RESEARCH ARTICLE

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Exploring the Cytotoxic Activity of *Dillenia serrata* Thunb. Leaf Extracts: An *In Vitro* and *In Silico* Investigation

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Abstract

Objective: *Dillenia serrata* Thunb. an endemic plant from Sulawesi Island, has been used by the local community as medicine for some diseases. However, studies related to these plants are still limited to several diseases. This study intends to investigate the cytotoxic activity of *Dillenia serrata* Thunb. leaves extract as an anticancer. **Methods:** This study was preceded by gradual maceration and then subjected to phytochemical test to evaluate the contain of secondary metabolites such as alkaloid, flavonoid, tannin, steroid, terpenoid, and saponin, toxicity assay by BSLT method, cytotoxicity test against HeLa cell lines, further compound identification using GC-MS analysis and in silico analysis. **Results:** The phytochemical tests demonstrated the presence of tannins, steroids, alkaloids, flavonoids, and saponins. The toxicity test indicated that all three extracts were toxic for *Artemia salina* L. as the premier test before the cytotoxicity test using HeLa cell lines. The LC_{50} values for the n-hexane, ethyl acetate, and methanol extracts were 58.27±6.15, 11.06±1.70, and 9.30±1.13 µg/mL, respectively. After evaluating the extracts' cytotoxicity activity, the ethyl acetate extract has the strongest activity with 91.08±0.23 µg/mL, then this extract was further identified using GC-MS analysis and reveals 51 chemicals which is Phytol as the main components in the extract with %area about 25.64%. Molecular docking analysis of Phytol against Epidermal Growth Factor Receptor (EGFR) showed a good binding energy of around -5.08 kcal/mol. The molecular dynamics simulation supports this result. **Conclusion:** All extracts demonstrated intense toxicity levels. Out of all the extracts, ethyl acetate extract exhibited the strongest cytotoxic properties to HeLa cell lines with IC_{50} value 91.08±0.23 µg/mL. Ethyl acetate extract of *D. serrata* T. contains Phytol compounds which have a quite good affinity to the EGFR. According to this study, ethyl acetate extract has the potential to be used as an alternative to anticancer medication.

Keywords: Cytotoxicity- *Dillenia serrata* Thunb- HeLa cell line- Molecular docking

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Introduction

Dillenia serrata Thunb. also referred to as Dengen locally, is one of the possible therapeutic plants found across Celebes Island, Indonesia. Traditionally, almost every part of *Dillenia serrata* Thunb. has been utilized medicinally. The fruit juice is used for oral thrush, vomiting of blood, fever, and wound medicine [1]. The stem is also used for vomiting blood because of the content of polyphenol compounds [2]. In addition, the leaf part is also used as an antioxidant and anti-inflammatory [1]. This plant contains secondary metabolites, such

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as flavonoids, alkaloids, polyphenols, saponins, and terpenoids [3-5]. It is well-recognized that this plant has antimicrobial, anticholesterol, and antioxidant properties [6-8]. Concurrently, two plants in the same genus as *Dillenia serrata* T. that are *Dillenia indica* and *Dillenia pentagyna*, were known to possess anticancer bioactivity [6, 7]. The existence of coetjapic and betulinic acids in *Dillenia serrata* T. isolates, which have inhibitory effects on PGE2 synthesis with IC_{50} values of 1.05 and 2.59 μM [8], further supports this. In fact, several studies indicate that PGE2 lowers antitumor immunity, promotes tumour growth and invasion, decreases apoptosis, and enhances metastasis and angiogenesis [9]. Prostaglandin E2 plays a significant role in cancer progression by involving in promoting tumor growth, invasion, metastasis, and immune evasion [9, 10]. Given that 3.2% of women develop cervical cancer and that less successful standard treatments are leading to an increase in cell resistance, it would be interesting to look at *Dillenia serrata* T. as a possible novel agent for the disease [11]. Globally data showed that there were an estimated 604.127 cervical cancer cases and 341.831 deaths which is equivalent to 56.58% of deaths in 2020 [12]. In Indonesia, cancer incidence in females was almost twice that in males because of cervical and breast cancer [13]. Thus, this work aims to assess the anticancer activity of *Dillenia serrata* T. against HeLa cells both in vitro and in silico.

Overexpression of the epidermal growth factor receptor (EGFR) is frequently linked to cervical cancer. Increased EGFR expression in HeLa cells, a cervical cancer cell line, aids in developing and spreading cancer cells. The adhesion, migration, proliferation, and differentiation of cancer cells were all impacted by EGFR tyrosine kinase [14]. Therefore, a substance that can block EGFR is also required to treat cancer. Molecular docking analysis can show how a drug molecule inhibits the EGFR.

A combination of in silico and in vitro techniques is used to evaluate the cytotoxic potential of compounds. In vitro testing uses the resazurin reduction assay, while molecular docking and molecular dynamics simulation will be used for in silico. The resazurin reduction test is a more sensitive and reasonably priced technique than tetrazolium assays like MTT and MTS [15]. Concurrently, the in silico technique is employed to examine how ligands and receptors interact, assess the target action of these interactions, and comprehend the underlying mechanisms [16]. Therefore, this study investigates *Dillenia serrata* Thunb. leaves through in vitro analysis using HeLa cell lines and in silico analysis by conducting molecular docking and molecular dynamics simulation.

