a therapy for hypertension, diabetes, cholesterol, diarrhea and diet [17]. *L. speciosa* contains active compounds with various pharmacological activities, such as Lagerstroemin, Quercetin, Corosilic acid and so on [16]. Various studies have reported the anticancer activity of *L. speciosa* in various types of cancer. Previous research also mentioned that *L. speciosa* has a good anti-breast cancer effect [18]. However, no studies have revealed which compounds in *L. speciosa* have the most role in anticancer activity. Therefore, research is needed to analyze the active compounds in *L. speciosa* with the most anti-breast cancer role. Using a computational approach is the most suitable approach for the early screening of anticancer compounds in plants.

Computer-aided drug discovery (CADD) is a drug discovery research method using a computational approach [19]. Conventional drug discovery is often a time-consuming process of target identification, validation, lead compound discovery and optimization [20]. With computational analysis, this time can be cut. Calculations using accurate algorithms can replace cell lines or animal models [19]. Previous studies have shown compatibility between computational and experimental approaches in drug discovery studies [21]. Therefore, computational methods are very suitable for this study to find compounds in *L. speciosa* that have the most anticancer roles. This research aims to predict which compounds in *L. speciosa* have the most role as anticancer breast and computationally predict the mechanism.

Materials and methods

Data retrieval

The compounds contained in *L. speciosa* were obtained from previous studies [18,22-25]. Canonical SMILES and the 3-dimensional structures of the active compounds of *L. speciosa* were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). The 3D structure of the target protein for molecular docking was obtained from the Research Collaboratory for Structure Bioinformatics Protein Data Bank (RCSB PDB) database (https://www.rcsb.org) (EGFR: 4i23, SRC: 1y57, AURKA: 6gra). Inhibitors for each protein were obtained from inhibitor compounds bound to proteins in the RCSB PDB database.

Drug likeness, physicochemistry, membrane permeability and bioactivity screening

All compounds contained in *L. speciosa* were screened with drug-likeness, physicochemical, membrane permeability and bioactivity parameters to identify the most potential compounds for anti-breast cancer. Drug likeness and Adsorption, Distribution, Metabolism and Excretion (ADME) analysis was performed using the SWISS ADME web server (http://www.swissadme.ch/index.php); drug-likeness parameters used were Lipinski, Ghose, Veber and Egan [26-29]. The physicochemical parameters analyzed were lipophilicity, compound size, polarity, insolubility, unsaturation and molecular flexibility. The permeability analysis of the compound membrane was carried out using the PerMM web server (https://permm.phar.umich.edu) to estimate the ability of a compound to penetrate the lipid membrane. The environmental parameters were set at 310K and pH 7.4 [30]. Prediction of the bioactivity of compounds related to breast cancer was carried out using PASS Online (http://www.way2drug.com/passonline). Bioactivity parameters related to breast cancer refer to Widyananda's 2022 research [21].

Toxicity analysis

Compounds that had passed the selection for drug-likeness, physicochemistry, membrane permeability and bioactivity were continued for toxicity tests. Toxicity analysis was aimed to find the safest compounds. The toxicity of the compounds to normal and cancer cell lines was analyzed using the CLC pred web server (https://www.way2drug.com/Cell-line) [31]. The compounds toxicity to mice and rats organs was predicted using ROSC-pred (https://www.way2drug.com/ROSC) [32]. Toxicity class and toxicity properties was analyzed with the Pro-Tox II web server (https://tox-new.charite.de/protox_II) [33].

Protein target prediction

The target protein from Lagerstroemin was predicted using the SWISS Target Prediction web server (http://www.swisstargetprediction.ch). Breast cancer-related proteins were obtained from the DisGeNET database (https://www.disgenet.org). The identified proteins from SWISS Target Prediction and DisGeNET are the target proteins of Lagerstroemin. Among these target proteins, cBioportal database (https://www.cbioportal.org) was used to determine which ones had the most alterations in breast cancer patients. The samples used on cBioportal were "MSK, Cancer Cell 2018", MSK, Nature Cancer 2020" and "MSK, Cancer Discovery 2022" [34-36].

Protein-Protein Interaction (PPI) network and functional annotation

Target protein interactions with other proteins were analyzed using the STRING database (https://string-db.org) integrated into Cytoscape 3.7.2 with a cut-off value 0.4. These proteins were then entered into the Database for Annotation, Visualization and Integrated Discovery (DAVID) web server (https://david.ncifcrf.gov) for functional annotation analysis. Functional annotation focused on the Gene

Ontology domain Biological process and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway databases (https://www.genome.jp/kegg) [37].

