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Original Article

Cytotoxic Activity of *Shorea macrophylla* Root Fractions against HeLa and MCF-7 Cells: LC–MS Profiling and CDK2-Targeted Molecular Docking

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ABSTRACT

Natural products remain an important source of anticancer drug leads. *Shorea macrophylla* (Dipterocarpaceae), a tropical tree with limited reported bioactivity, has not been systematically evaluated for the cytotoxic potential of its roots. In this study, the root was extracted with methanol and partitioned into *n*-hexane, ethyl acetate, and methanol residue fractions. The chemical composition of these fractions was characterized by LC-MS, and their cytotoxic activities were assessed against HeLa (cervical) and MCF-7 (breast) cancer cell lines using a resazurin assay. The major constituents of the most active fraction were further investigated by molecular docking against cyclin-dependent kinase (CDK) to explore potential mechanisms of action. LC-MS analysis revealed polarity-dependent differences in composition: the *n*-hexane fraction was enriched in lipophilic terpenoids and flavonoid aglycones (predominantly quercetin and kaempferol), the ethyl acetate fraction contained moderately polar phenolics, flavonoids, and oligostilbenes, and the methanol fraction was dominated by polar flavonoid glycosides. Consistent with these findings, the *n*-hexane fraction exhibited the strongest cytotoxicity against HeLa cells (IC_{50} = 3.95 µg/mL), followed by ethyl acetate (IC_{50} = 80.94 µg/mL); while the methanol fraction was inactive (IC_{50} > 500 µg/mL). All fractions exhibited weak activity against MCF-7 cells (IC_{50} = 183.20–447.80 µg/mL). Molecular docking indicated favourable binding of quercetin and kaempferol within the ATP-binding pocket of CDK, with binding energies of –56.18 and –52.56 kcal/mol, respectively. These results demonstrate a polarity-dependent cytotoxic profile and suggest that lipophilic flavonoids contribute to the observed activity, highlighting *Shorea macrophylla* root as a promising source of bioactive compounds.

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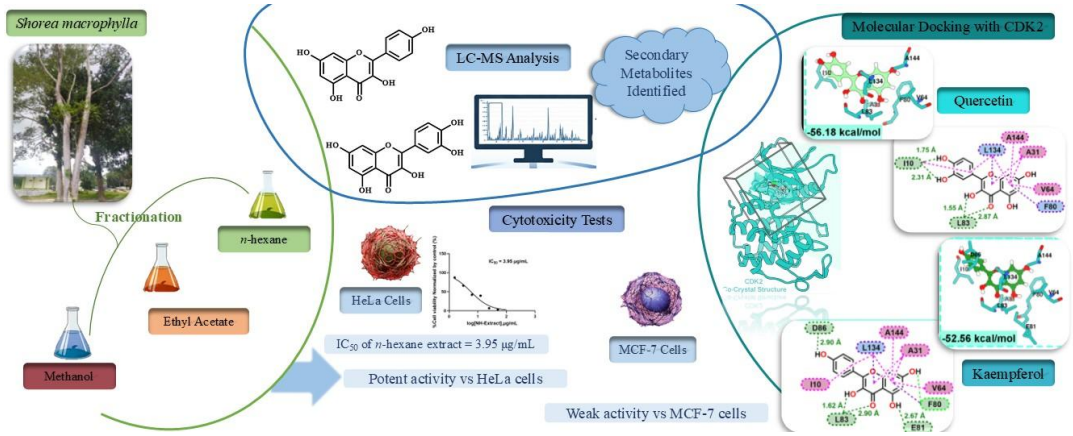
GRAPHICAL ABSTRACT



Introduction

Cancer remains one of the leading causes of death globally, accounting for nearly 10 million deaths in 2020, with cervical and breast cancers representing major contributors to morbidity and mortality among women [1]. Despite advances in chemotherapy and targeted therapies, clinical efficacy is often limited by drug resistance, off-target toxicity, and disease recurrence, underscoring the urgent need for novel therapeutic agents with improved selectivity and safety profiles [2]. Natural products have historically played a pivotal role in anticancer drug discovery, providing a vast chemical space enriched with structurally diverse secondary metabolites [1]. Plant-derived compounds such as terpenoids, flavonoids, and phenolic derivatives continue to be valuable scaffolds for the development of new therapeutics, often acting through multiple mechanisms that circumvent conventional resistance pathways [3].

The genus *Shorea* (family Dipterocarpaceae) is widely distributed across Southeast Asia and comprises approximately 150–200 species, many of which are recognized as rich sources of structurally diverse secondary metabolites [4]. These secondary metabolites have been reported to exhibit various biological activities, including antioxidant, anti-inflammatory, and anticancer effects [5]. *Shorea macrophylla* is a relatively under-studied species within this genus. Previous investigations have identified oligostilbenes (isohopeaphenol and hemsleyanol D) in the leaf extracts [6], but no comprehensive study has evaluated the cytotoxic activity of their root extracts against human cancer cell lines. Furthermore, no study has attempted to correlate the phytochemical composition of its extracts with biological activity in a polarity-dependent manner. Meanwhile, Liquid chromatography–mass spectrometry (LC–MS) enables rapid profiling of complex plant extracts and facilitates correlation of chemical fingerprints with bioactivities [7]. By integrating LC-MS-based metabolomics with *in vitro* cytotoxicity assays, compound classes likely responsible for pharmacological effects can be identified. To further explore potential mechanisms, molecular docking can be employed to predict the binding affinity of major constituents against key cancer-related targets, such as CDKs, which play critical roles in cell cycle regulation and are frequently dysregulated in cancer [8].