Materials and Methods

Materials

Chemicals solvent used in this research were n-hexane, ethyl acetate, methanol with technical grade and was refined with distillation. The solvents were used for phytochemical extraction of the *D. serrata* T. leaves. $FeCl_3$ (Pudak), magnesium powder (Merck), HCl (Merck), $CHCl_3$ (Merck), H_2SO_4 (Merck), and glacial acetate acid (Merck) for phytochemical test. Cisplatin (EDQM), DMSO (Merck), phosphate-buffered saline (Gibco), resazurin sodium salt (Sigma Aldrich), fetal bovine serum (Gibco), trypsin-EDTA (Gibco), trypan blue (Sigma Aldrich), and Roswell Park Memorial Institute Medium (RPMI, Gibco) were among the cytotoxicity assays.

Sample Collection and Identification

The sample was obtained from the village of Sorowako, East Luwu district, Indonesia, in March 2024 and recognized as *Dillenia serrata* Thunb. based on the morphological analysis of branches, leaves, flowers, and fruit in the Botanical Laboratory of the National Research and Innovation Agency, Indonesia.

Sample Extraction and Phytochemical Profiling

After being cleaned and allowed to dry in the room, the *D. serrata* T. leaves sample was blended until it reaches a mesh size of 20. Maceration was then used to extract the samples, progressively working through n-hexane, ethyl acetate, and methanol. The sample extracts were then evaporated until a crude extract was obtained. Each extract identified using alkaloids, flavonoids, tannin, steroid, terpenoid, and saponin test.

a. Alkaloid test

The alkaloid was identified using Wagner, Meyer, and Dragendorf reagents. The Wagner reagent produces a brown precipitate, the Meyer reagent yields a yellowish-white precipitate, and the Dragendorf reagent results in an orange to brownish-red precipitate, indicating the presence of an alkaloid. A few drops of each reagent were added to a 1 mL sample in separate test tubes, and precipitate formation was then observed.

b. Flavonoid test

Sample solution (1 mL) was treated with a few drops of $Pb(CH_3COO)_2$ solution and the precipitate was closely monitored. The development of a yellow precipitate serves as a clear indicator of the presence of flavonoid.

c. Tannin test

The identification of tannins can be confirmed through the vibrant color changes produced when a 1% $FeCl_3$ solution is introduced. By adding this solution to a 1 mL sample and observing the resulting agitation, we can monitor a spectrum of colors, including green, red, purple, blue, or black, as key indicators of tannin presence.

d. Terpenoid and steroid test

Sample solution (1 mL) was mixed with a few drops of Liebermann-Burchard reagent, and the color change was observed. A red or purple solution confirms terpenoids, while a green or blue solution indicates steroids.

e. Saponin test

Sample solution (1 mL) mixed with hot distilled water and shaken vigorously will produce stable foam of 1-3 cm high if saponins were present.

Toxicity Assay

Artemia salina Leach shrimp eggs are sprinkled in artificial seawater media (salinity 38 g/L) with aerial and lighting bulbs. The shrimp larva used for analysis is a larva that has been 48 hours old [17]. The sample of the *D. serrata* T. leaves extract was weighed at 24 mg and then dissolved with 200 µL DMSO and then diluted to a volume of 12 mL (the concentration of the extractive stem solution is 2000 µg/mL). The 2 mL *D. serrata* T. leaves extract stem solution was diluted with seawater gradually in a reaction tube, followed by adding two mL of seawater containing 15 shrimp larvae, which were randomly selected. A series of extracts containing the shrimp's larvae were then incubated for 24 hours under a lighting bulb. After the incubation, shrimp larvae's mortality rate (%) is calculated at each concentration and converted to probit values. LC_{50} values are calculated using the line equation obtained at the death curve (%) vs. sample concentration log. This analysis procedure generally refers to Meyer et al. (1982) with slight modifications [18].

Cytotoxicity Assay

The cytotoxicity test employs the resazurin reduction assay method to HeLa cancer cell lines (ATCC CCL-2) described by Sharma et al. [19] with a minor adjustment. The resazurin reduction test was chosen due to the more sensitive and reasonably priced technique. Trypan blue exclusion and resuscitation of cells with an end cell density of 170,000 cells/mL in the medium were used to quantify the number and vitality of cells. The cells were cultivated onto 96-well plates and then incubated at 37°C with 5% CO_2 gases until the cells confluence at least 70% of the time. To 90 µL of the medium, add 10 µL of sodium salt powder resazurin, and then incubate for one to two hours or until color changes are evident. The absorbance spectrum was used for resazurin and resorufin at 570 nm wavelengths using a multimode reader to measure the absorption, lower absorption indicates higher cell survival. Cisplatin was used as positive control, DMSO as negative control, and the extract concentration ranged from 7.81; 15.63; 31.25; 62.5; 125; 250; 500; 1000 µg/mL. According to the anticancer activity, the best extract will be identified using GC-MS instruments.

Molecular Docking Analysis

Molecular docking analysis was performed towards Phytol as the highest precent area in the GC-MS analysis in the ethyl acetate extracts of *Dillenia serrata* Thunb. and EGFR (PDB ID: 1M17) was chosen as the protein target due to the overexpression of these cells, which can cause the spreading of cancer cells. Molecular docking analysis was done with AutoDock4 and AutoDock Tools [20]. Chimera software (1.16 version) was used to produce proteins and ligands. Preparation was carried out by separating the EGFR-Erlotinib structure, and unnecessary molecules (such as water, ions, etc.) were removed. The EGFR structure was prepared using the DockPrep tool, and hydrogen atoms and charges were added to the separated ligand structure [21]. The analysis was carried out in the EGFR active site at the Coordinates of Central Grid Point of Maps: 21.697, 0.303, 52.093 using a 48 x 34 x 38 Å grid box, and 10 conformations were produced using the Lamarckian Algorithm [16]. Docking parameters were determined using a Genetic Algorithm (GA) with a population size of 300, and a maximum number of evals of 2500000 (medium) with a Lamarckian GA 4.2 output. The various parameters used in this docking process had previously been validated by re-docking a natural ligand (Erlotinib) and obtained an RMSD value of <2 Å (1.63 Å). The Discovery Studio visualizer was used for interaction visualization [22].