Molecular docking simulation

The Lagerstroemin and inhibitor 3D structure were optimized using the Open Babel 3.1.0 plugin in PyRx 8.0 software (https://pyrx.sourceforge.io/) [38]. The 3D structure of the target protein was prepared using the Biovia Discovery Studio 2019 software (Dassault Systèmes Biovia, San Diego, California, USA). Molecular docking between ligand and its target proteins was performed using the AutoDock Vina 1.2.3 software (https://vina.scripps.edu/) integrated into PyRx 8.0 with the specific docking method [39,40]. The grid coordinates were positioned at the active site of each protein AURKA (X: 20.1076 Å, Y: 22.3955 Å, Z: 25.00 Å), EGFR (X: 20.1076 Å, Y: 24.4201 Å, Z: 23.0480 Å) and SRC (X: 17.2831 Å, Y: 19.5081 Å, Z: 17.4841 Å). Molecular visualization was performed using Biovia Discovery Studio 2019 (Dassault Systèmes Biovia, San Diego, California, USA). The analysis was conducted by comparing the binding pose of Lagerstroemia and the inhibitor on each target protein.

Molecular dynamic simulation

Molecular dynamic simulation analysis was performed using Yet Another Scientific Artificial Reality Application (YASARA) with the AMBER14 forcefield [41]. The system's environmental conditions were adapted to human physiological conditions (temperature 37 °C, pH 7.4, 0.9 % salt content, 1 atm pressure). The system was arranged in the form of a cube with a volume 20 Å larger than the protein. System optimization was carried out for 250 ps. The simulation was run for 20 ns with autosave every 25 ps. The running simulation was conducted using the md_run macro program. The RMSD value of backbone protein, RMSD ligand movement and hydrogen bond between protein and ligand were analyzed using the md_analyze macro program. The binding energy values during the simulation were analyzed using the md_bindenergy macro program with the boundary fast method.

***Lagerstroemia speciosa* extraction**

The *L. speciosa* were obtained from UPT Laboratorium Herbal Materia Medica, Batu, East Java, Indonesia (7 ° 52'03"S 112 ° 31'09"E). Six g of powdered *L. speciosa* leaves were combined with 96 % ethanol in a 1:10 ratio and placed into an MAE (microwave-assisted extraction) vessel from Anton-Paar. The MAE process was conducted using specific parameters: Maintaining a temperature of 50 °C, a 5-minute warm-up period at 50 °C, a 10-minute holding time, a 5-minute cooldown and operated at 1500 W power. The resulting extract underwent filtration using Whatman filter paper and subsequent evaporation using a Buchi R-210 Rotavapor System at 50 rpm and 37 °C. The resulting extract was then stored at 4 °C.

Cell viability assay

The T47D breast cancer cell line was obtained from the Animal Physiology, Structure and Growth Laboratory, Brawijaya University. Cells were cultured in complete medium consisting of RPMI 1640 (Gibco, USA), fetal bovine serum (Gibco, USA) and penicillin-streptomycin (Gibco, USA). A total of 7500 T47D cells per well were grown in 96-well plates. Cells were treated with *L. speciosa* concentrations of 0, 31.25, 62.5, 125, 250 and 500 µg/mL then incubated at 37 °C and 5 % CO_2 for 24 h. The treatment medium was replaced with medium containing 5 % WST-1 (Sigma-Aldrich, USA). Absorbance readings were carried out at 450 nm using an ELx880TM microplate reader (BioTek, USA) [21].

Statistical analysis

The cell viability assay results in the figure represented the average of 3 replicates ± standard deviation. Significant differences between treatment and control (0 µg/mL) were analyzed using Student's *t*-test with $p < 0.05$, $p < 0.01$ and $p < 0.001$.

Results and discussion

Potential compounds that meet drug-likeness, physicochemistry, membrane permeability and bioactivity

Out of the 22 compounds, 4 exhibited drug-like characteristics based on the parameters of Lipinski, Ghose, Veber and Egan (**Figure 1(A)**). The physicochemical properties of the 4 compounds were presented in **Figure 1(B)**. The pink-colored zone indicated the suitable physicochemical space for oral bioavailability. Quercetin and Caffeic acid possessed high unsaturation numbers but still met most physicochemical parameters. All 4 compounds were able to penetrate the lipid bilayer membrane, with Lagestroemin being the easiest and Quercetin being the most difficult based on energy transfer values (**Figure 1(C)**). A simulation of compound's penetration through the lipid bilayer was shown in **Figure 1(D)**. Possible breast cancer-related bioactivities of the 4 compounds are shown in **Figure 1(E)**. Quercetin, Caffeic acid and Lagerstroemin had possible anticancer activity, while Dypirone has no potential as anti-breast cancer. Therefore, Quercetin, Caffeic acid and Lagerstroemin satisfied all the screening parameters and proceeded to the next step.