In this context, the present study aimed to evaluate the cytotoxic activity of *n*-hexane, ethyl acetate, and methanol fractions of *Shorea macrophylla* root against HeLa (cervical) and MCF-7 (breast) cancer cell lines using the resazurin assay, characterize the chemical composition of each fraction via LC-MS to identify major constituents; and perform molecular docking of the most abundant compounds from the active fraction against a CDK2 target to gain insight into their potential mechanism of action. This work represents the first systematic report on the polarity-dependent cytotoxicity and phytochemical profile of *Shorea macrophylla* root fractions, integrating experimental bioactivity with computational mechanistic insights to

provide a foundation for future bioactivity-guided isolation and drug development efforts.

Experimental

Chemicals and Reagents

Methanol, *n*-hexane, and ethyl acetate of analytical grade were used for extraction and solvent partitioning. LC–MS grade methanol and water were employed for chromatographic analysis. Dimethyl sulfoxide (DMSO) was used for sample preparation in cytotoxic assays. Resazurin sodium salt, BioReagent (Sigma Aldrich R7017) was used as a cell viability indicator. Roswell Park Memorial Institute Medium (RPMI) (Gibco 11875-093), Antibiotic (Sigma Aldrich P4333), fetal bovine serum (FBS) (Gibco 10270-106), phosphate-buffered saline (PBS) (Gibco 18912-014), Trypan Blue (Sigma Aldrich T-8154), and trypsin–EDTA (Gibco 25200-056) were used for cell culture. Human cervical cancer (HeLa) and breast cancer (MCF-7) cell lines were obtained from a certified cell repository. All chemicals and reagents were of analytical or cell culture grade and used without further purification.

Plant Material and Extraction

The fresh roots of *Shorea macrophylla* were collected and authenticated by Yayasan Generation Biology, Indonesia (voucher specimen no. 002/03.314JI.Genbinesia). The plant material was thoroughly washed, air-dried, and ground into a fine powder.

The powdered root material of *Shorea macrophylla* (1.5 kg) was extracted using methanol at room temperature. The solvent was removed under reduced pressure to obtain the crude methanolic extract. This extract was subsequently suspended in water and subjected to liquid–liquid partitioning with *n*-hexane followed by ethyl acetate to yield three fractions: *n*-hexane, ethyl acetate, and residual methanol (aqueous) fractions. Each fraction was concentrated under reduced pressure and stored for further analysis.

LC-MS Analysis

LC-MS analysis was performed on the *n*-hexane, ethyl acetate, and methanol fractions. The analysis was carried out using a Shimadzu LCMS-8040 system equipped with an electrospray ionization (ESI) source. Chromatographic separation was achieved on a Shim-pack FC-ODS column (150 mm × 2 mm, 3 µm particle size) maintained at 35 °C. The mobile phase consisted of 90% methanol delivered under isocratic conditions at a flow rate of 0.5 mL/min, with a total run time of 80 min. The injection volume was 1 µL.

Mass spectrometry was conducted in positive ionization mode. The capillary voltage was set at 3.0 kV, and the sampling cone voltage was 23.0 V. The collision energy was maintained at 5.0 V, with low-energy collision-induced dissociation (CID) for fragmentation. The source temperature was 100 °C, and the desolvation temperature was 350 °C with a desolvation gas (N_2) flow rate of 60 mL/hr. Mass spectra were recorded over a mass-to-charge ratio (*m/z*) range of 10-1,500 with a scan time of 0.6 s per scan. Compounds were putatively identified based on their retention times, molecular ion peaks, and comparison with literature data and available mass spectral databases.

Cell Culture

Human cervical adenocarcinoma (HeLa) and human breast adenocarcinoma (MCF-7) cell lines were used to evaluate the cytotoxic activity of the extracts. Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution. Cells were maintained in a humidified incubator at 37 °C with 5% CO_2. All experiments were conducted using cells in the logarithmic growth phase.

Preparation of Extract Solutions

Stock solutions of *n*-hexane, ethyl acetate, and methanol extracts were prepared by dissolving each extract in DMSO at a concentration of 1 mg/mL. Working solutions were prepared by serial dilution with culture medium to obtain final

concentrations ranging from 100 to 0.1 µg/mL. The final DMSO concentration in all treatment wells did not exceed 0.1% (v/v), which was confirmed to have no significant effect on cell viability.

Cytotoxicity Assay (Resazurin Assay)

Cytotoxicity was assessed using the resazurin reduction assay. Cells were harvested at approximately 70% confluence using trypsin-EDTA solution, centrifuged at 3,000 rpm for 5 min, and resuspended in fresh culture medium. Cell viability and density were determined by trypan blue exclusion using a hemocytometer. Cells were seeded into 96-well plates at a density of 1.7×10^4 cells per well (in 100 µL) and allowed to adhere for 24 h at 37 °C in 5% CO_2.