Molecular Dynamics Simulation

Molecular dynamics simulation was performed towards EGFR-Phytol complex compared to EGFR-Erlotinib using YASARA Structure (developed by Biosciences GmbH) with AMBER14 forcefield, lasting for 100 ns (recorded every 25 ps). The simulation was conditioned on physiological temperature 310K and pH 7.4 with Na^+ and Cl^- as counter ions. All trajectory data was used to analyze the root mean square deviation (RMSD), radius of gyration, and total of hydrogen bond by using md_analyze.mcr, while the root mean square fluctuation (RMSF) data was produced using md_analyzeres.mcr script.

Data analysis

The IC_{50} and LC_{50} were presented in means ± standard deviation of triplicate measurement. The significance of the data was considered by ANOVA between each extract ($p< 0.05$) using IBM SPSS Statistics 26.

Results

Sample preparation and extraction

Dillenia serrata T. was correctly identified in the Botanical Laboratory of National Research and Innovation Agency, Indonesia. Gradual maceration is the approach used to carry out the extraction. n-Hexane, ethyl acetate, and methanol were the solvents chosen for extraction. According to the like dissolves-like principle, each solvent was selected based on its polarization to dissolve the chemical at the same polarity level. Maceration was done to get the crude extract. The extract yields shown in Table 1 with the percentage of methanol, ethyl acetate, and n-hexane extracts were 9.07, 1.41, and 1.14%, respectively.

D. serrata T. was found to make up the majority of polar compounds, indicating that the highest yield of the obtained extract was methanol. The plant contains a variety of secondary metabolites identified by phytochemical screening, such as alkaloids, flavonoids, saponins, tannins, terpenoids, and steroids. The study conducted by Rahmawati et al. [23] revealed comparable content in *D. serrata* T. leaves.

Phytochemical Analysis

Phytochemical analysis revealed distinct chemical groups in every *D. serrata* T. leaf extract. Alkaloids, flavonoids, saponins, tannins, terpenoids, and steroids were all identified in *D. serrata* T. leaves. More detailed information can be found on Table 2.

Toxicity and Cytotoxicity Assay against Artemia salina

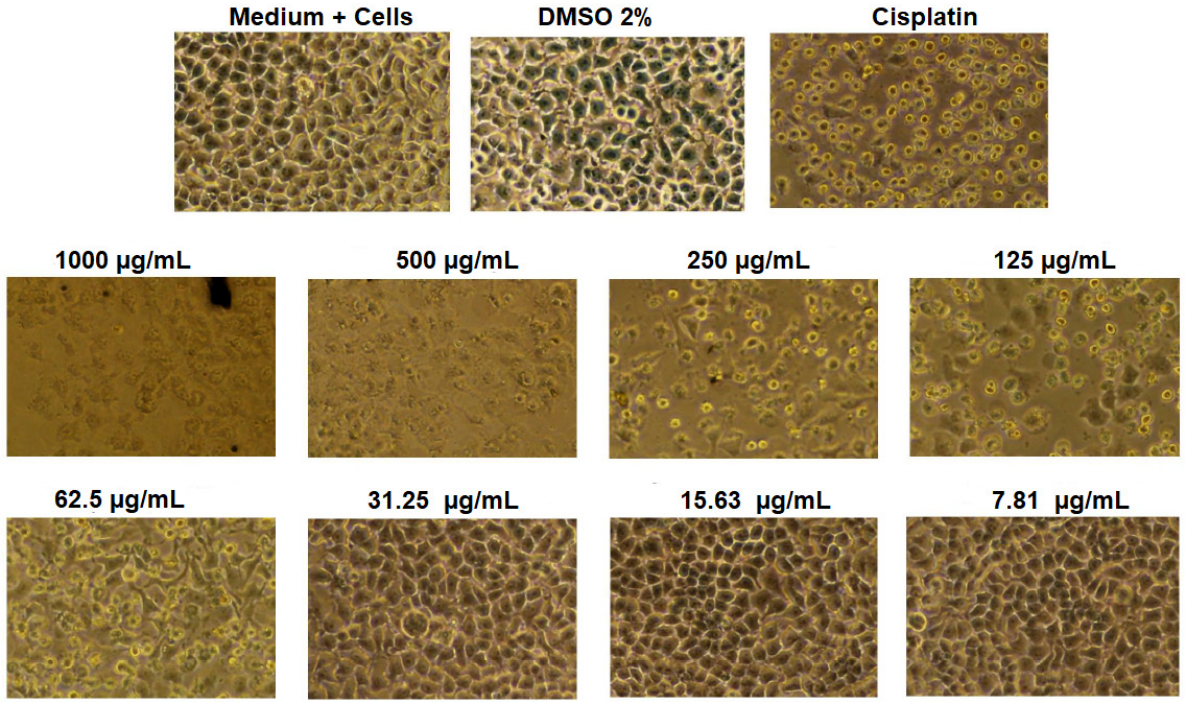

Figure 1. Morphology of HeLa Cells when Treated with Ethyl Acetate Extract of *Dillenia serrata* Thunb. comparing with Cisplatin as the positive control

