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Exploration of Anticancer Activity Potential and Molecular Docking Study of Essential Oil *Blumea balsamifera* Leaves Against Several Breast Cancer Cell Lines (T47D and MCF-7)

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ABSTRACT. The sembung plant (*Blumea balsamifera*) is a wild shrub traditionally used to treat various diseases, including cancer. However, comprehensive studies regarding the chemical composition, molecular docking interactions, and anticancer activity of its leaves essential oil against breast cancer cells are still limited. Therefore, this study aimed to explore the anticancer potential and molecular docking interactions of *B. balsamifera* leaves essential oil against estrogen receptor-positive breast cancer cell lines (T47D and MCF-7). The essential oil was isolated using hydrodistillation and analyzed using Gas Chromatography–Mass Spectrometry (GC-MS), which identified 36 chemical compounds. The major compounds were camphor (32.56%), caryophyllene (17.04%), 7-epi-silphiperfol-5-ene (5.33%), and gamma-eudesmol (5.01%). Preliminary cytotoxicity screening using the Brine Shrimp Lethality Test (BSLT) demonstrated strong cytotoxic activity with an LC_{50} value of 17.17 μg/mL. Molecular docking analysis against estrogen receptor alpha (ER-α) showed favorable binding affinity values ranging from -6.444 to -8.434 kcal/mol. Furthermore, the essential oil exhibited moderate anticancer activity against T47D and MCF-7 breast cancer cells, with IC_{50} values of 39.52 μg/mL and 30.18 μg/mL, respectively. These findings suggest that *B. balsamifera* leaves essential oil has potential as a natural source of anticancer compounds for further investigation.

Keywords: Anticancer, *Blumea balsamifera*, molecular docking, MTT assay

INTRODUCTION

Cancer cases around the world continue to increase, as are cancer cases in Indonesia. Data from the International Agency for Research on Cancer (IARC, 2024) shows that there were 19.97 million cancer cases worldwide, with 9.74 million deaths. In Indonesia, there were 408,661 cancer cases, with breast cancer being the most common type of cancer with 66,271 cases (16.2%)(IARC, 2024).

One plant believed to be effective as an anticancer drug is the sembung plant (*Blumea balsamifera*) (Pang et al., 2014; Wannes & Tounsi, 2021). Several studies have reported that fractions and extracts of this plant's leaves, such as ethyl acetate fraction, n-hexane fraction, water fraction, and ethanol extract, show significant cytotoxic activity against *Artemia salina* shrimp larvae (Rahmi et al., 2022;Rawati et al., 2023). The leaves essential oil also exhibits excellent cytotoxic activity against prawn larvae (Jiang et al., 2014). Regarding its anticancer activity, ethanol extract has anticancer activity against lung cancer cells (A549) (Nurlailiyah et al., 2025), chloroform extract against leukemia cells (HL-60) (Utami et al., 2023), and the methanol extract against hepatoma cancer cells (Norikura et al., 2008). These findings indicate that *B. balsamifera* contains bioactive compounds with promising cytotoxic and anticancer potential. However, previous studies mainly focused on extracts and fractions, while studies regarding the anticancer activity of *B. balsamifera* leaves essential oil against breast cancer cells are still limited.

Terpenoid compounds play a role in anticancer activity, including inducing apoptosis, inhibiting cell proliferation, and causing cell cycle arrest, with most of these compounds found in essential oils (Ulia et al., 2023;Onwuka et al., 2023). Estrogen receptor alpha (ER-α) was selected because it plays an important role in the growth and proliferation of breast cancer cells, while T47D and MCF-7 are estrogen receptor-positive breast cancer cell models commonly used in anticancer studies (Aka & Lin, 2012).

To date, there have been no reports regarding the anticancer activity of *B. balsamifera* leaves essential oil against T47D and MCF-7 breast cancer cells using combined GC-MS, molecular docking, BSLT, and MTT analysis. Therefore, molecular docking analysis was conducted in silico before in vitro testing to provide an overview of the interaction and potential anticancer activity of the major compounds in the essential oil, while BSLT and MTT methods were used to evaluate the cytotoxic and anticancer activity of the essential oil through LC_{50} and IC_{50} values.

EXPERIMENTAL SECTION

Material

The materials used in this study included fresh sembung leaves (*B. balsamifera*), distilled water, and anhydrous copper (II) sulfate. Seawater, *Artemia salina* shrimp larvae, dimethyl sulfoxide/DMSO (Merck), and Tween 80 for cytotoxicity testing using the BSLT method. T47D and MCF-7 breast cancer cells (Cell Culture Laboratory, Faculty of Biomedicine, Andalas University, and INALAB), RPMI-1640 medium for T47D cells, DMEM medium (Gibco, 2323000) for MCF-7 cells, fetal bovine serum/FBS (Gibco, 2445956RP), antibiotic and antimycotic/ABAM (Gibco, 2321098), phosphate buffer saline (PBS), and trypsin (Gibco, 2391466) for cytotoxic testing on cancer cells using the MTT method.

Equipment

The equipment and instruments used in this study include: a set of clevenger apparatus tools for isolating essential oils. GC-MS (Shimadzu GC-MS-TQ8050 NX) for analyzing chemical compound components. Glass boxes, micropipettes, and aerators for BSLT testing. Biosafety Cabinet (Thermo Scientific 1300 Series A2 Class II), CO_2 incubator (ARA P170), flask T25 (TPP 90026), 96-well plate (TPP, 92696), centrifuge tube (SPL, 61015), haemocytometer, inverted microscope (EVOS, AMEX1000), and microplate reader (iMax Biorad) for anticancer testing using the MTT method.

