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Isolation and Molecular Docking of Secondary Metabolites Medicinal Plant Legundi (*Vitex trifolia* L.) as MCF-7 Cell Anticancer Compounds

(Pengasingan dan Dok Molekul Metabolit Sekunder Tumbuhan Ubatan Legundi (*Vitex trifolia* L.) sebagai Sebatian Antikanser Sel MCF-7)

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ABSTRACT

Vitex trifolia L., known in Indonesia as 'Legundi', is a traditional plant commonly used as a remedy for coughing up phlegm, postpartum recovery, fever, inflammation, and diseases related to female reproductive organs, and has the ability to resist colorectal cancer and cervical cancer. This study aims to identify secondary metabolite compounds from the leaves of *V. trifolia* L. and their ability to inhibit breast cancer cells. In the investigation of the EtOAc fraction, two secondary metabolite compounds, Vitexicarpin and 4-hydroxybenzoic acid, have been successfully isolated. Both compounds exhibit strong cytotoxic abilities against MCF-7 cancer cells with IC_{50} values of 3.427 µg/mL and 8.298 µg/mL, respectively. The results of the docking study show that Vitexicarpin has a stronger binding interaction than 4-hydroxybenzoic acid to the three targeted receptors, with the docking score values on the Estrogen Receptor (ER) at -8.0259 kcal/mol, Progesterone Receptor (PR) at -7.4452 kcal/mol, and Human Epidermal Receptor 2 (HER2) at -8.3101 kcal/mol. The *in silico* values of Vitexicarpin and 4-hydroxybenzoic acid compounds correlate with the *in vitro* study results. Therefore, it can be concluded that the isolated compounds have the potential to be anti-cancer agents.

Keywords: Breast cancer; isolation; molecular docking; *Vitex trifolia* L.

ABSTRAK

Vitex trifolia L. dikenali di Indonesia sebagai 'Legundi', merupakan tumbuhan tradisional yang biasa digunakan sebagai ubat batuk kahak, pemulihan selepas bersalin, demam, keradangan, penyakit yang berkaitan dengan organ reproduktif wanita dan mempunyai keupayaan untuk menentang kanser kolorektal dan kanser serviks. Kajian ini adalah untuk mengenal pasti metabolit sekunder daripada daun *V. trifolia* L. dan keupayaannya untuk menghalang sel kanser payudara. Dalam kajian pecahan EtOAc, dua sebatian metabolit sekunder Vitexicarpin dan asid 4-hidroksibenzoat telah berjaya didapat dan kedua-duanya mempunyai keupayaan sitotoksik yang kuat terhadap sel kanser MCF-7 dengan nilai IC_{50} masing-masing 3.427 µg/mL dan 8.298 µg/mL. Hasil kajian dok menunjukkan Vitexicarpin mempunyai interaksi pengikatan yang lebih kuat berbanding asid 4-hidroksibenzoat kepada tiga reseptor sasaran dengan nilai doking pada Reseptor Estrogen (ER) ialah -8.0259 kcal/mol, Reseptor Progesteron (PR) dengan nilai dok sebanyak -7.4452 kcal/mol dan Reseptor Epidermis Manusia 2 (HER2) dengan nilai dok -8.3101 kcal/mol. Nilai *in silico* sebatian Vitexicarpin dan asid 4-hidroksibenzoat mempunyai korelasi dengan hasil kajian *in vitro*, sehingga dapat disimpulkan bahawa senyawa yang diperoleh berpotensi sebagai anti-kanser.

Kata kunci: Dok molekul; isolasi, kanser payudara; *Vitex trifolia* L.

INTRODUCTION

Cancer is one of the leading causes of death worldwide. Approximately 9.6 million deaths are attributed to this disease, with the number increasing annually. Thus, prompt treatment is crucial to prevent a rise in cancer-related mortality rates with minimal side effects (Naz et al. 2020; Ramchandani et al. 2020). Cancer can affect various parts and tissues of the body, including breast cancer (Firoozeh et al. 2019). The MCF-7 breast cancer cell line is a subtype of breast cancer cells, characterized by ER^+, PR^+, HER^{2-}, and falls into the luminal A molecular subtype. MCF-7 cancer cells are estrogen-sensitive and express transcription with high levels of Estrogen Receptor α (ER α) but low levels of Estrogen Receptor β (ER β) (Camarillo et al. 2014; Chusniasih & Tutik, 2020; Liambo et al. 2022). Breast cancer is a heterogeneous disease characterized by the overexpression or dysregulation of several key molecular targets, among which Human

Epidermal Growth Factor Receptor 2 (HER2), Estrogen Receptor (ER), and Progesterone Receptor (PR) are the most clinically significant. These receptors play central roles in the pathogenesis and progression of breast cancer through the activation of intracellular signaling pathways that promote cell proliferation, survival, and metastasis. HER2, a member of the EGFR family, is amplified in approximately 15-20% of breast cancers and is associated with aggressive tumor behavior (Liu et al. 2025). ER and PR, expressed in the majority of breast cancers, drive tumor growth in response to hormonal stimulation. Despite the availability of targeted therapies such as trastuzumab for HER2-positive tumors and endocrine therapy for hormone receptor-positive cases, drug resistance, tumor recurrence, and limited efficacy in triple-negative subtypes remain major challenges in current treatment. Therefore, these three receptors remain pivotal targets for therapeutic intervention and drug development in breast cancer (Cheng 2024).