Table 1. Yield of *Dillenia serrata* T. leaves Extract

Extract	Extract Weight (g)	Yield (%)
n-Hexane	7.28	1.14
Ethyl acetate	104.63	16.54
Methanol	73.27	13.87

L. and HeLa cell

The toxicity assay was employed to reveal the possibility of *D. serrata* T. leaves to inhibit HeLa cell lines' viability. This assay was conducted with the BSLT method and found that the toxicity ranged from 9.30±1.13 to 58.27±6.15µg/mL, categorized as toxic (LC_{50} < 100

Table 2. Phytochemicals Test Result of *D. serrata* T. Leaves Extract

Phytochemical test	*D. serrata* T. extract		
	n-Hexane	Ethyl acetate	Methanol
Alkaloid	-	++	+
Flavonoid	-	-	+
Tannin	-	-	+
Steroid	++	+	-
Terpenoid	+	-	-
Saponin	-	-	+

Note: (-), no reaction; (+), intense reaction; (++), higher intensity reaction

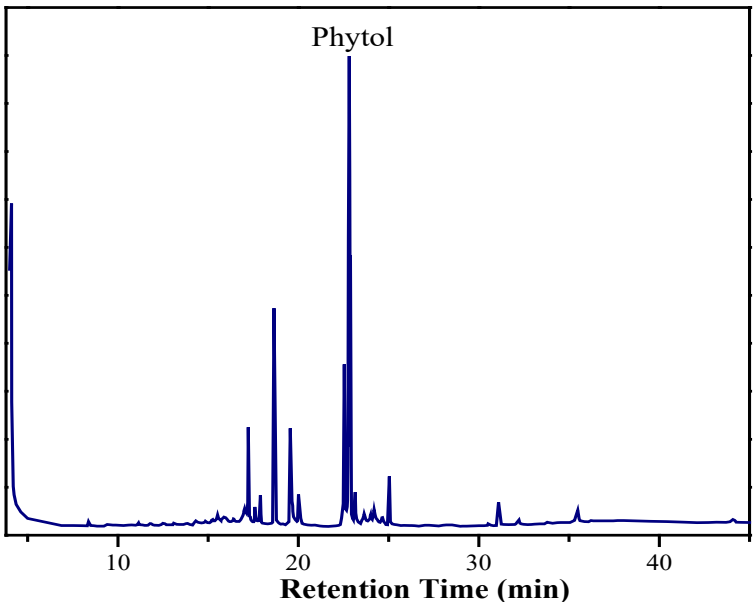

Figure 2. GC-MS Chromatogram of Ethyl Acetate Extract Containing Phytol as the Highest Percent area Compound

Table 3. Toxicity and Cytotoxicity test of *D. serrata* T. Leaves Extract

Extract	Concentration (µg/mL)	Larvae mortality (%)	LC_{50} (µg/mL)	IC_{50} (µg/mL)
Methanol	1000	100	9.30±1.13[a]	386.35±34.35[a]
	500	93		
	250	91		
	125	84		
	62.5	76		
Ethyl acetate	50	73	11.06±1.70[a]	91.08±0.23[b]
	25	62		
	12.5	56		
	6.25	42		
	3.125	27		
n-Hexane	500	100	58.27±6.15[b]	796.35±35.95[c]
	250	93		
	125	78		
	62.5	64		

Different letters on the same columns indicate mean values significantly differed ($p< 0.05$)

µg/mL) according to Nguta et al. [24]. Methanol extract had the highest level of toxicity, measuring 9.30±1.13 µg/mL, followed by ethyl acetate extract and n-hexane extract, which had toxicities values of 11.06±1.70 and 58.27±6.15 µg/mL, respectively. The data was shown more detail in Table 3.

The cytotoxic assay was designed to test the influence the extracts on the entire cell using resazurin reduction method. The cytotoxic assay was designed to evaluate the effects of the extracts on living cells using the resazurin reduction method. One of the main advantages of this assay is that it is relatively inexpensive and demonstrates greater sensitivity compared to tetrazolium assays [25]. The cytotoxicity of the extracts was evaluated in cervical cancer (HeLa) cells and compared to the standard drug Cisplatin and DMSO 2% as negative control. Weerapreeyakul et al. [26] state that extracts with a 50% cytotoxic impact at concentrations between 10 and 100 µg/mL are classified as strongly cytotoxic, and those with a concentration between 100 and 500 µg/mL as