Software

The software used in this study included: GraphPad Prism 10 for determining the IC_{50} value. AutoDock Vina Tools-1.5.7, PyMOL 3.1.6.1, and BIOVIA Discovery Studio 2021 for molecular docking tests.

Sample Preparation and Essential Oil Isolation from the Leaves of *Blumea balsamifera*

Fresh leaf samples of *B. balsamifera* were collected in the Pariaman area, West Sumatera Province. The samples were tested in a herbarium. Next, the cleaned leaves of *B. balsamifera* (± 2.85 kg) were isolated by hydrodistillation using a clevenger apparatus for ± 7 hours at a temperature of 100 °C. The essential oil produced was dehydrated using anhydrous copper (II) sulfate (Suryati et al., 2021).

Analysis of Essential Oil Compounds in the Leaves of *Blumea balsamifera*

Analysis was performed using a GC-MS system to determine the chemical composition of essential oils. Measurements were taken using an RTx-5MS capillary column (30 m × 0.25 mm × 0.25 µm) with helium as the carrier gas. A sample of 1.00 µL was injected with a split ratio of 1:250. The system temperature conditions were set: injector temperature 250 °C, interface temperature 280 °C, and ion source temperature 200 °C. The column oven temperature program started at 50 °C (maintained for 3 minutes) and then increased at a rate of 5 °C/minute until it reached 250 °C. Mass readings were taken in the m/z range of 33-1000 AMU. Compound identification was performed by comparing the spectrum data obtained with the National Institute of Standards and Technology database (NIST) 14 (Ulia et al., 2023).

Cytotoxicity Test of Essential Oils using the Brine Shrimp Lethality Test (BSLT) Method

The isolated essential oil (10 mg) was dissolved in DMSO (40 µL) and Tween 80 (10 µL) and diluted with seawater to obtain concentration variations of 80, 40, 20, 10, and 5 µg/mL. Twenty larvae of *A. salina* were introduced into each test solution. The test was performed in triplicate (n = 3). After 24 hours, the number of dead larvae was counted to determine the LC_{50} value using probit analysis and a regression equation with Microsoft Excel. The same procedure was also carried out for the negative control solution.

Molecular Docking of Breast Cancer Cells to Major Compounds

In the molecular docking test, the major compound (Camphor, caryophyllen, 7-epi-silphiperfol-5-ene, and gamma-eudesmol) was used as a ligand, and the receptor was estrogen alpha protein (ER-α) with PDB codes (3ERT, 5WGD, and 5U2D). This test used AutoDock Vina Tools, PyMOL, and BIOVIA Discovery Studio software. The receptor structure was downloaded from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (https://www.rcsb.org/), while the ligand structure was downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/). In the ER-α structure, water and non-essential molecules were removed, and the receptor and ligand were separated using PyMOL software. Next, the structure was given polar hydrogen additions, non-polar hydrogen bonding, and file format conversion to PDBQT using AutoDock Vina Tools software. Then, the docking process continued with ligand insertion and receptor active site search using the Grid Box menu. The grid box parameters for 3ERT, 5WGD, and 5U2D were 15 × 15 × 15, 14 × 14 × 14, and 18 × 18 × 18 points with center coordinates of (30.282, -1.913, 24.206), (61.699, -2.758, 62.170), and (-4.483, 19.765, 4.684), respectively. Once the ligand position is correct, docking can be performed by clicking Run Autodock. The docking results will be displayed in terms of binding affinity energy values. BIOVIA Discovery Studio software can visualize the docking results in 2D and 3D(Puspa et al., 2024).

Anticancer Testing of Essential Oils on T47D and MCF-7 Breast Cancer Cells

The isolated oil was dissolved in DMSO to obtain a stock solution of 1000 µg/ml. It was then diluted with medium to obtain test solution concentrations of 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL and 6.25 µg/mL. T47D cancer cells were grown in complete RPMI-1640 medium, while MCF-7 cancer cells were grown in complete DMEM medium supplemented with 10% FBS and 1% antibiotic-antimycotic. The cells were incubated at 37 °C, 95% humidity, and 5% CO_2 until they reached ≥ 80% confluence. Confluent cells were harvested and

seeded in a 96-well plate for 24 hours under the same conditions. Next, 20 µl of the test solution was added to each well and incubated for 48 hours. After incubation, the medium was discarded, and the cells were washed with 100 µl of PBS. MTT reagent (0.5 mg/mL) was added and incubated for 4 hours. The formazan crystals formed were dissolved with 100 µL DMSO, and the absorbance was measured at a wavelength of 570 nm using a microplate reader. GraphPad Prism software was used to calculate the percentage of cell viability and determine the IC_{50} value.

RESULTS AND DISCUSSION

Sample Preparation and Essential Oil Isolation from the Leaves of *B. balsamifera*

The taxonomic identification results of the samples based on letter number 333/K-ID/ANDA/VII/2025 show that the samples used in this study belong to the Asteracea family and the species *B. balsamifera* (L) DC. Isolation of essential oil from ± 2.85 kg of fresh leaves of *B. balsamifera* using the hydrodistillation method produced a pale yellow essential oil with a volume of 4.9 mL and a weight of 4.422 grams. Upon determination the specific gravity and yield, it was found that this essential oil has a specific gravity of 0.902 g/mL and a yield of 0.171% (w/w).