In recent years, the use of herbal plants to treat cancer has continued to be explored. Common treatment methods to prevent cancer include surgery, radiotherapy, and chemotherapy. Although these treatments are expensive, they still have serious side effects for patients such as hair loss and thinning of the digestive lining. Therefore, it is necessary to find other ways to treat cancer with fewer side effects (Naz et al. 2020). One of these methods is the use of herbal plants such as *V. trifolia* L.

V. trifolia L. is a medicinal plant in the Verbenaceae family and is typically found in tropical areas. Traditionally, various parts of the *V. trifolia* L. plant has been utilized by communities to treat conditions like coughs with phlegm, post-natal recovery, fever, wounds, inflammation, colds, irregular menstruation, and diseases related to the female reproductive organs (Djimabi et al. 2021; Resmi, Sri & Annisa 2018; Wibawa 2019).

Further analysis has shown that the phytochemical profile of the *V. trifolia* L. plant includes secondary metabolites such as terpenoids, flavonoids, alkaloids, phenolics, and steroids (Ifora, Aida & Sri 2022). Several components of secondary metabolite compounds isolated from *V. trifolia* L. plant are viterotulin A-D, abietatrien-3β-ol, 14-halimadien-6-one, and p-hydroxyacetophenone extracted from the fruit of *V. trifolia* L. (Djimabi et al. 2022, 2021). 3α-hydroxylanosta-8,24E-dien-26-oic acid and Ecdysone are derived from the leaves of *V. trifolia* L. (Ban et al. 2018; Thoa et al. 2018), while β-sitosterol is extracted from the stem bark of *V. trifolia* L. (Djimabi et al. 2022; Luo et al. 2017; Ojha & Jain 2021; Resmi et al. 2013).

Research indicates that the methanol extract of *V. trifolia* L. exhibits anti-inflammatory effects, cytotoxic effects, antioxidant properties, and antituberculosis H37Rv capabilities. Additionally, the ability of *V. trifolia* L. to inhibit the growth of colorectal cancer, cervical cancer, and breast cancer has been identified (Annamalai & Thangam 2022; Arneti, Ujang & Cylfyzha 2018; Indrayudha & Indah 2020; Jayuska et al. 2022; Ojha & Jain 2021; Tiwari et al. 2013). The potential of *V. trifolia* L. in addressing cancer lies in examining molecular interactions between ligands and macromolecules using computational simulations to predict chemical potency, determine bond conformations, and predict binding energies (Grinevicius et al. 2019). Docking methods can aid in discovering, designing, and developing new drugs and nutraceuticals from extracts, fractions, and pure compounds of plants such as *V. trifolia* L. Therefore, in this research, isolated secondary metabolite compounds from *V. trifolia* L. leaves were characterized to assess their potential as anti-breast cancer agents.

MATERIALS AND METHODS

GENERAL EXPERIMENTAL PROCEDURES

Reagents and solvents were purified using standard techniques. The melting point was determined in a capillary tube using a melting point apparatus (Stuart Scientific, Model SMP10). UV-Vis spectrum was measured using a Shimadzu UV-1800 UV/Visible Scanning Spectrophotometer, and the IR spectrum was determined using a Bruker Tensor 37 infrared spectrophotometer with KBr pellets. ^{1}H-NMR, ^{13}C-NMR, and 2D NMR spectra explaining the structure of pure compounds were performed using the BRUKER ASCEND 700MHz NMR spectrometer with acetone used as a solvent. Silica gel (Merck, 60–120 mesh) was used for column chromatography separation, and silica gel GF254 was used for thin-layer chromatography (TLC). Fractions were monitored using TLC and visualized by heating using a Lieberman Burchard (LB).

MATERIAL PLANT

Leaves of *V. trifolia* L. were collected from the Andalas Baruah Bukik village in West Sumatra Province, Indonesia, and the plant was identified at the Andalas University Herbarium (specimen number 488/K-ID/ANDA/XI/2022).

EXTRACTION AND ISOLATION

Samples (4.2 kg) of *V. trifolia* L. leaves were collected and dried before being extracted with methanol at room temperature. The resulting methanol extract was then mixed with water and subjected to partitioning with EtOAc. The EtOAc fraction (80 g) was further separated using Vacuum Liquid Chromatography (VLC) with elution carried out with n-hexane:ethyl acetate (10:0 - 0:10) and ethyl acetate:methanol (10:0 - 7:3) to yield seven fractions (Fr.1-Fr.7). Fraction 3 (3.1024 g) was then subjected to separation via silica gel column chromatography (60–120 mesh) with elution using hexane:ethyl acetate (10:0-0:10) and ethyl acetate:methanol (9.5:0.5), which resulted in the acquisition of seven sub-fractions (S.Fr.1-S.Fr.7). Sub-fraction 6 (S.F.6) was further purified using the trituration

method to isolate compound **1** in its pure form (35.7 mg), while sub-fraction 4 (S.F.4) underwent recrystallization with n-hexane/EtOAc (1:1) to yield pure compound **2** (41.1 mg).