Lagerstroemin meets the rules of drug-likeness based on Lipinski, Ghose, Veber and Egan, suggesting that Lagerstroemin has good ADMET (absorption, distribution, metabolism, excretion and toxicity) properties [42]. Lagerstroemin easily penetrates the lipid bilayer, as seen from its energy transfer value and changes in conformation when it penetrates the lipid membrane (**Figures 1(C)** and **1(D)**). Each molecule continuously adjusts its position to accommodate the hydrophilic and hydrophobic characteristics of the plasma membrane. While in motion, the nonpolar section of the compound will rotate and immerse itself into the lipid acyl chain region, while the more polar side will orient towards the membrane's boundaries [43]. Lagerstroemin's breast cancer anticancer bioactivity was obtained from the PASS Online database (**Figure 1(E)**). Lagerstroemin has a *Pa* value (probable activity) for several anticancer bioactivities, while the *Pa* value indicates a compound is predicted to have breast anticancer activity [44].

Toxicity of potential compounds in *L. speciosa*

Lagerstroemin emerged as the safest compound based on its toxicity profile. It was toxic to cancer cell lines and demonstrated lower toxicity to most normal cell lines (**Figure 2(A)**). This compound was also less toxic to various organs such as the urinary bladder, kidney, vascular system, stomach, small intestine, lung, ovary and pituitary gland. Meanwhile, Quercetin exhibited toxicity to the urinary bladder, kidney and small intestine, and caffeic acid is toxic to the kidney, stomach and lungs (2B). Lagerstroemin was classified into toxicity class 4 and had lower potential to cause hepatotoxicity, mutagenicity and cytotoxicity (**Figures 1(C)** and **1(D)**). Based on this toxicity analysis, Lagerstgroemin was determined to be the safest compound compared to Quercetin and Caffeic acid. The 2D structure of Lagerstroemin can be seen in **Figure 2(E)**.

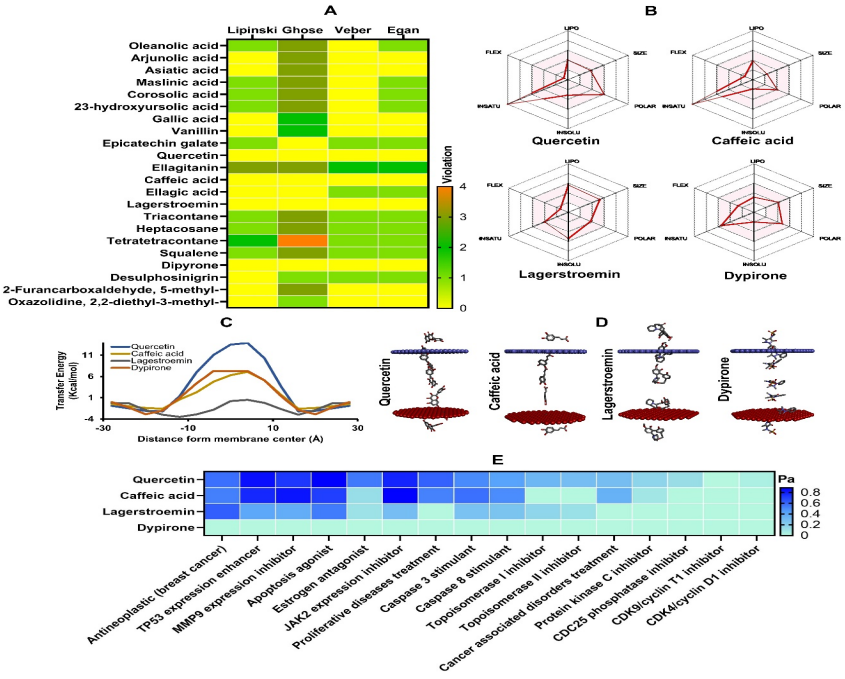

Figure 1 Drug likeness, physicochemistry, membrane permeability and bioactivity screening: (A) druglikeness with parameters Lipinski, Ghose, Veber and Egan, (B) physicochemical properties of Quercetin, Caffeic acid, Lagerstroemin and Dypirone, (C) the energy transfer value when the 4 compounds penetrate the lipid bilayer, (D) Simulation of compounds in penetrating the lipid bilayer and (E) Breast cancer-associated bioactivity.