Analysis of Essential Oil Compounds in the Leaves of *B. balsamifera*

Analysis of chemical compounds in essential oil from the leaves of *B. balsamifera* was performed using Gas Chromatography–Mass Spectrometry (GC-MS). The chromatogram profile (**Figure 1**) shows 36 peaks compared with the National Institute of Standards and Technology (NIST) database. The results of this comparison indicate the presence of 36 compound components in the essential oil of *B. balsamifera* leaves. The selected GC-MS data are compounds with a similarity index (SI) value of 76-98%. This is because the higher the similarity index value of a compound in the database, the greater its similarity to the compounds in the sample, as shown in **Table 1**.

Table 1 shows that the compounds found in the essential oil of *B. balsamifera* are dominated by terpenes, which include: hydrocarbon monoterpenes (2 compounds), oxygenated monoterpenes (5 compounds), sesquiterpene hydrocarbons (7 compounds), oxygenated sesquiterpenes (14 compounds), diterpene hydrocarbons (1 compound), and non-terpene compounds (7 compounds). The major compounds identified in the essential oil were camphor (32.56%) as an oxygenated monoterpene, caryophyllene (17.04%) and 7-epi-silphiperfol-5-ene (5.33%) as sesquiterpene hydrocarbons, and gamma-eudesmol (5.01%) as an oxygenated sesquiterpene.

Some of the chemical compounds in this study were almost the same as in previous studies. (Rasonabe et al., 2023) reported the main components of essential oil from *B. balsamifera* originating from the Philippines, including: γ-eudesmol (18.06%). Borneol (11.98%), Caryophyllene oxide (11.48%), and β-eudesmol (8.96%). (Thi et al., 2020) and, (Hai et al., 2024) also reported the main chemical components of the essential oil of this plant's leaves originating from Vietnam, including: Camphor (43.69%), Caryophyllene (12.71%), Caryophyllene oxide (5.98%), and β-Eudesmol (4.84%). The main components of essential oil from *B. balsamifera* originating from China were also reported (Wang & Yu, 2018) to include: borneol (57.7%), β-caryophyllene (7.6%), camphor (5.0%), and γ-eudesmol (2.0%). Overall, the chemical composition of essential oils from *B. balsamifera* plants is almost the same across different countries. The similarity in the main chemical components of these plants is because the same plants undergo the same biosynthetic pathways for secondary metabolites, which are genetically determined, but have different concentrations depending on their location and time of growth (Keefover-Ring, 2022; Gericke et al., 2021), the differences in the concentration of each essential oil are also influenced by temperature, rainfall, location, climate, and soil (Moghaddam et al., 2023).

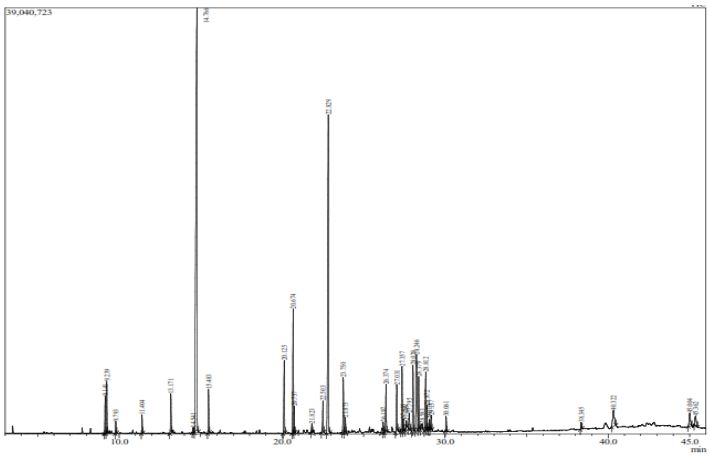

Figure 1. Chromatogram of gas chromatography–mass spectrometry (GC-MS) of essential oil from *B. balsamifera* leaves

Table 1. Chemical components of essential oil from *B. balsamifera* leaves

No	RT[a] (Minutes)	Compound	Molecular formula	Area (%)	SI[b] (%)
1	9.141	Beta-Pinene	$C_{10}H_{16}$	1.28	94
2	9.239	1-Octen-3-ol	$C_8H_{16}O$	1.73	98
3	9.793	3-Octanol	$C_8H_{18}O$	0.39	93
4	11.404	Beta-Ocimene	$C_{10}H_{16}$	0.63	95
5	13.171	Linalool	$C_{10}H_{18}O$	1.69	94
6	14.541	Chrysanthenone	$C_{10}H_{14}O$	0.32	87
7	14.766	Camphor	$C_{10}H_{16}O$	32.56	95
8	15.483	L-Borneol	$C_{10}H_{18}O$	1.69	96
9	20.125	Silphiperfol-5-ene	$C_{15}H_{24}$	2.86	96
10	20.674	7-epi-Silphiperfol-5-ene	$C_{15}H_{24}$	5.33	89
11	20.737	Cedrene-V6	$C_{15}H_{24}$	0.89	85
12	21.823	2-(1-Adamantyl)ethyl 1-adamantanecarboxylate	$C_{23}H_{34}O_2$	0.32	83
13	22.503	2,5-Dimethoxy-p-cymene	$C_{12}H_{18}O$	1.33	95
14	22.829	Caryophyllene	$C_{15}H_{24}$	17.04	95
15	23.750	Humulen	$C_{15}H_{24}$	2.21	92
16	23.875	(E)-2-epi-beta-caryophyllene	$C_{15}H_{24}$	0.62	93
17	26.197	Humulene epoxide II	$C_{15}H_{24}O$	0.45	82
18	26.374	Trans-Nerolidol	$C_{15}H_{26}O$	1.94	92
19	27.031	Ageratriol	$C_{15}H_{24}O_3$	2.06	87
20	27.357	Guaiol	$C_{15}H_{26}O$	2.84	93
21	27.435	Humulene epoxide I	$C_{15}H_{24}O$	0.47	95
22	27.609	Agarospirol	$C_{15}H_{26}O$	0.31	88
23	27.795	Rosifoliol	$C_{15}H_{26}O$	0.75	76
24	28.029	Gamma-Selinene	$C_{15}H_{26}$	2.87	88
25	28.246	Gamma-Eudesmol	$C_{15}H_{26}O$	5.01	92
26	28.379	Alpha.-Caryophylladienol	$C_{15}H_{24}O$	2.46	93
27	28.583	Pogostol	$C_{15}H_{26}O$	0.40	76
28	28.812	Alpha-epi-7-epi-5-Eudesmo	$C_{15}H_{26}O$	3.25	93
29	28.872	Neointermedeol	$C_{15}H_{26}O$	0.81	90
30	29.019	Bulnesol	$C_{15}H_{26}O$	0.38	90
31	29.137	14-Hydroxycaryophyllene	$C_{15}H_{24}O$	0.67	92
32	30.061	Pentadecanal	$C_{15}H_{30}O$	0.59	96
33	38.345	Neophytadiene	$C_{20}H_{38}$	0.28	92
34	40.322	2-Methylheptacosane	$C_{28}H_{58}$	1.68	93
35	45.004	Tetracosane	$C_{24}H_{50}$	1.07	92
36	45.362	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene	$C_{25}H_{42}$	0.83	90
Total				100	