*Compound **1** (Vitexicarpine)*

Yellow solid: UV-Vis (EtOAc) λmax 270 nm, 340 nm; IR (KBr) νmax 3431, 2918, 2848, 1732, 1651, 1588, 1554, 1514 cm^{-1}. ^{1}H dan ^{13}C data is shown in Table 1.

*Compound **2** (4-hydroxybenzoic acid)*

Soft brown solid: UV-Vis(EtOAc) λ_{max} 245 nm; IR (KBr) ν_{max} 3488, 3445, 2958, 2918, 2818, 1651, 1586, 1515, 1470, 1423 cm^{-1}. ^{1}H dan ^{13}C NMR data is shown in Table 1.

MTT ASSAY

In total, 100 µL of Michigan Cancer Foundation-7 (MCF-7) cells were cultured in each well of 96-well microtiter plates (three wells per sample) and incubated at 37 °C in a humidified atmosphere with 5% CO_2. To test the compounds, 100 µL of the test solution was dissolved in DMSO in DMEM medium with five concentrations ranging from 100 µg/mL to 6.25 µg/mL and incubated for 48 h. Subsequently, the cell media was removed, and 100 µL of 0.5 mg/mL MTT reagent, dissolved in DMEM medium, was added to each well (including control media). The cells were then incubated in a CO_2 incubator, following which 100 µL of stopper reagent (DMSO) was added. After 4 h of incubation, the absorbance of formazan crystals in each well was measured using an ELISA reader set at a wavelength of 595 nm. The IC_{50} value was determined by nonlinear regression analysis using GraphPad Prism 10.

MOLECULAR DOCKING

Molecular docking and binding modes of selected compounds were carried out using MOE 2022.02 software. The protein structure was obtained from the Protein Data Bank (https://www.rcsb.org/), and the molecular structure of the isolated compounds Vitexicarpin, 4-hydroxybenzoic acid, and lapatinib was downloaded from the website (https://pubchem.ncbi.nlm.nih.gov/). The structures of the compounds were prepared using Amber10EHT parameters and stored in the ligand database. The crystal structures of the proteins, which were estrogen receptor (ER) (ID: 2IOK), progesterone receptor (PR) (ID: 1A28), and Human Epidermal Receptor 2 (HER2) (ID: 3PP0), were downloaded from the PDB with Symmetry settings for Biomolecular Assembly and prepared using a Gradient of 0.001 RMS kcal/mol/A^2 and Forcefield CHARMM27. If the protein does not have a natural ligand, an active site search is performed via the menu Site Finder. The prepared proteins are validated using the re-docking method. Docking simulation was conducted using Ligand Atoms's 'Dock with Site' menu. The docking process was carried out using Triangle Matcher placement with the London dG score function and generated 30 poses. Subsequently, refinement was conducted using the Rigid Receptor method with the GBVI/WSA dG score function. Docking results were tabulated, containing Docking Score (S) and Root Mean Square Deviation (RMSD). Both 2D and 3D visualizations of all poses were considered to select the best pose.

RESULTS AND DISCUSSION

CHEMISTRY

Isolated compounds (Figure 1), Vitexicarpine (35.7 mg) is a yellow solid with the molecular formula $C_{19}H_{18}O_8$, melting at 187-188 °C. The IR spectrum of compound **1** shows absorption at 3431 cm^{-1}(OH), 2918 cm^{-1} (C-H), 1732 cm^{-1} (C=O) and 1651 cm^{-1} (C=C). The H-NMR and C-NMR spectra identified were in good agreement with the literature (Han et al. 2007; Mesaik et al. 2009) ^{1}H NMR indicates (700 MHz, Acetone) δ ppm: the discovery of 4 methoxy signals (-OCH_3), at δH 3.800 - 3.985 ppm with respective shift values are 3.985 ppm (3H, s, 7-OCH_3), 3.952 ppm (3H, s, 4'-OCH_3), 3.800 ppm (3H, s, 3-OCH_3), 3.884 ppm (3H, s, 6-OCH_3). As a result, the proton signals indicating the aromatic ring structure at δH 6.825 - 8.012 ppm with shift values of 8.012 ppm (1H, s, 3'-OH), 7.652 ppm (1H, d, J = 3 Hz, H-2'), 7.697 ppm (1H, dd, J = 3 Hz, 3.0 Hz, H-6'), 7.123 ppm (1H, d, J = 8. 0 Hz, H-5'), 6.825 ppm (1H, s, H-8), as the proton signal indicating the presence of hydroxyl groups that have hydrogen bonds at a shift of 12.695 ppm (1H, s, 5-OH) where the signal is typical for protons from OH groups that have hydrogen bonds with carbonyl groups. ^{13}C NMR showed 18 carbon signals (175 MHz, Acetone) δ ppm: the existence of four methoxy subtituents on the structur, four methines and eleven quaternary carbons. Four methoxy carbons at δC 55.94 ppm (6-OCH_3), 55.44 ppm (3-OCH_3), 59.66 ppm (7-OCH_3), 59.32 ppm (4'-OCH_3). Methine carbon signals at δC 90.84 ppm (C-8), 111.25 ppm (C-2'), 106.17 ppm (C-5'), 114.92 ppm (C-6'). Quaternary carbon signals at δC 132.29 ppm (C-1'), 138.62 ppm (C-6), 146.51 ppm (C-3), 120.98 ppm (C-3'), 150.05 ppm (C-4'), 155.83 ppm (C-2), 152.66 ppm (C-5), 152.31 ppm (C-9), 178.93 ppm (C-4), 159.27 ppm (C-7).