After the attachment period, the culture medium was replaced with 100 µL of fresh medium containing varying concentrations of each extract. The final concentration of DMSO in all treatment wells did not exceed 0.1% (v/v), with cisplatin serving as a positive control. Untreated cells, which received only culture medium was used as the negative control, while cells treated with 0.1% DMSO (vehicle) served as the vehicle control. Cells were incubated for 48 hours under standard culture conditions. Following treatment, the medium was removed, and 100 µL of resazurin solution was added to each well. Resazurin is reduced by metabolically active cells to resorufin, producing a fluorescent signal proportional to viable cell number. Plates were incubated for 1-2 h, after which absorbance was measured at 570 nm with a reference wavelength of 600 nm using a multimode microplate reader. All experiments were performed in triplicate, with at least three independent replicates.

Receptor and Ligand Preparation

The target protein selected was cyclin-dependent kinase 2 (CDK2), and its co-crystal structure was retrieved from the Protein Data Bank (PDB ID: 6GUB) [9]. Missing amino acid residues were reconstructed using homology modeling with Modeller 9.21 software. Protonation at pH 7.0 was performed using the H++ online service. In the crystal complex, Alvocidib (PDB ligand code: F9Z) served as the native inhibitor for CDK2 and was used as a reference for the ATP-binding pocket. The docking process utilized native ligand coordinates extracted from the co-crystal, employing the Chimera 1.13 package. The potential inhibitors investigated in this study were quercetin and kaempferol, which were identified as the major compounds in the *n*-hexane fraction of *Shorea macrophylla* root based on LC-MS analysis. To compute the electrostatic potential (ESP) charges of quercetin and kaempferol, the Gaussian 16 program was used with density functional theory at the B3LYP/6-311++G(d,p) level of theory. Various parameters, including bonded, nonbonded, and restrained electrostatic potential (RESP) charges, were calculated using the antechamber and Amber force field (FF14SB).

Molecular Docking

The Dock6 package was used for molecular docking [8]. To identify the active site of CDK2, a redocking step was performed. Several essential parameters were considered for the redocking process, including grid_spacing, center coordinates, and dimensions. Specifically, for CDK2, the grid_spacing was 0.3 Å, the center coordinates were (x: −8.63, y: −22.54, z: 21.91), and the dimensions were (x: 26.62, y: 26.68, z: 26.22). A spherical cluster selection method was employed to prepare the grid box, and the functional grid score was used to calculate the energy of the flexible conformation and identify the ligand–receptor interactions. Additionally, the validation criterion was a ligand root-mean-square displacement value ≤ 2.0 Å. In the gas state, the functional grid-based scoring procedure was examined, taking into account variables such as van der Waals energy (E_{vdW}) and electrostatic energy (E_{ele}). The grid score was calculated as follows:

Grid-score = $E_{vdW} + E_{ele}$

Statistical Analysis

Results are expressed as mean ± standard error of the mean (SEM) of three independent

experiments, as calculated by the testing laboratory. Percentage cell viability was determined relative to untreated control cells. The percentage inhibition and half-maximal inhibitory concentration (IC_{50}) values were calculated from dose response curves based on three independent experiments by nonlinear regression analysis using Microsoft Excel 2021 based on dose–response curves. Moreover, for complex mixtures such as the *n*-hexane, ethyl acetate, and methanol fractions, cytotoxic activity is conventionally expressed as the half-maximal inhibitory concentration (IC_{50}) in µg/mL.

Results and Discussion

Cytotoxic Activity Against Cancer Cell Lines

The cytotoxic effects of the *n*-hexane, ethyl acetate, and residual methanol fractions obtained from the sequential partition of the crude extract were evaluated against HeLa (cervical) and MCF-7 (breast) cancer cell lines using the resazurin assay. Cell viability was measured after 48 h of treatment, and the half-maximal inhibitory concentration (IC_{50}) values were calculated from dose-response curves [10].

The *n*-hexane fraction exhibited potent concentration-dependent cytotoxicity against HeLa cells, with an IC_{50} value of 3.95 µg/mL (Figure 1). Complete growth inhibition (≥99%) was observed at concentrations ≥12.5 µg/mL (Figure 2). The ethyl acetate fraction showed moderate activity (IC_{50} = 80.94 µg/mL), while the residual methanol fraction was essentially inactive (IC_{50} > 500 µg/mL). Meanwhile, all three fractions showed considerably weaker activity against MCF-7 breast cancer cells than against HeLa cells. The *n*-hexane fraction gave an IC_{50} of 183.20 µg/mL, followed by ethyl acetate (392.20 µg/mL) and methanol (447.80 µg/mL) (Figure 3). Even at the highest tested concentration (1,000 µg/mL), complete cell death was not achieved for the ethyl acetate and methanol fractions, indicating lower sensitivity of MCF-7 cells to these extracts.