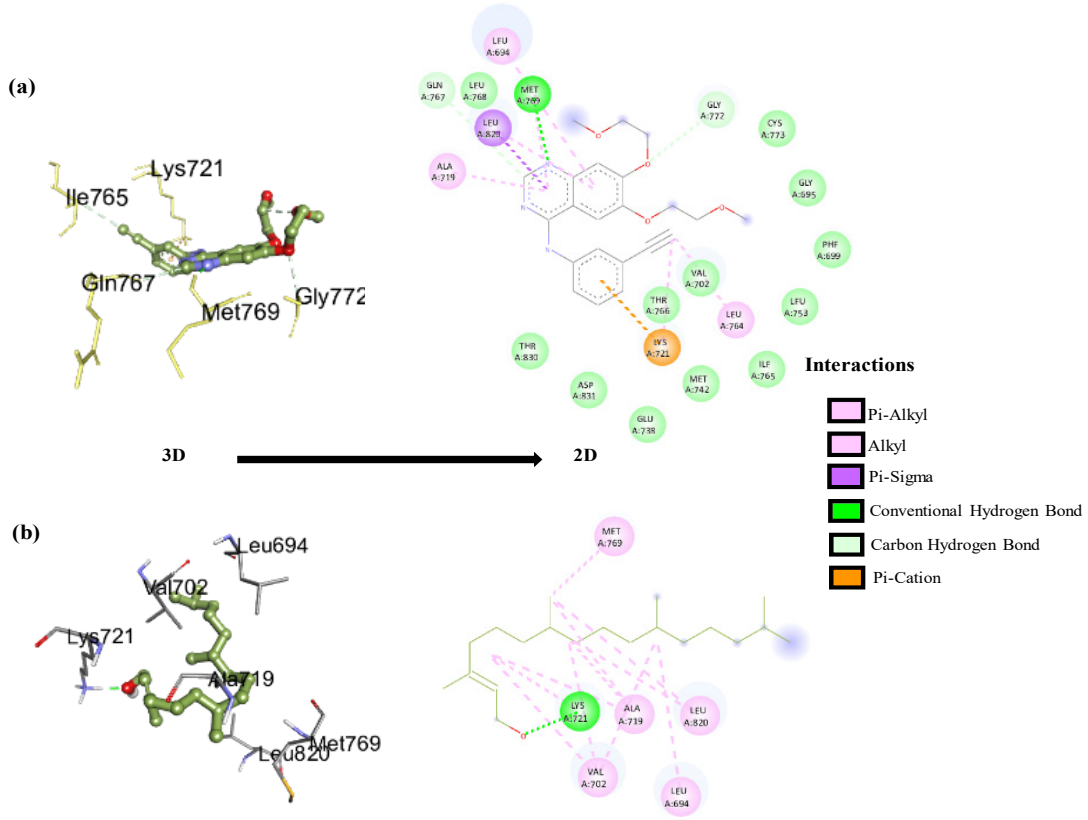

Figure 3. 3D and 2D Visualization and Docking Poses of Erlotinib as Native Ligand (a) and Phytol (b) in the Binding Site of EGFR

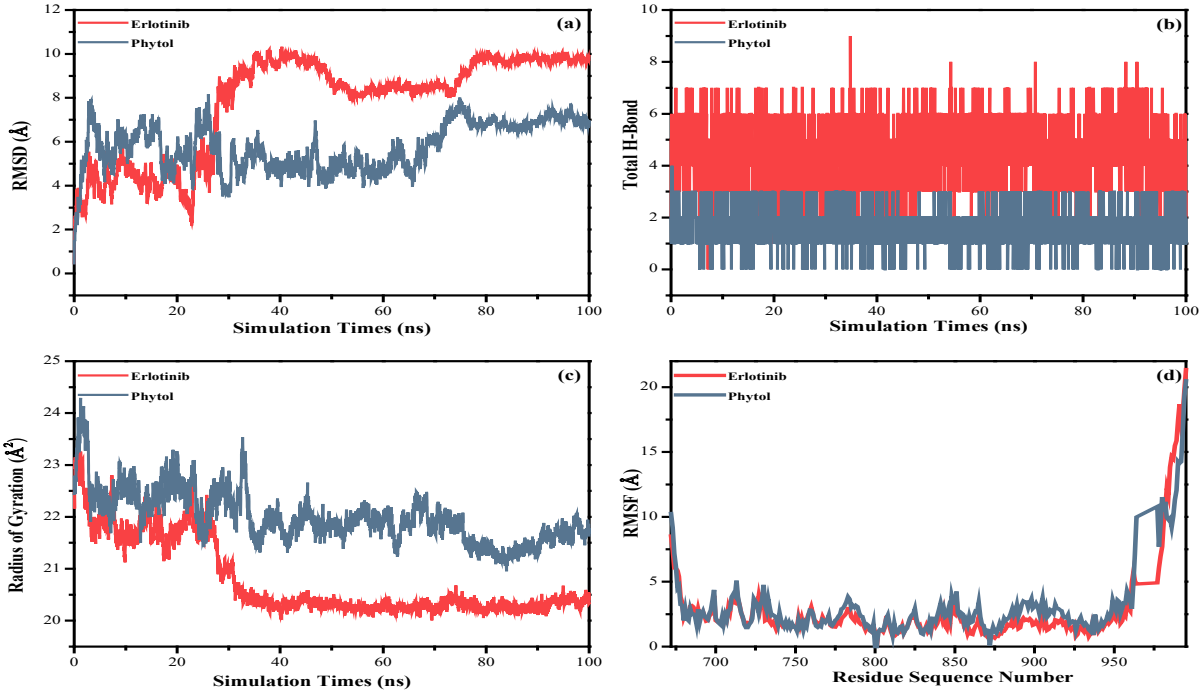

Figure 4. Dynamics Properties of EGFR-Phytol Compare to EGFR-Erlotinib Complex; (a) RMSD, (b)Total H-Bond, (c) RG, and (d) RMSF

Table 4. The Anticancer Bioactivity of Plants from the Genus of Dillenia

Plant	Cancer Cell	IC_{50} (µg/mL)	Reference
Dillenia serrata Thunb.	Breast Cancer (MCF-7)	493.17	[23]
	Cervical Cancer (HeLa)	91.08±0.23	Present Study
Dillenia suffruticosa Martelli	Hepatocellular	52.39	
	carcinoma (HepG2)	88.52	[31]
	Cervical Cancer (HeLa)	25.07	
Dillenia philippinensis	MCF-7	15.59	
	HCT 115	14.23	

moderately cytotoxic. The IC_{50} values of the methanol and n-hexane extracts were 250.90 and 380.20 µg/mL, respectively, indicating moderate cytotoxic activity. On the other hand, the ethyl acetate extract demonstrated high cytotoxic activity with an IC_{50} value of 91.08±0.23 µg/mL as presented in Table 3 and Figure 1.