4-hydroxybenzoic acid (41.1 mg) was discovered as a soft brown solid with the molecular formula $C_7H_6O_3$ and has a melting point of 198 °C. The IR spectrum of compound **2** shows absorption at 3488 cm^{-1}(OH), 2958 cm^{-1} (C-H), and 1651 cm^{-1} (C=C). The H-NMR and C-NMR spectra identified were in good agreement with the literature (Hudia Umami et al. 2022). Spectra identified ^{1}H NMR (700 MHz, Acetone) δ ppm: 7.913 (1H, tt, H-1), 6.912 (1H, tt, H-4), the spectrum of that chemical shifts indicated aromatic protons. ^{13}C NMR showed five carbon signals (175 MHz, Acetone) δ ppm: 116.01 (C-3), 122.72

(C-1), 132 (C-2), 162.67(C-4), 167.68 (C-5). The signal at chemical shift 167.68 (C-5) indicates the carboxylate signal, and the other chemical shifts indicate the value of the aromatic carbon.

MTT ASSAY

The MTT assay of Vitexicarpin and 4-hydroxybenzoic acid demonstrated a correlation between the percentage of viability and variations in the concentration of the isolated compounds utilized. The percentage of cell viability with concentration (Figure 2). It was observed that the compound Vitexicarpin, at a concentration of 6.25 µg/mL, resulted in a viability percentage of 45.19%, indicating that 54.81% of the cells could not survive. This suggests that this concentration inhibited approximately 50% of the cancer cells tested, with the viability percentage continuing to decline up to a concentration of 100 µg/mL, where the viability percentage was 37.34%. This indicates that cell growth progressively diminishes as the sample concentration increases.

Similar trends were observed for the compound 4-hydroxybenzoic acid, with cell growth increasingly inhibited as the concentration of the test sample rose. However, 4-hydroxybenzoic acid's ability to inhibit around 50% of cancer cells was observed at concentrations between 25 µg/mL and 100 µg/mL. Through value conversion utilizing nonlinear regression analysis (GraphPad Prism 10), it was determined that the isolated compounds Vitexicarpin and 4-hydroxybenzoic acid exhibit strong cytotoxic abilities against MCF-7 cancer cells, with IC_{50} values of 3.427 µg/mL and 8.298 µg/mL, respectively. A low IC_{50} value indicates a strong cytotoxic capacity of a compound (Naishima et al. 2023).

MOLECULAR DOCKING

Vitexicarpin and 4-hydroxybenzoic acid, compounds that were obtained, were docked with three valid receptor proteins for re-docking: ER (PDB ID: 2IOK), HER2 (PDB ID: 3PP0), and PR (PDB ID: 1A28). The initial interaction observed was between the isolated compounds and the ER protein. Estrogen Receptor (ER) is a receptor in body cells, particularly in breast tissue and female reproductive organs. Estrogen receptors are essential transcription regulators and impact the hormone estrogen's biological function. These receptors are known to play a role in the development and progression of luminal A and B breast cancer (Fauziah et al. 2023). The 2D and 3D interaction images depicting the best interaction between the isolated compounds and ER are shown in Figure 3.

The strongest binding of the Vitexicarpin compound was observed with the 2IOK protein, with a docking score of -8.0259 kcal/mol, binding to two types of amino acids, Leucine (LEU 1346) and Phenylalanine (PHE 1404). The initial interaction occurs due to the electron density of the double bond (π) in the C ring of the Vitexicarpin compound, which binds to the hydrogen atom of the amino acid Leucine (LEU 1346), forming a strong and stable bond at a distance of 4.34 Å. The subsequent interaction occurs between the aromatic ring B group of the Vitexicarpin compound and the hydrogen atom of Phenylalanine (PHE 1404), forming a stronger hydrophobic bond with a distance of 3.79 Å.

4-Hydroxybenzoic acid binds weakly to the 2IOK protein compared to compound **1**, with a docking score of -4.8904 kcal/mol. It binds to two types of amino acids, namely Glutamic acid (GLU 1353) and Phenylalanine (PHE 1404). Strong interaction was also observed in isolated compounds with Human Epidermal Receptor 2 (HER2). HER2 is a protein responsible for the growth, proliferation, and differentiation of breast cells and the activation of the HER signaling pathway. Abnormal HER2 expression can lead to tumorigenesis, necessitating the inhibition of HER2 activity (Fauziah et al. 2023). The 2D and 3D interactions of the isolated compound with HER2 can be observed in Figure 4. Regarding the compound Vitexicarpin, the strongest binding occurred with a docking score of -8.3101 kcal/mol, binding to the amino acid Valine (VAL 734) and forming a bond distance of 3.77 Å.

The compound 4-hydroxybenzoic acid exhibited a lower interaction than compound **1**, with a docking score of -4.7153 kcal/mol. It binds to the amino acid Phenylalanine (PHE 864), forming a bond between the π electron of the aromatic group in 4-hydroxybenzoic acid and the H atom of the amino acid Phenylalanine (PHE 864) at a bond distance of 4.47Å.