[a] Retention time
[b] Similarity index

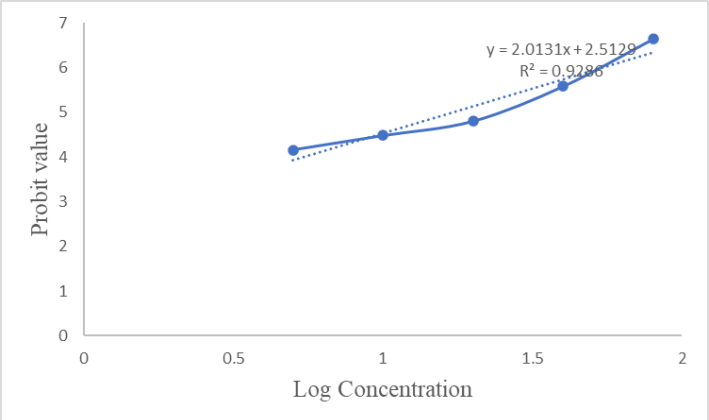

Figure 2. Relationship between log concentration and BSLT test probit value

Table 2. Three major compounds in *B. balsamifera* leaves essential oil that have cytotoxic activity

No	Compound	Cancer cells line	IC_{50}	Literature
1	Camphor	A549 (lung)	265.22 μg/mL	(Wu et al., 2016)
		MCF-7 (breast)	-	(Wu et al., 2016)
		OVCAR-3 (ovarian)	12.56 μg /mL	(De Lima et al., 2014)
2.	Caryophyllen	HCT-116 (colorectal)	19 μM	(Di Sotto et al., 2020)
		Colorectal	-	(Dahham et al., 2021)
		MDA-MB-231 & MCF-7 (breast)	27.6 μM and 4.4 μM	(Gbadebo et al., 2025)
		MG-63 (bone)	-	(Annamalai et al., 2020)
		Hepatoma & fibroblasts	63.7 μg /mL & 195.0 μg /mL	(Neta et al., 2017)
		KB (mouth)	-	(Ramachandhiran et al., 2022)
3	Gamma-Eudesmol	B16-F10 (melanoma) & K562 (leukemia)	8.86 ± 1.27 μg/mL & 5.15 ± 1.06 μg/mL	(Bomfim et al., 2013)

The compounds contained in an essential oil can influence the biological activity of a plant. Terpene compounds in the form of monoterpenes, sesquiterpenes, and diterpenes have been reported to play an important role in cytotoxic activity against several cancer cells (Contreras Martínez et al., 2023; Voon et al., 2022). This group of terpene compounds is the dominant composition in the essential oil of *B. balsamifera* leaves.

Cytotoxic Activity of Essential Oils Using the Brine Shrimp Lethality Test (BSLT) Method

The Brine Shrimp Lethality Test (BSLT) was conducted as a preliminary test to determine the cytotoxic activity of essential oil from the leaves of *B. balsamifera*. The LC_{50} value was determined based on the percentage of test animal mortality, namely *Artemia salina* shrimp larvae. The mortality rate of shrimp larvae varied according to the concentration used; the higher the concentration, the higher the percentage of test animal mortality. This was related to the composition of active compounds in the test solution used. The cytotoxic test results are attached in **Figure 2**.

The results of the LC_{50} value analysis in **Figure 2** show that the essential oil of *B. balsamifera* leaves has an LC_{50} value of 17.17 μg/mL and is therefore classified as highly toxic. This cytotoxic classification is based on the reported Mayer and Clarkson toxicity index (Zakwan et al., 2023), where compounds are categorized as highly toxic if the LC_{50} value is < 30 μg/mL, toxic in the range of 30–1000 μg/mL, and considered non-toxic if the $LC_{50} > 1000$ μg/mL. Based on the LC_{50} value, the essential oil of *B. balsamifera* leaves showed potential cytotoxic activity and may be considered for further anticancer evaluation against breast cancer cells. However, BSLT is only a preliminary general toxicity screening and does not specifically indicate anticancer activity.