The morphology of HeLa cells presented in Figure 1 shows that HeLa cells that have not been given the addition of extract have an irregular shape due to abnormal proliferation or cell division. Changes in cell morphology are an early sign of apoptosis induction. The higher the extract concentration, the fewer cells developed, and the HeLa cells began to decrease significantly at 62.50 µg/mL. Comparison of several anticancer studies of plants from genus Dillenia was shown in Table 4.

GC-MS Characterization

Gas Chromatography and Mass Spectrometry characterization was conducted to the most active extract due to IC_{50} value resulted in the cytotoxicity analysis. The chromatogram of ethyl acetate extract exhibited Phytol as the compound with highest percent area (25.64%) that presents in Figure 2.

Molecular Docking Analysis Through EGFR

The strategy to know the interaction mechanism of active compounds and protein receptors related to specific diseases is by conducting molecular docking analysis. EGFR as the protein receptor target was chosen due to the relation of EGFR overexpression with the adhesion, migration, proliferation, and differentiation of cancer cells (14). Figure 3 presents the 3D and 2D interaction of Phytol compared with a native ligand (erlotinib) in the active site of EGFR.

Molecular Dynamics Simulation

Molecular dynamics simulations showed that the EGFR-Phytol complex exhibited lower Root Means Square Deviation (RMSD) fluctuations than EGFR-Erlotinib (Figure 4a). This figure shows that the EGFR-Phytol complex underwent significant conformational changes after the simulation lasted 70 ns, but not as high as the EGFR-Erlotinib complex. Meanwhile, the EGFR-Erlotinib complex experienced higher conformational changes after the simulation lasted 20 ns, indicated by

the increase in RMSD value. High fluctuations in the EGFR-Erlotinib complex were also observed in the Radius of Gyration (RG) analysis but tended to stabilize after the simulation lasted 30 ns (Figure 4c). The stability is supported by the high number of hydrogen bonds during the simulation (Figure 4b). A comparison of the RMSF analysis of the two complexes shows a similar fluctuation pattern (Figure 4d), indicating that the complex formed has a similar stabilizing effect on the amino acids that make up the EGFR structure.

Discussion

Dillenia serrata T. was found to make up majority by polar compound, indicates by the highest yields of the obtained extract was methanol. The plant contains variety of secondary metabolite identified by phytochemical screening, such as alkaloids, flavonoids, saponins, tannins, terpenoids, and steroids. The study conducted by Rahmawati et al. [23] revealed comparable content in *D. serrata* T. leaves.

Bioactivity assay of *D. serrata* T. leaves extract with BSLT identified that the toxicity increased with the order of the extracts' polarity. The strongest toxicity activity (lowest LC_{50}) exhibited by the methanol extract, which becomes weaker as its polarity decreases (LC_{50} increased). The findings of this investigation were consistent with those of a study by Wakeel et al. [27], which found a correlation between an extract's or compound's polarity and toxicity. Previous studies have reported similar toxicity activities, with *D. serrata* T. leaves ethanol extract having an LC_{50} of 18.34 μg/mL [28].

The analysis also revealed a positive relationship between the sample's concentration and the larvae's mortality. Table 3 provides additional specific findings. In addition to the toxicity test, this method of analysis was also one of the common approaches to know the potential pharmacological effects of an extract or compound [29]. The high concentration of polyphenol in the extract was one of the variables, according to the study, that contributed to its toxicity value. Based on the findings in this analysis, it can be concluded that *D. serrata* T. leaves extract potentially has excellent pharmacological effects.

Research on the anti-cancer properties of the genus Dillenia has been documented previously (Table 4). The study conducted by Rahmawati et al. [23] found that the ethanol extract of *D. serrata* T. leaves exhibited moderate cytotoxicity against breast cancer cell lines (MCF-7), with an IC_{50} value of 493.17 μg/mL. This indicates a moderate level of cytotoxicity. The observed cytotoxicity is associated with steroid and alkaloid compounds, which aligns with our research identifying these phytochemicals. Additionally, in vitro analysis of HeLa cancer cell lines showed that *D. serrata* T. leaves have the potential to be developed into a future chemotherapeutic agent (IC_{50} 91.08±0.23 of ethyl acetate extract).

The ethyl acetate extract with the best anti-HeLa activity is known to contain phytol (25.64%), as shown by the GC-MS spectrum in Figure 2. This terpenoid compound is a secondary metabolite with diverse biological activities, one of which is an anticancer [30]. This result is in line with the molecular docking analysis carried out, namely that the Phytol compound was able to show a quite good affinity for the EGFR protein even though the Erlotinib (native ligand), an approved EGFR inhibitor, was still better. Respectively, the energy produced by these compounds when interacting with the EGFR protein is -5.08 and -6.77 kcal/mol. The Erlotinib complex with EGFR shows a hydrogen bond with the key amino acid that makes up the protein, namely Met796. Meanwhile, the Phytol has hydrogen bonds with the amino acid residue Lys721. The amino acid Met796 is also found in this complex but with a different type of interaction, namely Alkyl interaction.

The molecular dynamics simulation of the EGFR-Phytol complex demonstrated its stability. This stability is reflected in the lower fluctuations of the RMSD (Root Mean Square Deviation) and RG (Radius of Gyration) values. The stability of the EGFR-Phytol complex is quite similar to that of the EGFR-Erlotinib complex, as indicated by the RMSF (Root Mean Square Fluctuation) profile; however, it exhibits a lower number of hydrogen bonds.