The latest interaction observed was the compound's interaction with the Progesterone Receptor (PR), which is crucial in regulating the cell's response to the hormone progesterone. The 2D and 3D interactions of the compound with the Progesterone Receptor (PR) are depicted in Figure 5. In the Vitexicarpin compound, the most substantial binding occurred with a docking score of -7.4452 kcal/mol, binding to the amino acid Methionine (MET 801). The interaction involves the formation of bonds between the electronegative O atoms from the hydroxyl group in ring A of the Vitexicarpin compound and the amino acid Methionine (MET 801) at a bond distance of 3.99Å.

Regarding the compound 4-hydroxybenzoic acid, an interaction was found on protein 1A28 with a docking score of -4.7499 kcal/mol. It binds to three types of amino acids: Leucine (LEU 718), Arginine (ARG 766), and Phenylalanine (PHE 778). In Leucine (LEU 718), hydrogen bonding occurs between the O atom of the carboxylic acid group (COOH), resulting in a bond distance of 2.96 Å. In Arginine (ARG 766), hydrogen bonds form between the O atom of the hydroxyl group of 4-hydroxybenzoic acid and the H atom of the amine group (-NH2) in Arginine (ARG 766) with a bond distance of 3.04Å. Meanwhile, in the amino acid Phenylalanine (PHE 778), a hydrophobic bond was established between the π electrons of the aromatic group in 4-hydroxybenzoic acid and the H atom of the amino acid Phenylalanine (PHE 778) at a bond distance of 4.45Å.

TABLE 1. Spectrum data of ^{1}H NMR (700 MHz) and ^{13}C NMR (175 MHz) from compounds **1** and **2** in $CDCl_3$ (δ in ppm)

Position	**1**		**2**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1				122.72
2		155.83	7,913(tt)	132.77
3		146.51	6,912(tt)	116.01
4		178.93		162.67
5	12,695(s)	152.66		167.68
6		138.62		
7		159.27		
8	6.825 (s)	90.84		
9		152.31		
10		123.24		
1’		132.29		
2’	7.652 (d)	111.25		
3’	8.012 (s)	120.98		
4’		150.05		
5’	7,123 (d)	106.17		
6’	7.697 (dd)	114.92		
(7- OCH_3)	3.985 (s)	59.66		
(4’- OCH_3)	3.952 (s)	59.32		
(6- OCH_3)	3.884 (s)	55.94		
(3- OCH_3)	3.800 (s)	55.44		

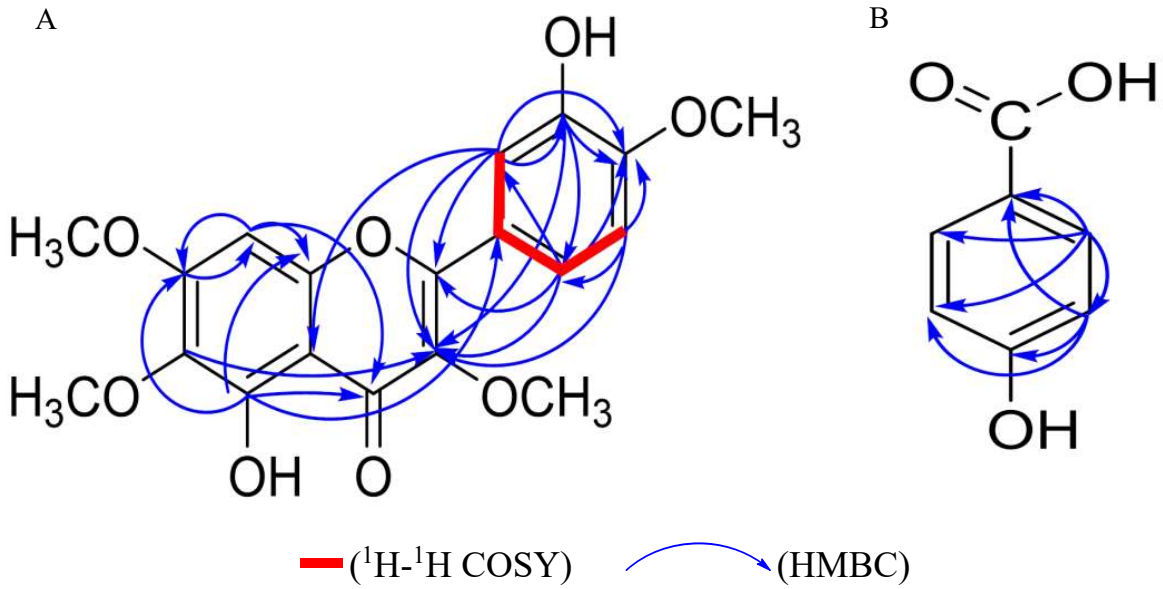

FIGURE 1. Structure and 2D NMR Correlations of (A)Vitexicarpin dan (B)4-hydroxybenzoic acid