The high cytotoxic activity of *B. balsamifera* leaves essential oil is influenced by its chemical content, which is dominated by terpenes. (Ulia et al., 2023) explains that the terpene content in essential oils plays a significant role in the cytotoxic activity of a test sample. Several major compounds in the essential oil of *B. balsamifera* leaves that have been reported to have cytotoxic activity are shown in **Table 2**.

The data in **Table 2** show that three of the four major compounds with the largest composition in *B. balsamifera* leaves essential oil have been reported to have cytotoxic activity against several cancer cells. This indicates that these compounds are likely to be the main contributors to the cytotoxic activity of *B. balsamifera* leaves essential oil.

The cytotoxic mechanism of terpenoid compounds in the BSLT method on *A. salina* L. brine shrimp larvae is related to their lipophilic properties, which enable them to penetrate cell membranes. This causes ion leakage and loss of homeostasis, damaging the larvae's cellular components, such as lipids, proteins, and DNA. As a result, the metabolic processes and biological functions of the larvae are disrupted, leading to their death (Pohan et al., 2023).

Molecular Docking of Breast Cancer Cells to Major Compounds

Molecular docking tests were conducted as a preliminary study to examine the cytotoxic potential of the major compound in essential oils against breast cancer cell proteins in silico. This test aims to predict the bioactivity of compounds through their interaction with the estrogen receptor alpha (ER-α). The estrogen receptor alpha (ER-α) was selected because this protein regulates breast growth and the development of breast cancer cells, both in MCF-7 and T47D cancer cells (Jia et al., 2016). In this study, ER-α proteins with PDB codes (3ERT, 5WGD, and 5U2D) were selected. These PDB-coded proteins have X-ray structures with a resolution ≤2.5 Å, providing adequate atomic interaction quality for statistical analysis c (Ferreira & Schapira, 2017). The use of X-ray structures with PDB resolution ≥ 2.5 Å resulted in less accurate molecular

docking results due to less accurate predictions of bonds, ligand orientation, and binding poses (Chakraborti et al., 2021). The redocking validation results are presented in **Table 3**, while the molecular docking results are shown in **Table 4.**

Table 3 shows that all compounds have a Root Mean Square Deviation. The RMSD value is used to measure how far the position of the docking ligand atom deviates from the position of the ligand in the crystal structure, where the smaller the RMSD value, the more similar the ligand pose is to the original crystal pose. (Ramirez & Caballero, 2018) Explains several RMSD value classifications, including: docking results are good if the RMSD value is ≤ 2 Å, acceptable if the RMSD value is between 2 Å and 3 Å, and poor if it is ≥ 3 Å. Therefore, it can be concluded that the docking results in this study are promising. **Table 4** also shows different binding affinity values for each compound and PDB code, indicating the strength of the interaction between the ligand and the target protein. The more negative the binding affinity value, the more stable the ligand and protein complex formed. These binding affinity values are also categorized into several categories: ≥ -5 is classified as weak, -5 to -7 is categorized as moderate activity, ≤ -7 is categorized as strong, ≤ -8 is very strong, and -9 to -12 is categorized as very strong and better than the control (Sharma et al., 2022; Ivanova & Karelson, 2022).

For the compounds camphor, caryophyllen, and gamma-eudesmol, the PDB code 3ERT is used, while for the compound 7-epi-Silphiperfol-5-ene, the PDB code 5WGD is used. This selection is also based on each protein PDB code's best binding affinity values. The camphor compound shows a moderate binding affinity value (-6.444), while the other three major compounds, caryophyllene, 7-epi-Silphiperfol-5-ene, and gamma-eudesmol, show binding affinity values ≤ -8, which are categorized as very strong and can be used as potential candidates for further anticancer testing. The interactions between the ligands and the target proteins are shown in **Figures 3-6**.

Figure 3 shows the interaction of camphor with the 3ERT receptor through van der Waals bonds (green) with residues Glu353, Leu384, and Met388, alkyl/π-alkyl interactions (purple), and hydrogen bonds (green lines) with residue Arg394. In addition, hydrophobic residues such as Leu346, Ala350, Leu349, Leu391, Leu387, Leu3, and Phe404 may also contribute to the stability of the complex. The 3D ligand-receptor complex shows the three-dimensional orientation of the ligand within a binding pocket surrounded by α-helices (red) and β-sheets (cyan). The 3D structural model shows a surface representation where the ligand is embedded in a pocket that enhances binding affinity.

Figure 4 shows the interaction of the caryophyllene compound with the 3ERT receptor. This interaction is hydrophobic (alkyl/π-alkyl) with residues Leu349, Leu346, Leu391, Leu387, Met388, and Ala350, which stabilize the position of the ligand in the receptor binding pocket. In addition, there are weak interactions with residues Met343, Gly521, and Phe404 through van der Waals forces. These interactions indicate that the overall stabilization of the complex depends on non-polar residues within the binding pocket.

Table 3. Results of the docking method validation

PDB ID	Native ligand	RMSD (Å)	Binding affinity (kcal/mol)	Validation
3ERT	OHT	0.992	-9.899	Valid
5WGD	EST	0.72	-11.11	Valid
5U2D	OBH	0.452	-11.69	Valid

Table 4. Molecular docking results of the major compound of essential oils

Ligands	PDB ID	Binding affinity (kcal/mol)
Camphor	3ERT	**-6.444**
	5WGD	-6.122
	5U2D	-5.794
Caryophyllen	3ERT	**-8.434**
	5WGD	-8.129
	5U2D	-8.056
7-epi-Silphiperfol-5-ene	3ERT	-7.876
	5WGD	**-8.127**
	5U2D	-7.48
Gamma-Eudesmol	3ERT	**-8.358**
	5WGD	-7.958
	5U2D	-7.632