Through this study, *Dillenia serrata* Thunb. leaves extract presents the bioactivity through inhibition on HeLa cell lines as in vitro analysis, especially the ethyl acetate extract, and exhibited Phytol as main components in the extract. Supported by the in silico analysis through molecular docking and molecular dynamics simulation, phytol has a good binding affinity and stability to the EGFR as the target protein. The in silico analysis provides insights into the potential of *Dillenia serrata* Thunb, especially ethyl acetate extract as an anticancer. The extract can inhibit HeLa cancer cell lines, mainly through its Phytol compound, which acts by inhibiting the EGFR protein. Inhibition of EGFR can potentially reduce the proliferation of cancer cells so that it can interfere with the signals necessary for cancer cell growth. However, isolating the bioactive compound components from the ethyl acetate extract of *Dillenia serrata* Thunb is necessary. for further research. It is important because the cytotoxic activity observed is the combined effects of all bioactive compounds in the extract, which may interact antagonistically or synergistically.

In conclusion, the cytotoxic and anticancer properties of *D. serrata* T. leaves extract have been investigated. All extracts demonstrated strong toxicity levels against *Artemia salina* L. as preliminary test with LC_{50} values ranging from 9.30±1.13 to 58.27±6.15 μg/mL. Out of all the extracts, ethyl acetate extract exhibited the strongest anticancer properties around and 91.08±0.23 μg/mL. Furthermore, Phytol as a main component in the extract presents the binding energy about -5.08 kcal/mol against EGFR as protein receptor target. With additional testing by using numerous cancer cells, the ethyl acetate of *Dillenia serrata* T. leaves extract certainly shows potential as a future anticancer agent.

Author Contribution Statement

Herlina Rasyid, Nunuk Hariani Soekamto, Arniati Labanni, contributed to design the research, writing the

manuscript, and overall supervision. Bulkis Musa and Syadza Firdausiah contributed to literature search and data processing. Siswanto Siswanto and Nur Hilal A. Syahrir contributed to compound clustering and data collection. Artania A. T. Suma and Harno Dwi Pranowo contributed to involved in field works/analysis and critical review. Bahrun Bahrun, Kadek Susi Badrawati and Mohammad Taufik Yusuf contributed to writing the manuscript, involved in field works/analysis.

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Ethical Declaration

Both human and animals are not used as research participants in this study.

Conflict of interest

The authors declare that they have no competing financial interest of personal relationships.

References

1. Safira F, Handayani V, Pratama M. Penetapan kadar flavonoid total pada ekstrak buah dengen (*Dillenia serrata* Thumb). Makassar Nat Prod J. 2023;1:260-267.
2. Sinala S, Ibrahim I, Dewi STR. Arisanty development of emulgel product from standardized dengen steam bark extract (*Dillenia serrata*). Res J Pharm Technol. 2024;17:188-196.
3. Illing I, Jelita ML. Identifikasi senyawa metabolit sekunder ekstrak buah dengen (*Dillenia serrata*). Pros Semin Nasiona. 2018;4(1):260-266
4. Sinala S, Ibrahim I, Salasa AM. Profile total polyphenol of the ethanol extract from Dengen (*Dillenia serrata*) leaf and stem bark which comes from Malangke City Luwu District. Interprofessional Proc Collab Urban Heal. 2019;2(1) 838-5.
5. Carla Wulandari Sabandar, Jalil J, Ahmat N, Aladdin NA, Kamaruddin HS, Wahyuningrum R. Aktivitas antioksidan dan penghambatan xantin oksidase kulit batang songi (*Dillenia serrata* Thunb.). Galen J Pharm. 2020;6:151-9. https://doi.org/10.22487/j24428744.2020.v6.i1.15008
6. Agus IP, Wibawa H, Lugrayasa IN. Potential study of *Dillenia serrata* Thunb. fruit extract from Bali Botanical Garden's collection. Berk Penelit Hayati. 2021;26(2):79-84. https://doi.org/10.23869/bphjbr.26.1.20215
7. Kamal SE, Asrina R, Lau SHA, Farid M, Patandung G, Kadang Y, et al. Potential of Antihypercholesterol Extract of Sempur Fruit Peel (*Dillenia serrata*) with In Vivo Study on Male White Rats (Rattus novergicus). Health Notions. 2021 Sep 30;5(9):317-21.
8. Jalil J, Sabandar CW, Ahmat N, Jamal JA, Jantan I, Aladdin NA, et al. Inhibitory effect of triterpenoids from dillenia serrata (dilleniaceae) on prostaglandin e2 production and quantitative hplc analysis of its koetjapic acid and betulinic acid contents. Molecules. 2015;20(2):3206-20. https://doi.org/10.3390/molecules20023206.
9. Finetti F, Travelli C, Ercoli J, Colombo G, Buoso E, Trabalzini L. Prostaglandin e2 and cancer: Insight into tumor progression and immunity. Biology (Basel). 2020;9(12). https://doi.org/10.3390/biology9120434.
10. Wilson DJ, DuBois RN. Role of prostaglandin e2 in the progression of gastrointestinal cancer. Cancer Prev Res (Phila). 2022;15(6):355-63. https://doi.org/10.1158/1940-6207.Capr-22-0038.
11. Hashem S, Ali TA, Akhtar S, Nisar S, Sageena G, Ali S, et al. Targeting cancer signaling pathways by natural products: Exploring promising anti-cancer agents. Biomed Pharmacother. 2022;150:113054. https://doi.org/10.1016/j.biopha.2022.113054.
12. Singh D, Vignat J, Lorenzoni V, Eslahi M, Ginsburg O, Lauby-secretan B, et al. Articles Global estimates of incidence and mortality of cervical cancer in 2020 : A baseline analysis of the WHO global cervical cancer elimination initiative. Lancet Glob Heal. 2020;11(2):197-206. https://doi.org/10.1016/S2214-109X(22)00501-0
13. Gondhowiardjo S, Christina N, Ganapati NPD, Hawariy S, Radityamurti F, Jayalie VF, et al. Five-year cancer epidemiology at the national referral hospital: Hospital-based cancer registry data in indonesia. JCO Glob Oncol. 2021;7:190-203. https://doi.org/10.1200/go.20.00155.
14. Alnoman RB, Parveen S, Khan A, Knight JG, Hagar M. New quinoline-based BODIPYs as EGFR/VEGFR-2 inhibitors: molecular docking, dft and in vitro cytotoxicity on HeLa cells. J Mol Struct. 2022;1247:131312. https://doi.org/10.1016/j.molstruc.2021.131312
15. Özenver N, Sönmez N, Yüzbaşioğlu Baran M, Uz A, Demirezer LÖ. Structure-activity relationship of anticancer drug candidate quinones. Turkish J Chem. 2024;48:152-65. https://doi.org/10.55730/1300-0527.3647
16. Muttaqin FZ. Molecular docking and molecular dynamic studies of stilbene derivative compounds as sirtuin-3 (Sirt3) histone deacetylase inhibitor on melanoma skin cancer and their toxicities prediction. J Pharmacopolium. 2019;2(2):112-121. https://doi.org/10.36465/jop.v2i2.489
17. Geethaa S, Thavamany PJ, Chiew SP, Thong OM. Interference from ordinarily used solvents in the outcomes of Artemia salina lethality test. J Adv Pharm Technol Res. 2013;4(4):179-82. https://doi.org/10.4103/2231-4040.121411
18. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med. 1982;45(5):31-34. https://doi.org/10.1055/s-2007-971236
19. Sharma N, Arya G, Kumari RM, Gupta N, Nimesh S. Evaluation of anticancer activity of silver nanoparticles on the A549 human lung carcinoma cell lines through alamar blue assay. Bio Protoc. 2019;9:e3131. https://doi.org/10.21769/BioProtoc.3131
20. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. J Comput Chem. 2009;30:2785-91. https://doi.org/10.1002/jcc.21256
21. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. Ucsf chimera--a visualization system for exploratory research and analysis. J Comput Chem. 2004;25(13):1605-12. https://doi.org/10.1002/jcc.20084.
22. Biovia DS. Discovery Studio Visualizer v.20.1.0.19295, Dassault Systèmes. San Diego, USA; 2019.
23. Rahmawati, Zulkifli, Nuringtyas TR, Jenie RI, Nugroho LH. Antioxidant and anticancer activity of *Dillenia serrata* Thunb ethanol extract against MCF-7 breast cancer cell line. Indones J Cancer Chemoprevention. 2023;13(3):175-183. https://doi.org/10.14499/indonesianjcanchemoprev13iss3pp175-183
24. Nguta JM, Mbaria JM, Gakuya DW, Gathumbi PK, Kabasa