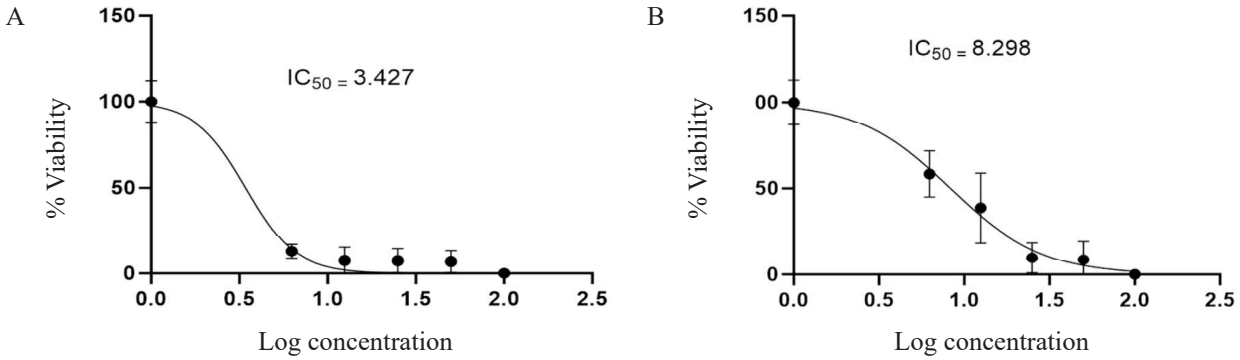

FIGURE 2. Cytotoxicity essay (A)Vitexicarpin dan (B) 4-hydroxybenzoic acid of MCF-7 cells

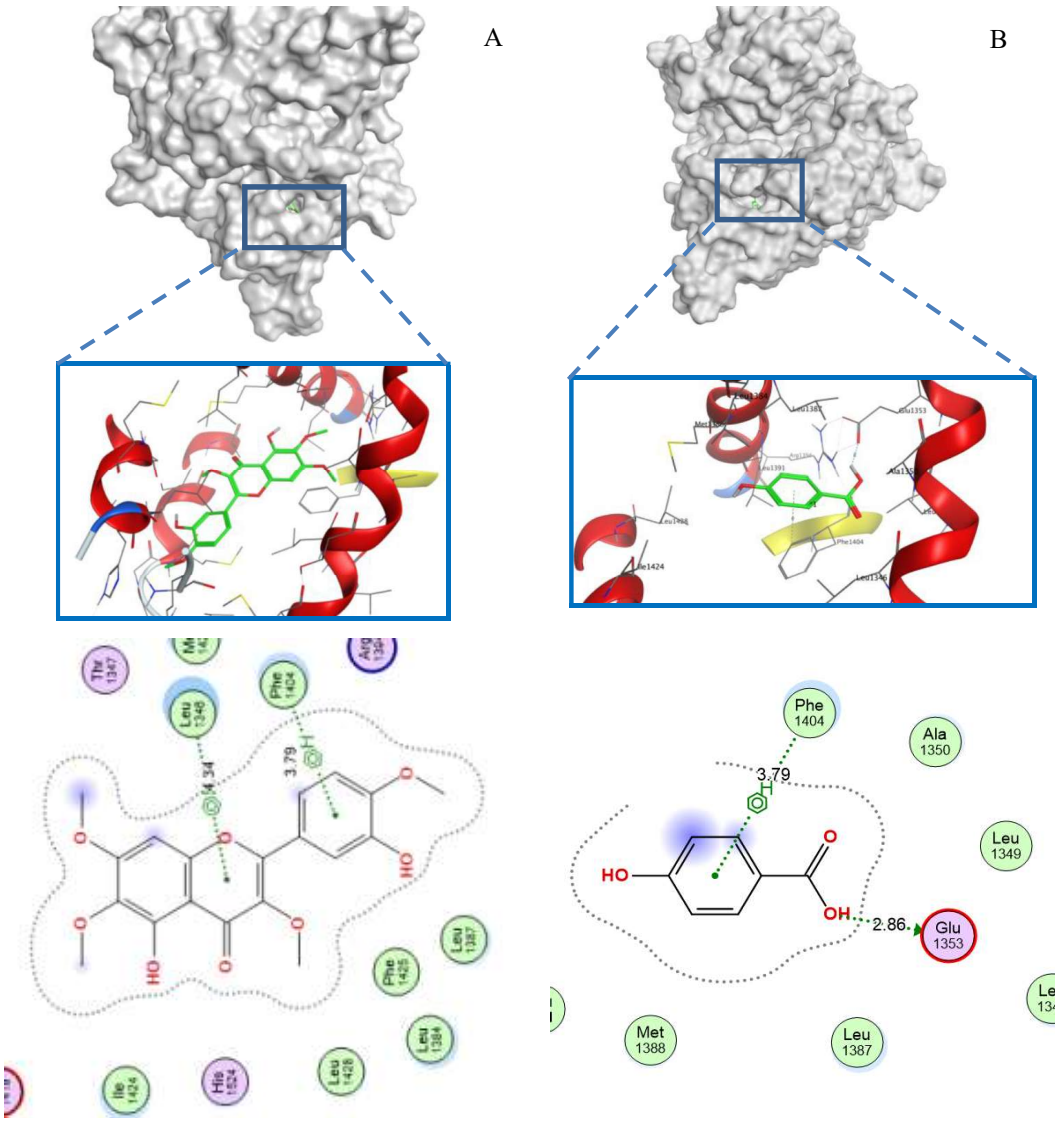

FIGURE 3. Interaction 2D and 3D with *Estrogen Receptor* (ER) of *Vitexicarpin* (A) and *4-hydroxybenzoic acid* (B)

The docking process was also compared to Lapatinib (Table 2) as a reference compound that has been found to be effective as a breast cancer drug both pre-clinically and clinically (Faizan et al. 2024). Based on the two compounds obtained, the Vitexicarpin compound showed a binding affinity value close to the standard drug compounds, especially ER and HER2. However, concerning PR, the docking value of the isolated compound was better than that of the Lapatinib compound.