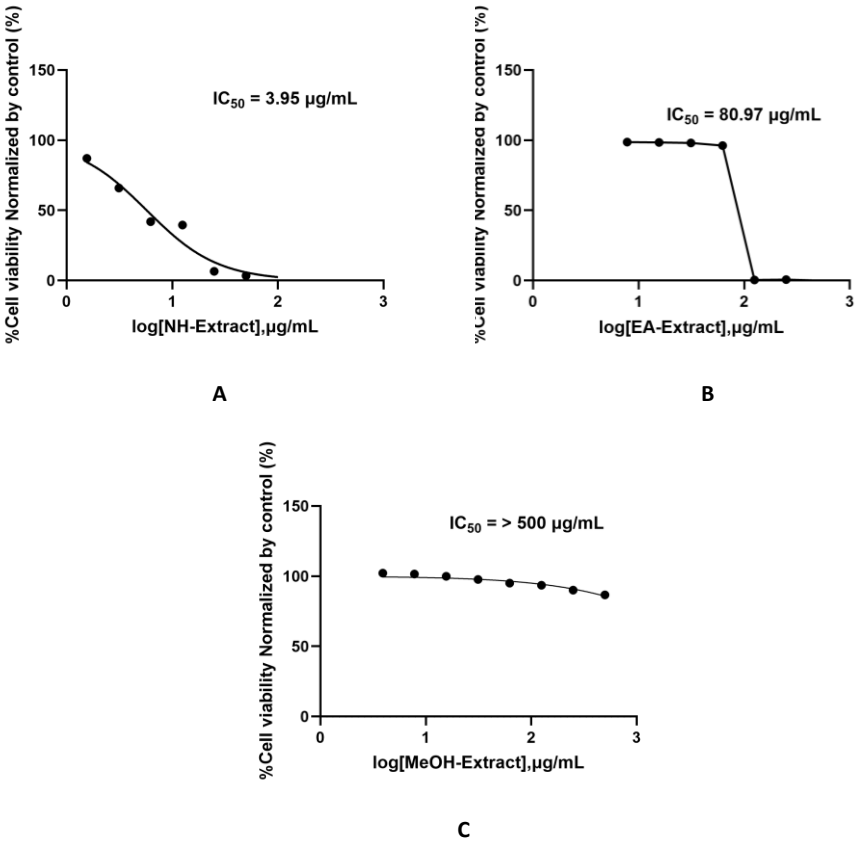


Figure 1: Cytotoxic activity (IC_{50} in µg/mL) of *Shorea macrophylla* root fractions A: *n*-hexane, B: ethyl acetate, and C: methanol against HeLa cells

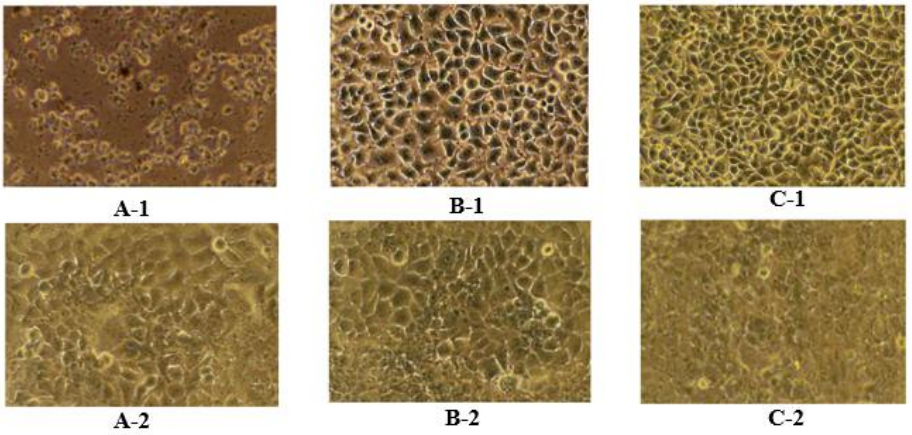


Figure 2: Anticancer activity on *HeLa* cell line in A-1; *n*-hexane, B-1; ethyl acetate, and C-1; methanol, MCF-7 cell line in A-2; hexane, B-2; ethyl acetate, and C-2; methanol extracts of *Shorea macrophylla* root