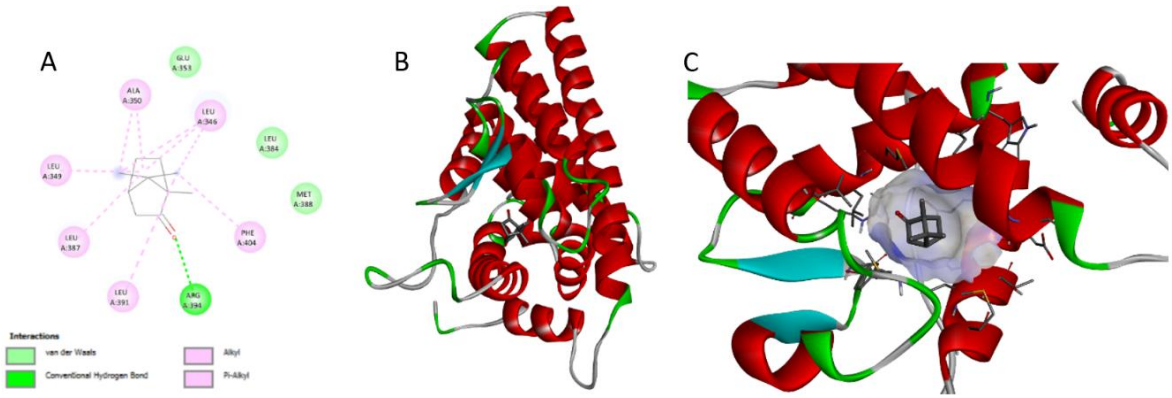

Figure 3. Interaction of camphor ligand with 3ERT receptor (**A**) 2D Interaction Diagram (**B**) 3D ligand-receptor complex (C) View of binding pocket surface

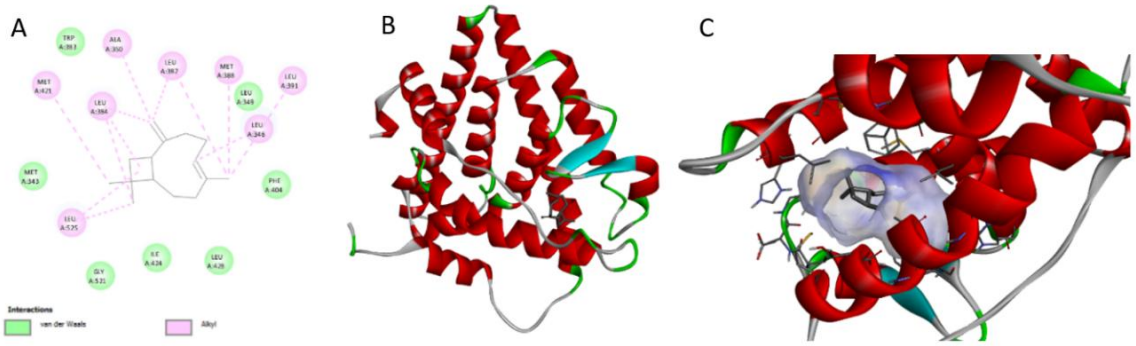

Figure 4. Interaction of the caryophyllene ligand with the 3ERT receptor (**A**) 2D Interaction Diagram (**B**) 3D Ligand-Receptor Complex (**C**) View of the binding pocket surface

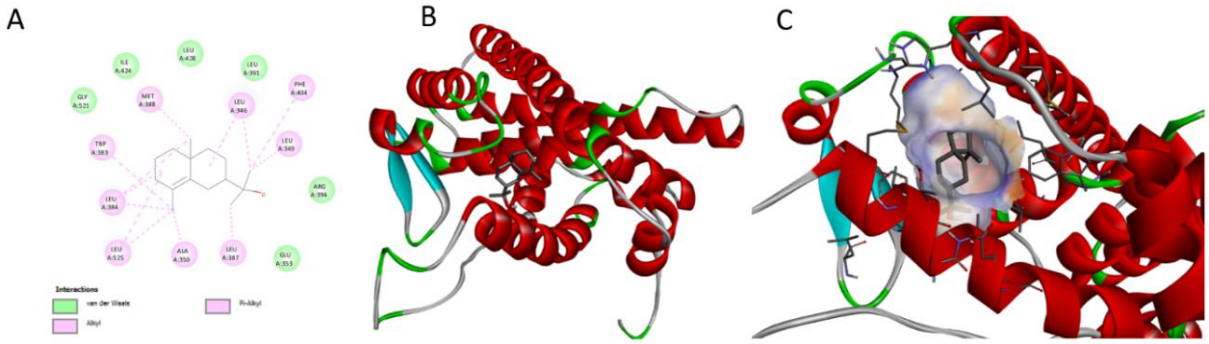

Figure 5. Interaction of the Gamma-Eudesmol ligand with the 3ERT receptor (**A**) 2D Interaction Diagram (**B**) 3D Ligand-Receptor Complex (**C**) View of the Binding Pocket Surface