JD, Kiama SG. Evaluation of acute toxicity of crude plant extracts from Kenyan biodiversity using brine shrimp, Artemia salina L. (Artemiidae). Open Conf Proc J. 2012;3:30-34. https://doi.org/10.2174/2210289201203010030
25. Riss TL, Moravec RA, Niles AL, Duellman S, Benink HA, Worzella TJ, Minor L,. Cell Viability Assays. Promega Corporation: Eli Lilly & Company and the National Center for Advancing Translational Sciences, Bethesda (MD); 2004.
26. Weerapreeyakul N, Nonpunya A, Barusrux S, Thitimetharoch T, Sripanidkulchai B. Evaluation of the anticancer potential of six herbs against a hepatoma cell line. Chin Med. 2012;7(1):15. https://doi.org/10.1186/1749-8546-7-15.
27. Wakeel A, Jan SA, Ullah I, Shinwari ZK, Xu M. Solvent polarity mediates phytochemical yield and antioxidant capacity of isatis tinctoria. PeerJ. 2019;7:e7857. https://doi.org/10.7717/peerj.7857.
28. Dali N, Dali S, Chairunnas A, Ayu H, Amalia M, Ayu S, Puspitasari A. Extraction of the chemical components of Dengen leaves (*Dillenia serrata* Thunb) by MAE method and activity test as antioxidant and toxicity. Indones J Chem Res. 2022;10(2):74-82.
29. Carballo JL, Hernández-Inda ZL, Pérez P, García-Grávalos MD. A comparison between two brine shrimp assays to detect in vitro cytotoxicity in marine natural products. BMC Biotechnol. 2002;2:17. https://doi.org/10.1186/1472-6750-2-17.
30 Islam MT, Ali ES, Uddin SJ, Shaw S, Islam MA, Ahmed MI, et al. Phytol: A review of biomedical activities. Food Chem Toxicol. 2018;121:82-94. https://doi.org/10.1016/j.fct.2018.08.032.
31. Rahayu S, Chang J, Supiyani A, Prasetiyo A. Anticancer activity of simpor (Dillenia suffruticosa Martelli) leaf extracts from belitung against HeLa cervical cancer cells. Hasanuddin University; 2024.
32. Dante RAS, Ferrer RJE, Jacinto SD. Leaf Extracts from Dillenia philippinensis Rolfe exhibit cytotoxic activity to both drug-sensitive and multidrug-resistant cancer cells. Asian Pac J Cancer Prev. 2019;20(11):3285-90. https://doi.org/10.31557/APJCP.2019.20.11.3285

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