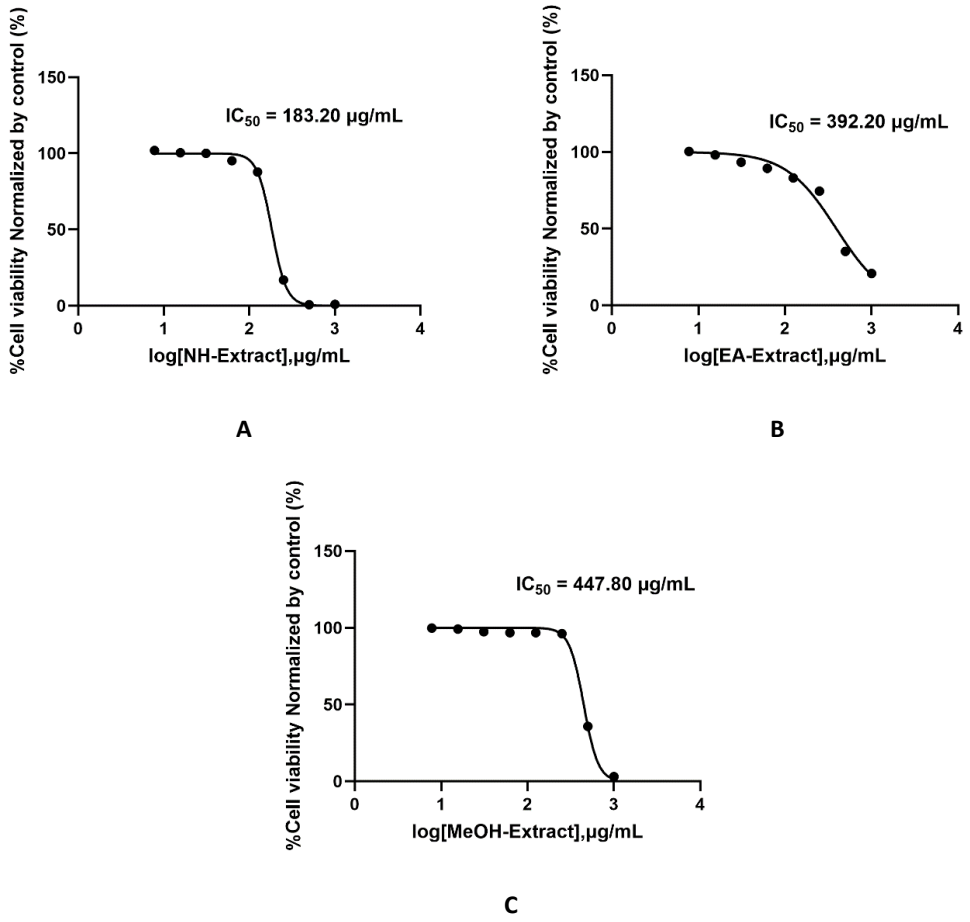


Figure 3: Cytotoxic activity (IC_{50} in µg/mL) of *Shorea macrophylla* root fractions A; *n*-hexane, B; ethyl acetate, and C; methanol against MCF-7 cells

A clear polarity-dependent trend was observed where the non-polar *n*-hexane fraction was the most potent, while the polar methanol fraction was the least active. Notably, all fractions showed selectivity toward HeLa cells over MCF-7 cells, with selectivity indices of 46.4 for *n*-hexane, 4.8 for ethyl acetate, and approximately 0.9 for methanol (Table 1). The *n*-hexane fraction was approximately 46-fold more active against HeLa cells than against MCF-7 cells, suggesting a cell line-specific sensitivity. The full concentration-response data (mean % inhibition ± SEM) are provided in Supplementary Tables S1 (HeLa) and S2 (MCF-7).

Table 1: *In vitro* cytotoxic activity of *Shorea macrophylla n*-hexane, ethyl acetate, and methanol extracts in MCF-7 cancer cell line

Extracts	IC_{50} (µg/mL) HeLa cancer cell line	IC_{50} (µg/mL) MCF-7 cancer cell line	Selectivity index (MCF 7/HeLa)
n-Hexane	3.95	183.20	46.4
Ethyl Acetate	80.94	392.20	4.8
Methanol	>500	447.80	~0.9

LC–MS Chemical Profiling

The *n*-hexane, ethyl acetate, and methanol fractions of *Shorea macrophylla* roots were analyzed by LC–MS to characterize their phytochemical compositions. Each fraction exhibited a distinct metabolite profile consistent with the polarity of the extraction solvent. Identifications were based on retention times, mass spectra, and comparison with literature and databases.

Table 2: The top three compounds identified by LC–MS in each fraction of *Shorea macrophylla* roots

Fraction	Rank	RT (min)	*m/z* $[M+H]^+$	Proposed compound	Composition (%)
n-Hexane	1	11.427	302.0427	Quercetin (1)	2.96
	2	10.322	286.0477	Kaempferol (2)	2.67
	3	2.249	168.1514	4-Methoxythujane (3)	2.47
Ethyl acetate	1	31.171	538.0900	8-[5,7-Dihydroxy-2-(4-hydroxyphenyl)-4-oxochromen-6-yl]-5,7-dihydroxy-2-(4-hydroxyphenyl) chromen-4-one (4)	2.01
	2	33.056	566.1213	7,4″-Di-*O*-methylagathisflavone (5)	1.96
	3	31.843	552.1056	7′-*O*-Methylagathisflavone (6)	1.86
Methanol	1	25.944	506.1060	(+)-*α*-viniferin-13b-*O*-*β*-glucopyranoside (7)	2.46
	2	11.427	302.0427	Hemsleyanoside C (8)	2.48
	3	10.322	286.0477	Vaticanol B (9)	2.34

**Identifications are putative, based on mass spectral matching and literature comparison. Percentages represent relative abundance based on peak area.*

The top three most abundant compounds in each fraction are summarized in Table 2. The *n*-hexane fraction was dominated by the free flavonoid aglycones' quercetin (1) and kaempferol (2), together accounting for >5.6% of the total ion current, along with the terpenoid 4-methoxythujane. The ethyl acetate fraction was characterized by biflavonoids, specifically Agathis flavone derivatives, with the highest relative abundance observed for the compound 8-[5,7-dihydroxy-2-(4-hydroxyphenyl)-4-oxochromen-6-yl]-5,7-dihydroxy-2- (4-hydroxyphenyl) chromen-4-one (4) (2.01%). Notably, quercetin was also detected in the ethyl acetate fraction (1.55%), while it was completely absent in the methanol fraction. The methanol fraction was enriched in oligostilbene glycosides, including (+)-α-viniferin-13b-O-β-glucopyranoside (7), hemsleyanoside C (8), and vaticanol B (9). The distribution of quercectin across the fractions mirrors the observed cytotoxicity: the *n*-hexane fraction (highest quercetin content, 2.96%) was the most potent (IC_{50} = 3.95 µg/mL), the ethyl acetate fraction (moderate quercetin, 1.55%) showed moderate activity (IC_{50} = 80.94 µg/mL), and the methanol fraction (no quercetin) was inactive (IC_{50} > 500 µg/mL). This correlation, together with the strong binding affinity of

quercetin to CDK2 in molecular docking, strongly suggests that quercetin is a key contributor to the anticancer activity of the *n*-hexane fraction. The presence of quercetin and kaempferol as the most abundant compounds in the *n*-hexane fraction, which exhibited the strongest cytotoxicity, made them prime candidates for subsequent molecular docking studies to explore their potential mechanism of action. The structure of the top three abundant compounds from each extract is demonstrated in Figure 4.