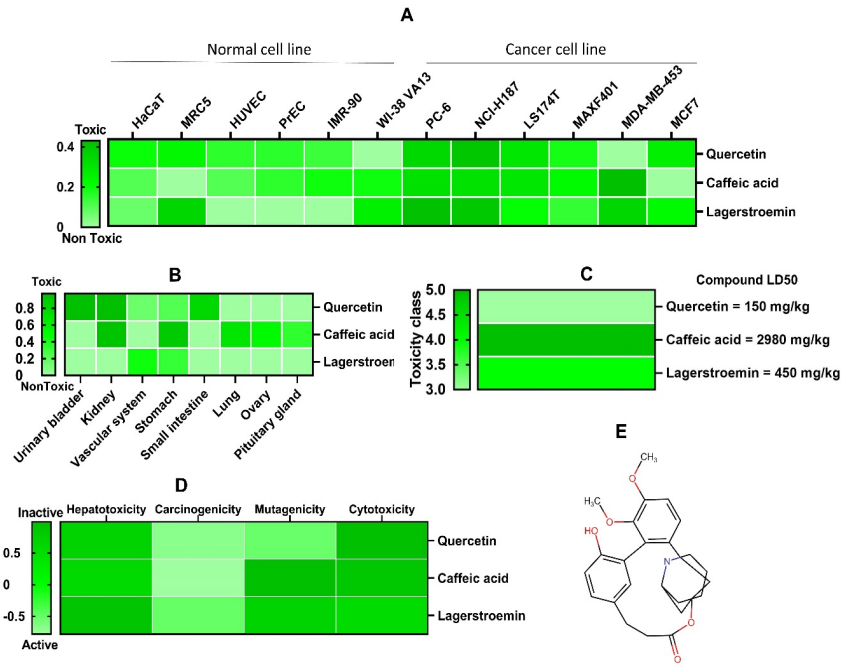

Figure 2 Potential compound toxicity: (A) toxicity of the compound to cancer and normal cell lines, (B) toxicity of the compound to organs, (C) toxicity class of each compound, (D) the compound's potential to cause hepatotoxicity, carcinogenicity, mutagenicity and cytotoxicity and (E) structure of the Lagerstroemin compound.

Protein target of Lagerstroemin, PPI network and functional annotation related to breast cancer

Based on the SWISS Target Prediction database, Lagerstroemin had possible interactions with 73 proteins in humans. However, based on the DisGeNET database, only 6 proteins were related, namely CYP2D6, EGFR, SRC, AURKA, NOS2 and MAOA (**Figure 3(A)**). Based on analysis with the cBioportal database, EGFR, SRC and AURKA were the most altered proteins in breast cancer patients (**Figure 3(B)**).