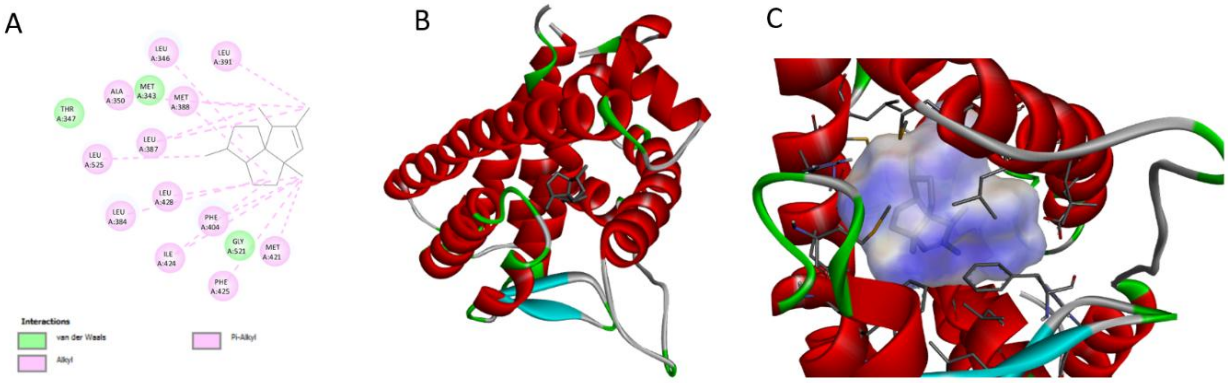

Figure 6. Interaction of the 7-epi-Silphiperfol-5-ene ligand with the 5WGD receptor (**A**) 2D Interaction Diagram (**B**) 3D ligand-receptor complex (**C**) View of the binding pocket surface

Figure 5 shows the interaction of the gamma-eudesmol compound with the 3ERT receptor with hydrophobic residues such as Leu349, Leu346, Leu391, Leu387, Ala350, and Met388 through alkyl and π-alkyl bonds. Weak binding through van der Waals bonds also occurs through residues Gly521, Ile424, Arg394, and Glu353. These interactions also indicate that the stability of the gamma-eudesmol-3ERT complex is supported by hydrophobic interactions, while other residues provide additional contributions through weak interactions.

The results of the interaction between 7-epi-silphiperfol-5-ene and the 5WGD receptor are shown in **Figure 6.** This interaction occurs through hydrophobic bonds (alkyl and π-alkyl) with residues Leu346, Leu391, Leu387, Leu428, Leu384, Met388, Met421, Ala350, Phe404, and Phe425. In addition, there are weak van der Waals interactions with residues Met343, Thr347, and Gly521, which contribute to the stability of the complex. The 3D image of the surface pocket also confirms that the 7-epi-silphiperfol-5-ene compound is stably bound in the hydrophobic cavity with a lock-and-key fit.

This in silico molecular docking test shows that essential oil from the leaves of *B. balsamifera* has potential as an anticancer drug candidate. This potential is indicated by the binding affinity value, RMSD, and interactions between the compound and amino acid residues through hydrogen bonds, hydrophobic (alkyl and π-alkyl) bonds, and van der Waals forces. Therefore, further in vitro MTT research is needed to confirm this anticancer activity

Anticancer Potential of Essential Oils Against T47D and MCF-7 Breast Cancer Cell

The Anticancer potential of essential oil from the leaves of *B. balsamifera* was determined using the MTT (Microculture tetrazolium) method to determine the ability of essential oils to inhibit cell growth, through the percentage of cell viability from the reduction of tetrazolium salt into formazan crystals by the enzyme succinate dehydrogenase (Ismaryani *et al.*, 2018). The absorbance measurement results are shown in **Figure 7**.

Figure 7 shows that there is a relationship between concentration and absorbance value. The higher the concentration, the lower the absorbance obtained. The higher the concentration, the more active compounds can inhibit cell growth. As a result, the number of living cells will decrease, so the succinate dehydrogenase enzyme needed to reduce tetrazolium salt into formazan crystals will also decrease. Therefore, the absorbance value decreases as the concentration of the test sample increases. The absorbance values of these various concentrations can be used to calculate the percentage of cell viability, which is the number of cells that survive after exposure to a test sample. The results are shown in **Figure 8**.

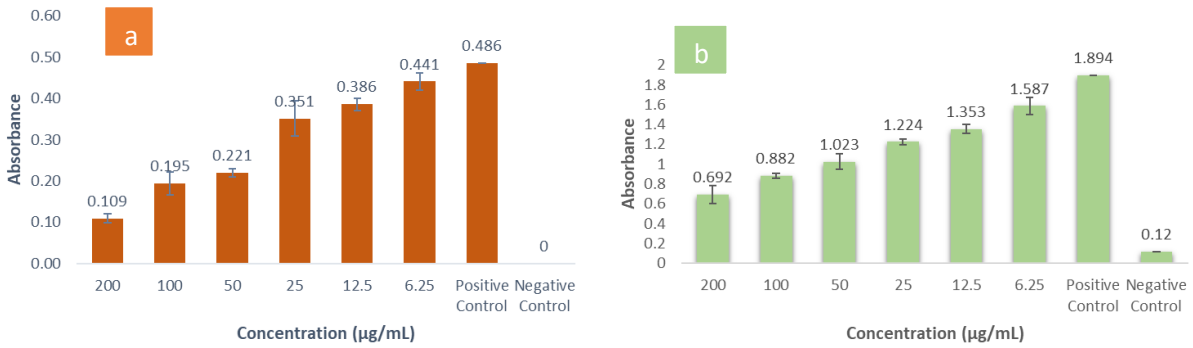

Figure 7. MTT assay absorbance values of essential oil against (**a**) T47D and (**b**) MCF-7 breast cancer cells at various concentrations. Data are presented as mean ± SD.