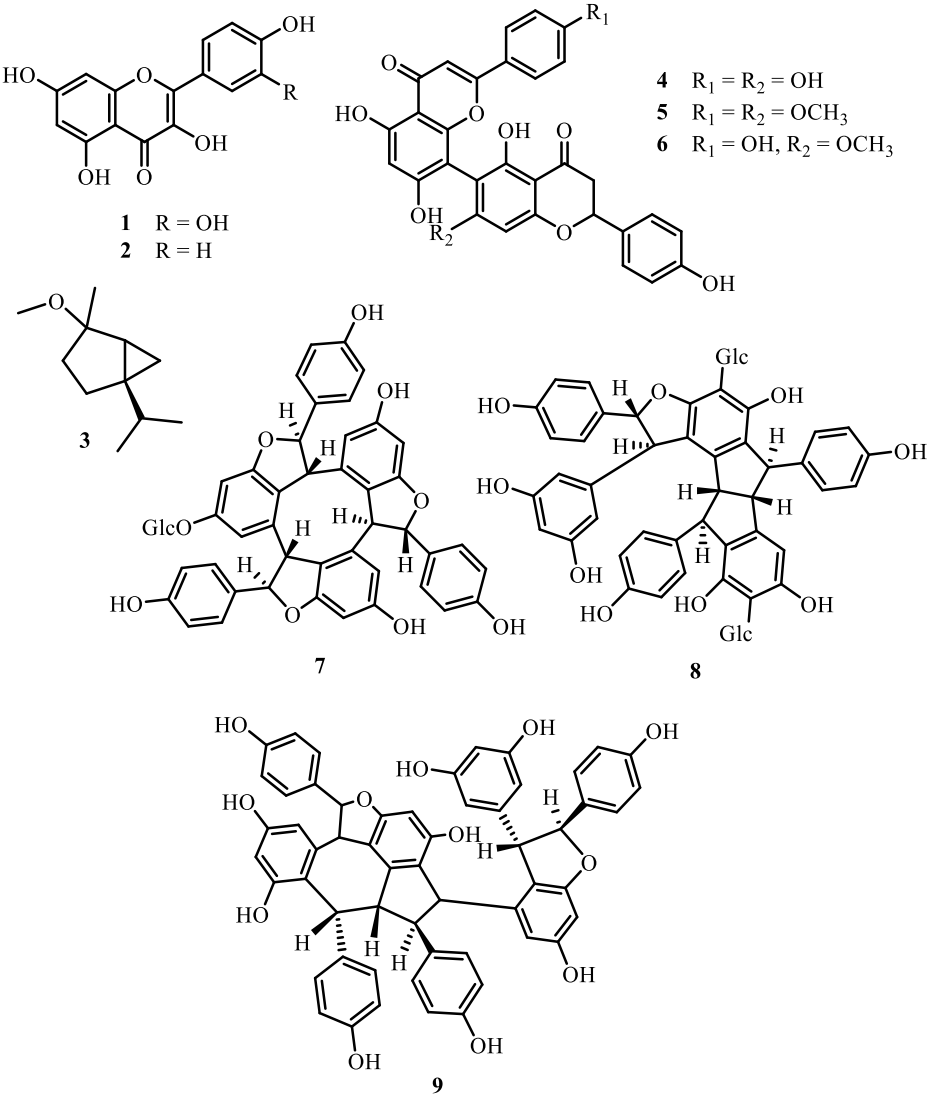


Figure 4: Chemical structures of the major compounds identified in *Shorea macrophylla* root fractions

Molecular Docking

Molecular docking is a technique that helps understand the active site coordinates of the target protein. To gain insight into the potential mechanism of action of the major compounds from the *n*-hexane fraction, molecular docking was performed against CDK2. The native co-crystal ligand, Alvocidib (PDB ligand code: F9Z), was used as a reference for the ATP-binding pocket. A redocking procedure was initially carried out to validate the docking protocol. The redocked F9Z superimposed well on the co-crystal coordinates and also met the necessary conditions, with an RMSD value of 0.48 Å and a grid-score of -65.92 kcal/mol, indicating a close alignment with the co-crystal coordinates (Figure 5). The potential inhibitor docking procedure was then performed using the same parameters as the redocking step.