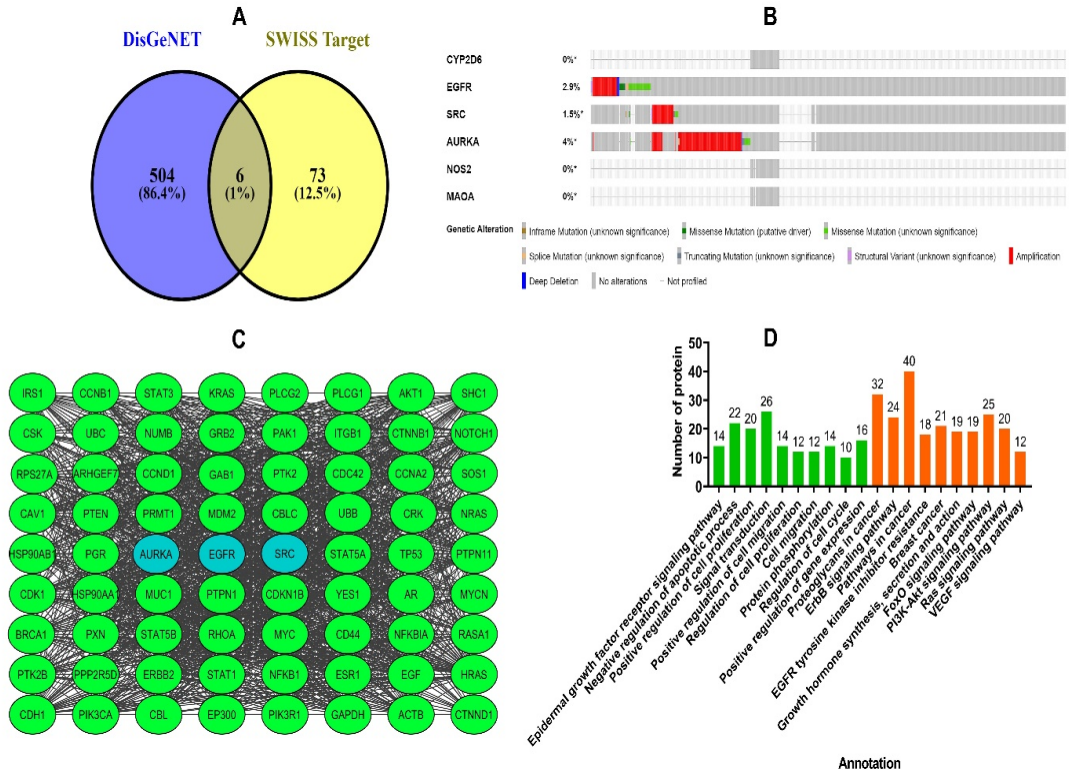

Figure 3 Lagerstroemin's target protein and functional annotation analysis: (A) Lagerstroemin target protein based on SWISS Target Prediction and DisGeNET, (B) protein-protein interaction network based on 3 target proteins, (C) functional annotation based on Gene Ontology and KEGG pathway databases (green histogram represents Gene Ontology domain Biological Process, orange represents KEGG pathway database).

The protein-protein interaction network showed that AURKA, EGFR and SRC interacted with cell proliferation-related proteins such as AKT1, ERα, STAT1, PI3K, CDK1, MYC and so on (**Figure 3(C)**). Based on the functional annotation, proteins interacting with AURKA, EGFR and SRC had roles in biological processes and cancer-related pathways (**Figure 3(D)**). Based on the Gene Ontology database, out of 72 proteins, 14 had a role in biological processes related to the EGFR signalling pathway, 22 proteins in the regulation of apoptosis, 20 in cell proliferation and so on. Based on the KEGG pathway, 40 proteins played a role in the pathway in cancer, 32 proteins in proteoglycans in cancer, 25 proteins in the PI3K-Akt signaling pathway and so on.

Target protein analysis stated that AURKA, EGFR and SRC were protein targets for Lagerstroemin. AURKA is a protein that plays an important role in the cell cycle's G2 to M phase transition. In the late G2 phase, AURKA will accumulate in the centromere and recruit various proteins such as Ajuba, Arpc1b,

calmodulin, CEP192 and nucleophosmin to activate the centromere [45]. AURKA also supports the activation of CDK1/cyclin B complex to start nuclear entry by phosphorylating CDK-activating phosphatase CDC25B [46]. In addition, AURKA also has an application in metastasis and drug resistance in breast cancer [47]. Previous research reported that AURKA inhibition can inhibit breast cancer growth [48]. Other studies have suggested that AURKA inhibition can induce autophagy in breast cancer cells [49]. EGFR is a receptor that activates various pathways in the development of breast cancer. After binding to EGF, EGFR will activate PI3K, JAK and RAF, which then induce cell proliferation, differentiation, transformation and survival [10]. Previous research noted that EGFR inhibition can inhibit the growth of liver and breast cancer cell lines and reduce CDK7 expression [50]. SRC is a proto-oncogene involved in cell proliferation, differentiation, survival and migration [51]. SRC is activated by integrins, receptor tyrosine kinases and G-protein receptors. Once activated, SRC activates the RAS protein and induces the transcription of genes related to cancer cell proliferation and metastasis [52]. Previous studies reported that inhibition of SRC inhibited metastasis of murine breast cancer models [13]. Other studies reported that SRC inhibition can inhibit the ERK pathway, which results in decreased expression of LGUT1, which causes cancer cells to lack energy and is induced to carry out apoptosis [53]. Previous studies have suggested that inhibition of AURKA, EGFR and SRC activity can kill breast cancer cells. This study provides promising findings because it predicts that Lagerstroemin can inhibit these 3 proteins.

Molecular interactions between Lagerstroemin and its 3 target proteins

The molecular docking results were shown in **Figure 4** and **Table 3**. These results indicated that Lagerstroemin interacted with its target protein in the same position as the inhibitor compound. The interaction between AURKA and Lagerstroemin produced a binding affinity value of –7.2 kcal/mol and shared the same interaction sites with the AURKA-Inhibitor complex, namely on Lys143, Leu263 and Ala273 (**Figure 4(A)**). The EGFR-Lagerstroemin complex (–7.1 kcal/mol) had the same 3 interactions sites as the EGFR-inhibitor on Leu718, Val726 and Lys745 (**Figure 4(B)**). The SRC-Lagerstroemin complex (–7.3 kcal/mol) had the same 2 interactions with SRC-Inhibitors on Val281 and Leu393 (**Figure 4(C)**). The identical interaction position with inhibitors suggested that Lagerstroemin was predicted to have an activity similar to the inhibitor compounds. Furthermore, the stability of the protein-ligand interaction was analyzed using molecular dynamic simulation.