On the Estrogen Receptor (ER), the difference in ΔG value between Vitexicarpin and Lapatinib compounds was 0.4873 kcal/mol; on the HER2 protein, it was 2.5787 kcal/mol. This variance in value is attributed to the varying biological activities between the isolated compounds and standard drugs. Lapatinib is recognized as a tyrosine kinase inhibitor that selectively inhibits epidermal growth factor receptors (Bilancia et al. 2007; Faizan et al. 2024).

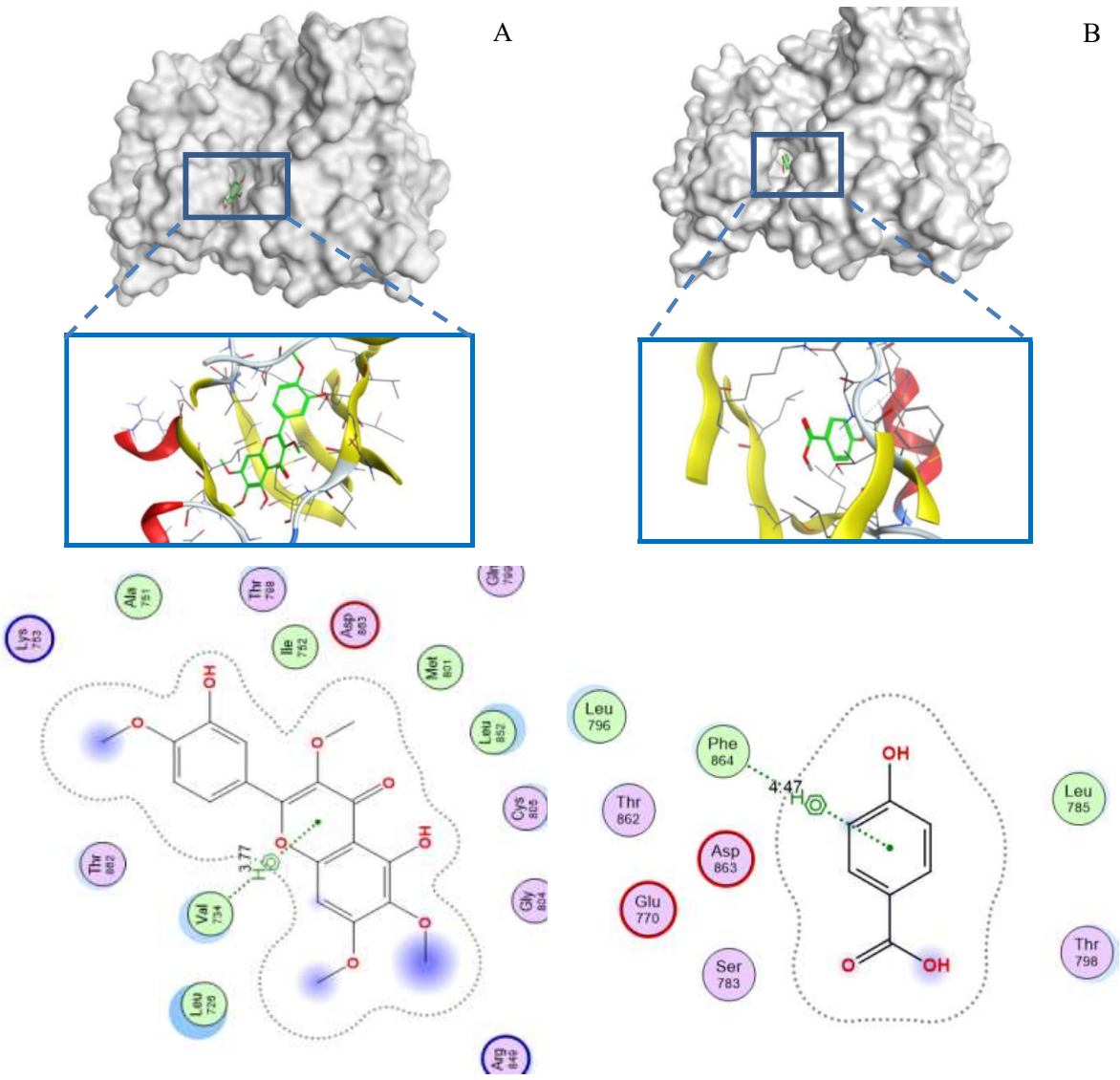

FIGURE 4. Interaction 2D and 3D with Human Epidermal Receptor 2 (HER2) of Vitexicarpin (A) and 4-hydroxybenzoic acid (B)