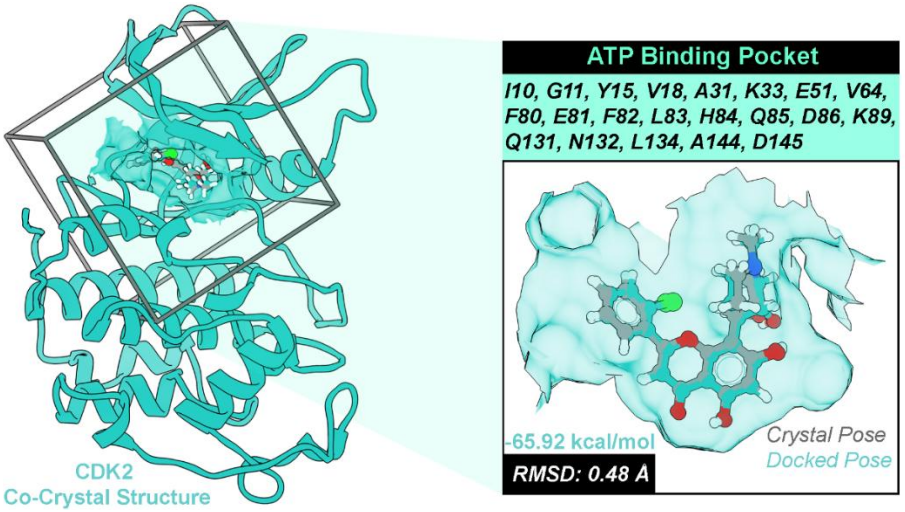


Figure 5: The ATP binding pocket of CDK2 was targeted using a native ligand (F9Z) coordinated through a re-docking step. The superposition of the native ligand between the crystal (light see green) and docked (gray) conformation. The grid score was performed in kcal/mol units.

It is important to highlight that molecular docking parameters measure the initial gas-phase conformations and provide insights into binding interactions of isolated compounds with CDK2. However, the precise molecular mechanisms of how these compounds exert their anticancer effects remain to be fully elucidated. Additional studies, including molecular dynamics simulations and experimental validation, are required for a more comprehensive understanding. Nonetheless, molecular docking is an effective tool for determining the initial binding coordinates and for rationalizing the observed bioactivity (Figure 6).

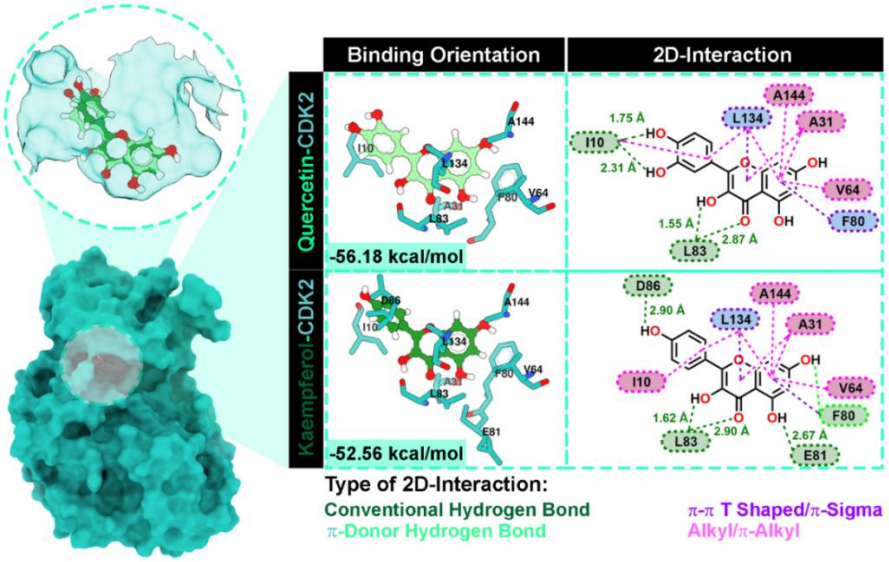


Figure 6: The modeled compounds are well-suited within the ATP-binding pocket of CDK2. The ligand-CDK2 interaction was presented by grid score (GS in kcal/mol units), 3D binding orientation, and 2D interaction types.

The pronounced cytotoxic activity of the *n*-hexane fraction against HeLa cells (IC_{50} = 3.95 µg/mL), compared to its relatively weak effect on MCF-7 cells, supports the contribution of lipophilic bioactive constituents identified in this fraction. The strong binding affinities of quercetin and kaempferol toward CDK2 further reinforce this observation, suggesting that inhibition of CDK2-mediated cell cycle progression may be associated with the observed antiproliferative activity. The higher binding affinity of quercetin, together with its greater relative abundance, is consistent with

the potent activity of the *n*-hexane fraction against HeLa cells. In contrast, the weaker response in MCF-7 cells indicates that additional cellular factors may influence sensitivity to these compounds. Overall, the docking results support the hypothesis that lipophilic constituents, particularly flavonoid aglycones, play a significant role in the cytotoxic activity of *Shorea macrophylla* root through potential CDK2 targeting.

Although, CDK2 is a key regulator of the cell cycle and a recognised anticancer target. The molecular docking results suggest that quercetin and kaempferol are capable of binding to the ATP pocket of CDK2. However, this computational finding does not, by itself, prove that CDK2 inhibition is responsible for the observed cytotoxicity. Experimental validation, such as CDK2 kinase activity assays and cell cycle analysis, would be required to confirm this mechanism in future studies.