Based on the molecular docking results, Lagerstroemin interacted with AURKA, EGFR and SRC at the same binding sites as the inhibitors. Specifically, Lagerstroemin bound to AURKA at the ATP-binding site around the Tyr122, Leu173 and Ala183 residues. The ATP binding site of AURKA was 100 % conserved, suggesting minimal resistance development in AURKA due to mutations [54]. Lagerstroemin also binds to Lys75 and Cys797 residues on the EGFR which were previously identified as important residues in ATP binding [55]. Therefore, Lagerstroemin's high-potency suggested its potential to act as an EGFR inhibitor. Furthermore, Lagerstroemin emerged as a potential inhibitor of ATP from SRC. The SRC ATP binding site was located in the Met341 of the hinge region, Leu273 of the glycine-rich or P loop and Leu393 of the N-terminal loop [56]. Lagerstroemin formed bonds with these important residues in ATP binding, namely Val21 and Leu393.

Table 1 Details of interactions between Lagerstroemin and its target proteins.

Protein	Compound	Binding affinity (kcal/mol)	Position of chemical interaction	
			Hydrogen bond	**Hydrophobic interaction**
AURKA	Inhibitor (BRD-7880)	–10	Lys141, Val174, Thr217, Asn261, Lys162, Lys143	Leu178, Leu169, Phe144, Arn220, Ala273, Leu263
	Lagerstroemin	–7.2	Lys143, Glu260, Asn251	Lys143, Tyr219, Leu263, Ala273
EGFR	Inhibitor (Dacomitinib)	–8.2	Met793	Leu718, Val728, Ala743, Lys745, Leu788
	Lagerstroemin	–7.1	Ser720, Gly724	Leu718, Val726, Lys745, Cys797, Leu844
SRC	Inhibitor (MPZ)	–8.6	Met341	Ala293, Leu393, Val281
	Lagerstroemin	–7.3	-	Leu273, Val281, Lys295, Leu393, Ala403

Note: The underscore '_' indicates the similarity of the interaction position between the inhibitor and Lagerstroemin.

Interaction stability between Lagerstroemin and its 3 target proteins

The stability of the interaction between Lagerstroemin and its target protein was analyzed by molecular dynamics simulation (**Figure 5**). The results showed that the structural stability of AURKA, EGFR and SRC after interacting with Lagerstroemin tended to be stable. This was evident from the RMSD values, which did not differ significantly from the protein-inhibitor structure. As previously reported a more stable protein structure is associated with reduced fluctuations in the protein RMSD value [57]. The stability of Lagerstroemin when interacting with its target proteins can be seen from the RMSD ligand, the number of hydrogen bonds between protein ligands and the binding energy value. Lagerstroemin exhibited a higher RMSD Ligand value compared to the inhibitors; however, it remained within a stable trend. The number of hydrogen bonds between AURKA-Lagerstroemin and SRC-Lagerstroemin was lower than AURKA-Inhibitor and SRC-Inhibitor. Meanwhile, EGFR-Lagerstroemin had a similar value to EGFR-Inhibitor. AURKA-Lagerstroemin and SRC-Lagerstroemin complexes were higher than protein-inhibitors. The stability of the interaction increases with a greater number of hydrogen bonds between the protein and the ligand [58]. The EGFR-Lagerstroemin complex demonstrated a lower binding energy than the control, remaining within a stable trend. Conversely, the Lagerstroemin-AURKA and Lagerstroemin-SRC complexes exhibited higher binding energy values than the control, suggesting more stable interactions. An increase in molecular dynamic binding energy has been reported to correlate with enhanced stability in molecular interactions [59]. Overall, the simulations indicated that the structures of the 3 proteins stabilized during interaction with Lagerstroemin. Lagerstroemin interacted stably with AURKA, EGFR and SRC and did not demonstrate to a higher level of stability compared to than inhibitors.