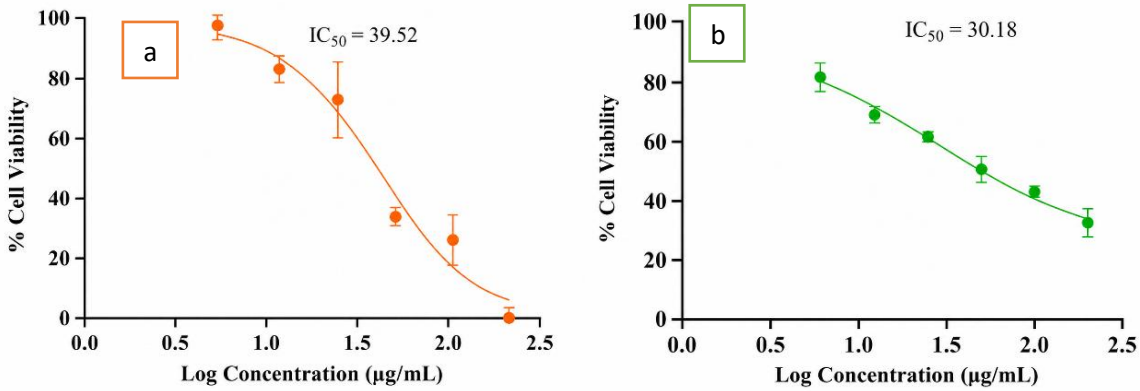

Figure 8. Relationship between log concentration of essential oil and percentage of cell viability against (**a**) T47D and (**b**) MCF-7 breast cancer cells. Data are presented as mean ± SD.

From **Figure 8.a**, the viability of T47D breast cancer cells can be determined. At the highest concentration (200 μg/mL), cell viability was 22.48%, indicating that at this concentration, approximately 77.52% of cells could not survive. Thus, cell growth could not be completely inhibited at this concentration. At a concentration of 50 μg/mL, cell viability was 45.38%, indicating that at this concentration, more than 50% of cells could not survive. Meanwhile, at the lowest concentration of 6.25 μg/mL, cell viability was 90.69%, indicating that only about 10% of cells could survive. Thus, at this concentration, the essential oil of *B. balsamifera* leaves was not yet able to effectively inhibit the growth of T47D cancer cells.

Furthermore, **Figure 8.b** also shows the viability of MCF-7 breast cancer cells. The highest concentration of 200 μg/mL, cell viability was only 32.26%, indicating that approximately 67.74% of the cells were killed. However, complete inhibition of cell growth was not achieved at this concentration. At 50 μg/mL, cell viability was 50.87%, suggesting that nearly half of the cells had lost their ability to survive. In contrast, at the lowest concentration of 6.25 μg/mL, cell viability remained high at 82.67%, with only about 18% of the cells unable to survive.

The cytotoxic results using the MTT method produced an IC_{50} value, which represents the concentration required to inhibit cell proliferation by 50%. Based on the results, the essential oil of *B. balsamifera* leaves showed IC_{50} values of 39.52 μg/mL (95% CI: 34.13–45.80 μg/mL) against T47D cells and 30.18 μg/mL (95% CI: 16.46–54.48 μg/mL) against MCF-7 cells. The IC_{50} values in both cancer cells were categorized as moderately cytotoxic and have the potential to be further developed as anticancer drugs. This classification refers to the criteria of the National Cancer Institute (NCI), which stipulates that highly cytotoxic compounds have an $IC_{50} \leq 20$ μg/mL, moderately cytotoxic compounds are in the range of 21–100 μg/mL, weakly cytotoxic compounds are 100–200 μg/mL, and non-cytotoxic if $IC_{50} > 200$ μg/mL (Zulkipli et al., 2024).

The cytotoxic activity of *B. balsamifera* leaves essential oil is influenced by the chemical components contained in the oil. Based on the GC-MS analysis, this essential oil is dominated by terpene compounds, which have been reported to possess various pharmacological activities, including anticancer activity (Kamran et al., 2022). Several major compounds identified in this study, such as camphor, caryophyllene, 7-epi-silphiperfol-5-ene, and γ-eudesmol, have also been reported to exhibit cytotoxic activity against various cancer cell lines, as presented in **Table 2.**

The activity against T47D and MCF-7 cells observed in this study may be related to the presence of these compounds. Molecular docking analysis also showed favorable interactions with ER-α, which plays an important role in the growth and proliferation of breast cancer cells. Previous studies reported that caryophyllene may induce apoptosis and inhibit cell proliferation through ROS-related pathways and caspase activation (Fidyt et al., 2016; Dahham et al., 2021). Camphor has also been reported to exhibit anticancer activity through apoptosis induction and increased ROS production (Agus et al., 2018; Zhang et al., 2019). In addition, γ-eudesmol has also been associated with apoptosis induction through mitochondrial dysfunction and caspase activation. Therefore, the cytotoxic activity observed in this study may be related to the potential activity of these terpenoid compounds. However, further studies are still needed to confirm the specific molecular mechanism.

CONCLUSIONS

Essential oil from the leaves of *B. balsamifera* contains 36 chemical compounds, with the main components being camphor (32.56%), caryophyllene (17.04%), 7-epi-silphiperfol-5-ene (5.33%), and gamma-eudesmol (5.01%). This essential oil has very strong cytotoxic activity against *A. salina*. shrimp larvae with an LC_{50} value of 17.17 μg/mL. Molecular docking tests showed moderate to strong binding affinity, where caryophyllene, 7-epi-silphiperfol-5-ene, and gamma-eudesmol showed stronger binding affinity than camphor. In addition, the anticancer potential test using the MTT method also showed promising activity against breast cancer cells, with IC_{50} values of 39.52 μg/mL in T47D cells and 30.18 μg/mL in MCF-7 cells. Further studies are still needed to evaluate the anticancer activity in vivo and to confirm the specific molecular mechanism of the major compounds in this essential oil.

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