Structure–Activity Relationship

The LC-MS chemical profiling of the three fractions, combined with their cytotoxic activities, reveals a clear polarity-dependent structure–activity relationship. The non-polar *n*-hexane fraction, which was most potent against HeLa cells (IC_{50} = 3.95 µg/mL), contained the free flavonoid aglycones quercetin (2.96%) and kaempferol (2.67%) as its major constituents. These lipophilic, low-molecular-weight compounds readily diffuse across cell membranes, enabling rapid intracellular accumulation and interaction with key targets such as mitochondria and DNA [11,12]. Quercetin and kaempferol are well-known for their antiproliferative effects, including cell cycle arrest and induction of apoptosis [13]. Their high abundance in this fraction strongly correlates with the observed potent cytotoxicity. The semi-polar ethyl acetate fraction (IC_{50} = 80.94 µg/mL) was dominated by biflavonoids (agathisflavone derivatives) and also contained quercetin (1.55%). Biflavonoids are larger and more polar than monomeric flavonoids, which may reduce membrane permeability and direct cytotoxicity. Nevertheless, they have been reported to exhibit moderate anticancer activity through mechanisms such as inhibition of angiogenesis and modulation of signalling pathways [14]. The lower potency of this fraction compared to the *n*-hexane fraction is consistent with the reduced abundance of quercetin and the presence of larger, less permeable biflavonoids. The polar methanol fraction (IC_{50} > 500 µg/mL) contained oligostilbene glycosides such as (+)-α-viniferin-13b-*O*-β-glucopyranoside, hemsleyanoside C, and vaticanol B, but no detectable quercetin or kaempferol. These glycosylated compounds are highly hydrophilic and poorly penetrate cell membranes, resulting in minimal direct cytotoxicity. Their presence is consistent with the inactivity of this fraction, despite the known antioxidant and chemopreventive properties of oligostilbenes [15,16].

Molecular docking further supports the role of quercetin and kaempferol as key active constituents. Quercetin showed a strong binding affinity to the ATP-binding pocket of CDK2 (grid score = –56.18 kcal/mol), forming multiple hydrogen bonds and hydrophobic contacts with key residues. Kaempferol also bound (–52.56 kcal/mol) but with a lower score. The superior binding energy of quercetin aligns with its higher abundance in the most active fraction and its stronger cytotoxicity. These results suggest that quercetin may act as an ATP-competitive inhibitor of CDK2, contributing to the antiproliferative effect of the *n*-hexane fraction. Taken together, the data establish a consistent relationship: cytotoxic potency decreases with increasing polarity and glycosylation, and the most abundant lipophilic flavonoids (quercetin and kaempferol) in the *n*-hexane fraction are likely responsible for the observed activity, with quercetin playing a predominant role based on its distribution and docking score. The *n*-hexane fraction exhibited a 46-fold selectivity for HeLa cells over MCF-7 cells, indicating a marked cell line-specific sensitivity. Such differential responses are not uncommon and may arise from inherent differences in receptor expression, drug efflux pump activity, or apoptotic pathway integrity between cervical and breast cancer cell lines. While the exact mechanism underlying this selectivity requires

further investigation, the potent and selective activity of the *n*-hexane fraction against HeLa cells highlights its potential for further development.

A limitation of this study is the lack of isolated pure compounds. While LC-MS identified quercetin and kaempferol as major components of the active *n*-hexane fraction, the observed cytotoxicity may result from synergistic effects of multiple compounds or other unidentified constituents. Definitive attribution of activity to specific compounds requires bioactivity-guided fractionation and testing of isolated compounds in future studies.

Conclusion

This study provides the first systematic evaluation of the cytotoxic activity and phytochemical composition of *Shorea macrophylla* root fractions in relation to solvent polarity. The *n*-hexane fraction exhibited potent and selective cytotoxicity against HeLa cells (IC_{50} = 3.95 µg/mL) with 46-fold selectivity over MCF-7 cells, while the ethyl acetate fraction showed moderate activity (IC_{50} = 80.94 µg/mL), and the methanol fraction was inactive (IC_{50} > 500 µg/mL). LC-MS profiling of the three fractions revealed distinct chemical profiles: the *n*-hexane fraction was enriched in quercetin and kaempferol, the ethyl acetate fraction in biflavonoids, and the methanol fraction in oligostilbene glycosides. A clear polarity-dependent structure–activity relationship was established, demonstrating that cytotoxic potency decreases with increasing polarity and molecular complexity. Molecular docking further suggested that quercetin, the most abundant compound in the active fraction, binds favourably to CDK2, providing a plausible mechanism for its antiproliferative effect. The observed selectivity toward HeLa cells highlights the potential of the *n*-hexane fraction for further investigation. Although these findings are preliminary and require confirmation through isolation of individual compounds, they suggest that the *n*-hexane fraction of *Shorea macrophylla* roots contains lipophilic flavonoids that merit further investigation. Future work should focus on bioactivity-guided isolation of the active constituents such as quercetin and kaempferol from the *n*-hexane fraction, elucidation of their mechanisms of action, and evaluation of their safety profiles.

Conflict of Interest

The authors declared no conflicts of interest regarding the publication of this paper.

Consent for Publications

No applicable.

Availability of Data and Materials

The data supporting the findings of this study are included within the article and its supplementary materials.

Authors' Contributions

Theint Su Wai: conceptualization, methodology, investigation, data curation, and writing – original draft. Nanik Siti Aminah: funding acquisition, resources, and supervision. Muhammad Ikhlas Abdjan: methodology, investigation, formal analysis, visualization, and writing – review and editing. Salar Hafez-Ghoran: formal analysis and writing – review and editing: Abdullahi Musa: investigation and formal analysis. Rico Ramadhan: supervision and review. Alfinda Novi Kristanti: writing – review and editing and validation. Ahmad Saiful Ilah As Shofi: software, data analysis, and validation. Yoshiaki Takaya: NMR analysis and data interpretation. Yosephine Sri Wulan Manuhara: formal analysis and visualization. Alinanuswe Joel Mwakalesi: validation and review. Mizanur Rahman: software and investigation.

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