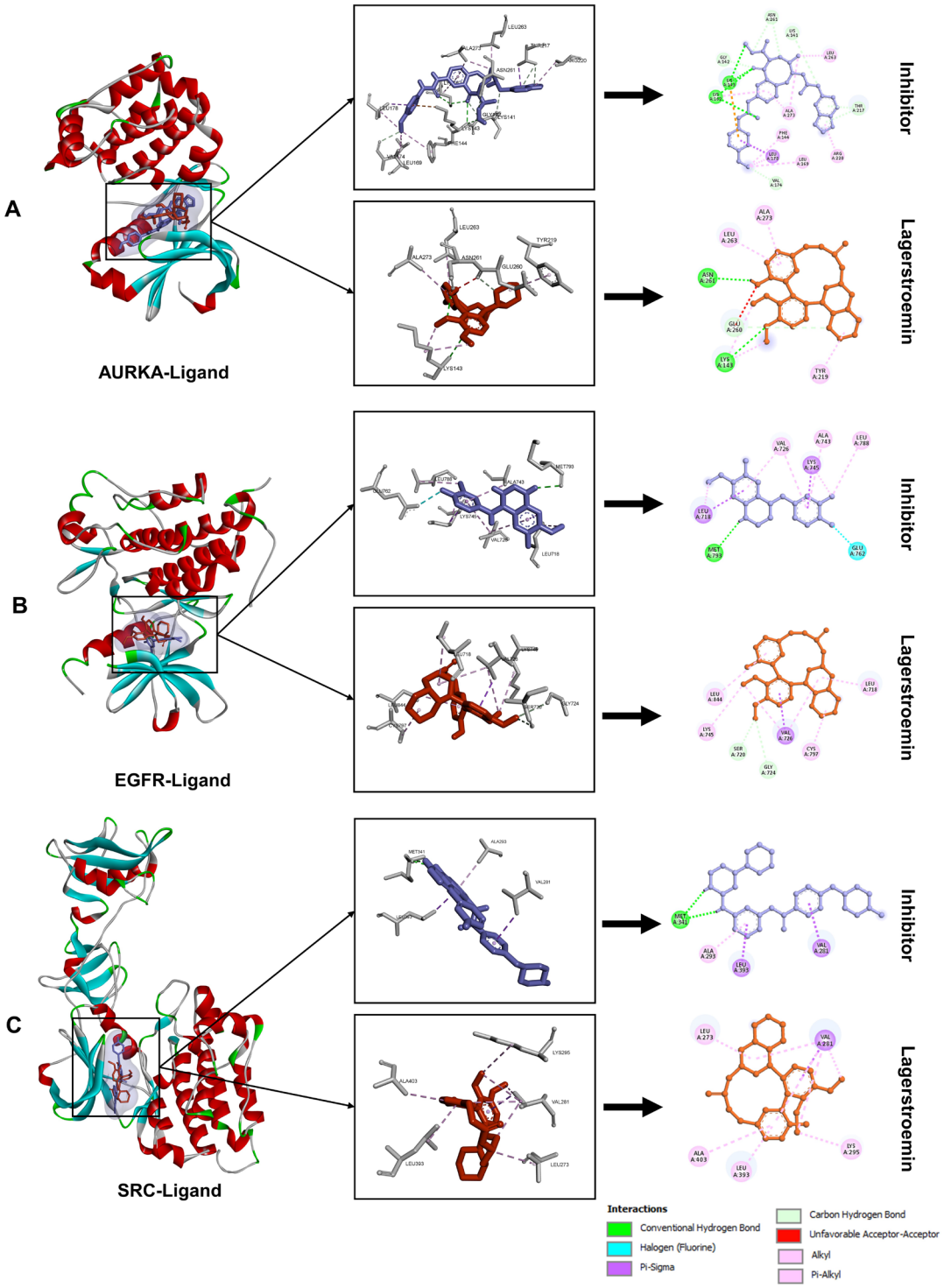

Figure 4 Interaction between Lagerstroemin and its target protein: (A) AURKA-Lagerstroemin, (B) EGFR-Lagerstroemin and (C) SRC-Lagerstroemin.