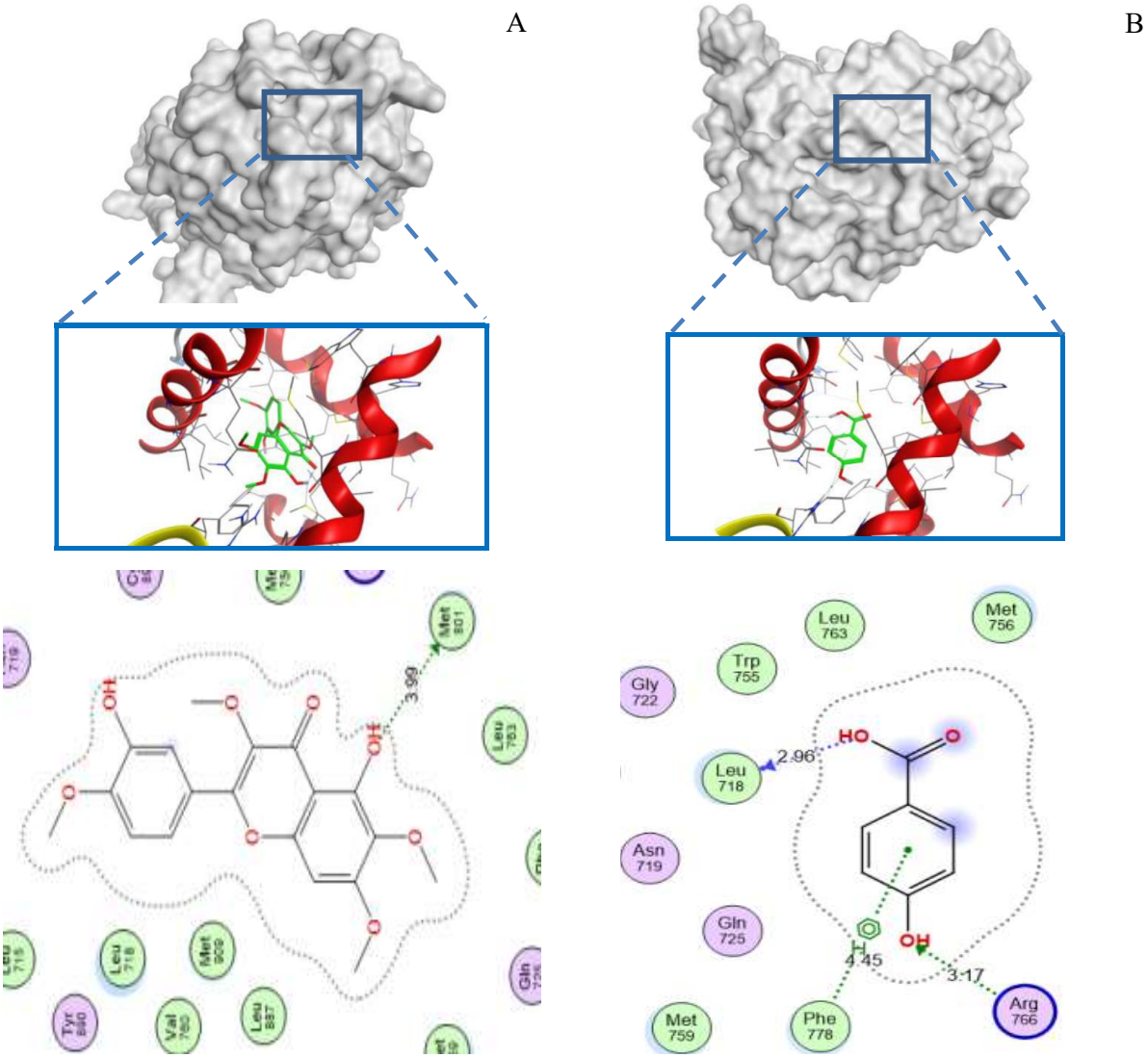

FIGURE 5. Interaction 2D and 3D with Human Epidermal Receptor 2 (HER2) of Vitexicarpin (A) and 4-hydroxybenzoic acid (B)

TABLE 2. Lapatinib docking with ER, HER2, and PR proteins

NO	Medicinal compound (ligand)	PDB	DS (kcal/mol)	RMSD	Bonding distance (Å)	Type of bond	Amino acids
1	Lapatinib	2IOK	-8,5132	1,8023	3,16	H- acceptor	CYS 1530
					3,04	H-acceptor	LYS724
2		3PP0	-10,888	1,9793	3,12	H-acceptor	MET801
					3,74	π–H	LEU726
3		1A28	-0,5721	2,801	3,41	H-donor	MET 801
					3,21	H-donor	CYS 891

CONCLUSIONS

These results indicate the potential of *V. trifolia* L. as an alternative plant for breast cancer prevention. Vitexicarpin and 4-hydroxybenzoic acid were successfully isolated and demonstrated strong cytotoxic bioactivity with IC_{50} values of 3,427 µg/mL and 8,298 µg/mL, respectively. The silico results using the molecular docking method for ER protein showed that Vitexicarpin exhibited stronger binding with a docking score value of -8.0259 kcal/mol compared to 4-hydroxybenzoic acid with a docking score value of -4.8904 kcal/mol. For protein HER2, Vitexicarpin showed stronger binding with a docking score of -8.3101 kcal/mol compared to 4-hydroxybenzoic acid (-4.7153 kcal/mol). In relation to protein PR, Vitexicarpin also demonstrated stronger binding than 4-hydroxybenzoic acid, with docking score values of -7.4452 kcal/mol and -4.7499 kcal/mol, respectively.

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