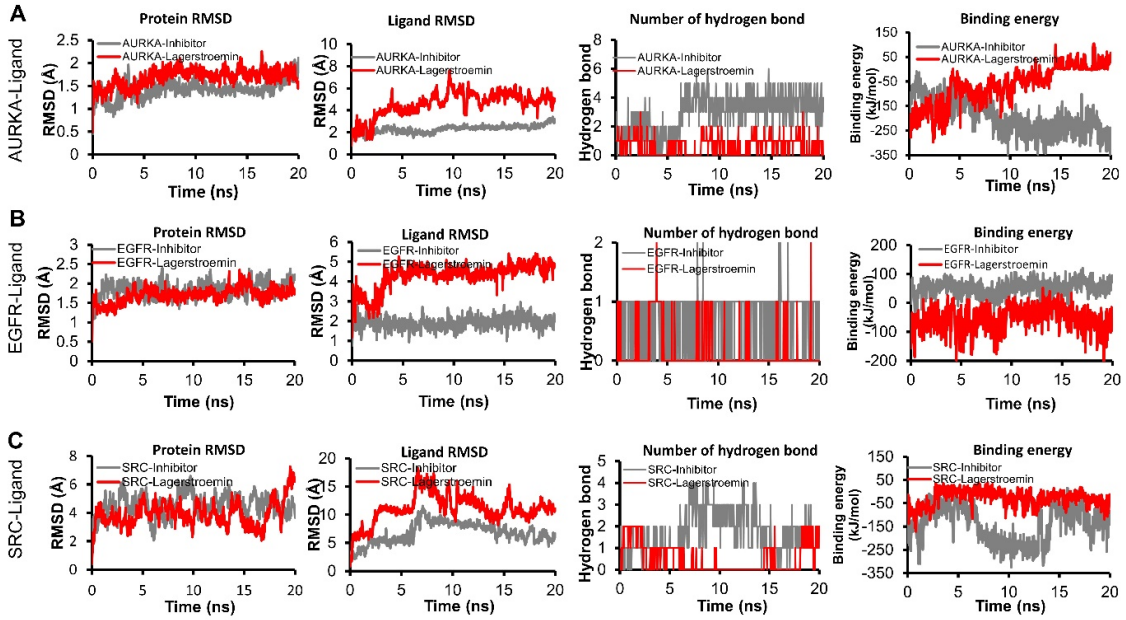

Figure 5 The stability of the protein-ligand complex interaction can be seen from the RMSD protein, RMSD ligand, number of hydrogen bonds between protein and ligand, and binding energy value: (A) AURKA-Ligand, (B) EGFR-Ligand and (C) SRC-Ligand.

Ethanol extract of *L. speciosa* reduces the viability of breast cancer cell line

The viability assay aimed to analyze the effect of *L. speciosa* extract on the viability of T47D breast cancer cells. The results of the viability test were shown in **Figure 6**, indicating that increasing the extract dose significantly reduced the viability of T47D cells. Therefore, *L. speciosa* extract exhibited dose-dependent reduction in cancer cell viability. The IC50 value was obtained as 284.72 ± 19.79 µg/mL, indicating that the extract could reduce the viability of T47D cells by half at this dose. Previous studies had suggested that the decrease in cancer cell viability is caused by mechanisms such as apoptosis, cell cycle arrest and inhibited proliferation [60]. This study confirmed that the decreased viability of T47D breast cancer cells is likely due to the presence of Lagerstroemin, the main compound in *L. speciosa* [16], which inhibited proteins related to cancer growth, such as AURKA, EGFR and SRC.

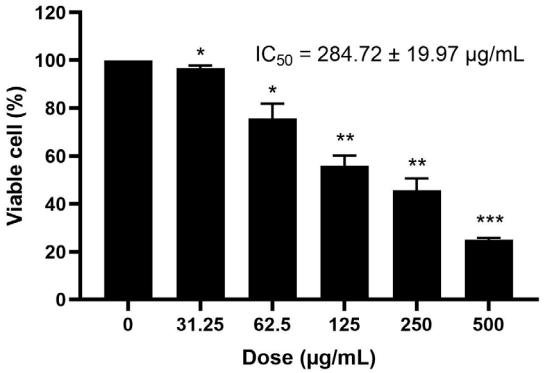

Figure 6 T47D cell viability significantly decreased with increasing doses of *L. speciosa* extract administered. The cell viability was measured in triplicate and is shown as a means ± standard deviation (SD). *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ versus 0 µg/mL.

Lagerstroemin, found in *L. speciosa*, exhibits high potential as an anti-breast cancer agent. *L. speciosa* reduces the cell viability of breast cancer cell lines, likely due to the role of Lagerstroemin. This compound targets 3 proteins simultaneously: AURKA, EGFR and SRC, all of which play crucial roles in breast cancer progression. This multi-targeting approach renders Lagerstroemin more effective than synthetic chemotherapy drugs currently in use. Synthetic drugs typically target a single protein, leading to the development of resistance [61]. Previous research has shown that the chemotherapy drug Doxorubicin induces resistance in breast cancer by activating alternative signaling pathways [62]. Similar phenomena occur with the chemotherapy drug Cisplatin [63]. Lagerstroemin's ability to target multiple proteins makes it superior, reducing the likelihood of drug resistance.

Conclusions

The physicochemical properties of Lagerstroemin made it have good ADMET and could easily penetrate the lipid bilayer membrane. Lagerstroemin could bind stably at the ATP binding site of AURKA, EGFR and SRC, so it had the potential to become an ATP inhibitor for these proteins. The experimental analysis confirm that *L. speciosa* extract decrease the viability of breast cancer cell line.

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